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Schrier's Diseases of the Kidney

VOLUME I

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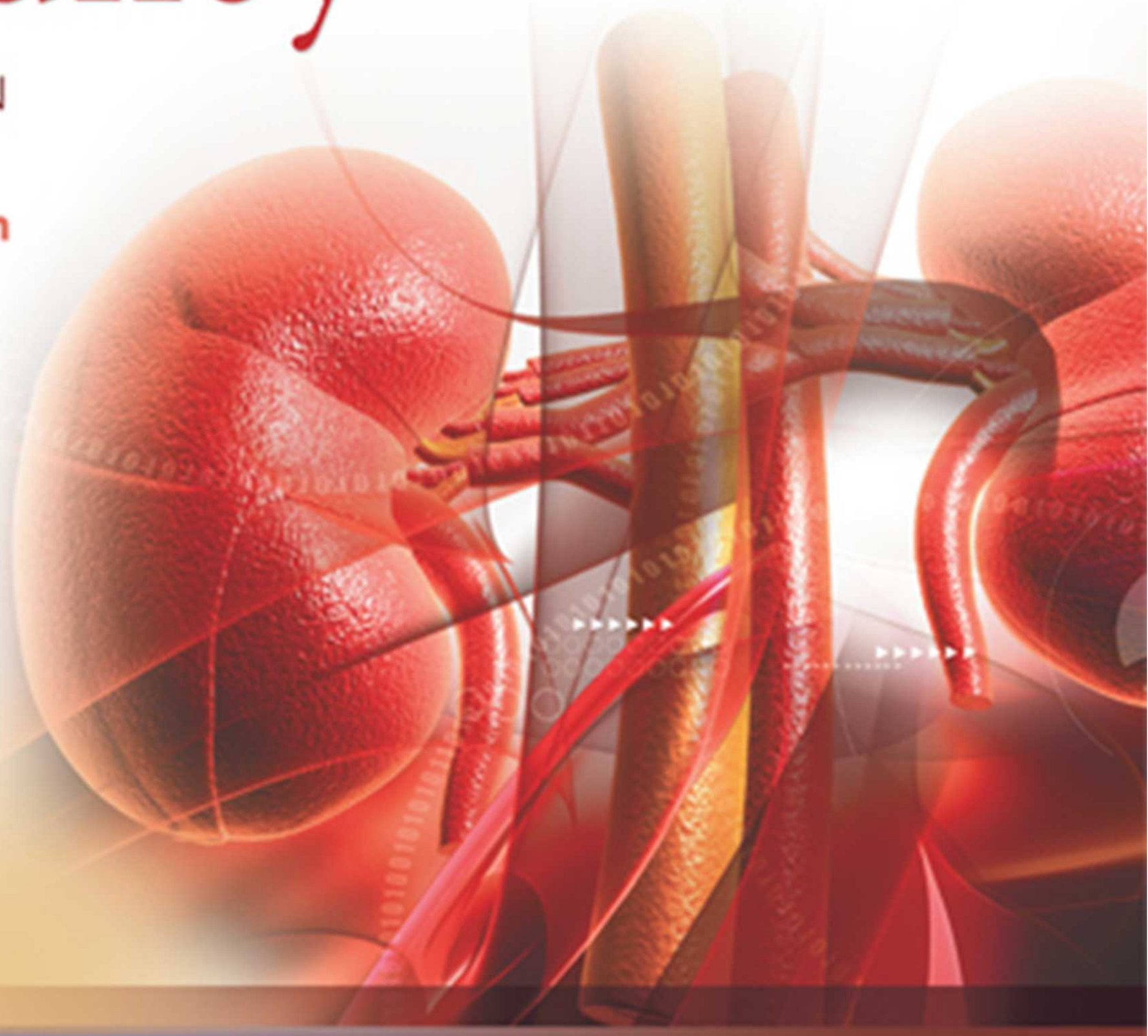

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SCHRIER'S DISEASES OF THE KIDNEY

NINTH EDITION

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VOLUME I

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CONTENTS

**Deceased.*

PREFACE

The recent advances in many aspects of kidney diseases have mandated a new edition of Schrier's Diseases of the Kidney. As in previous editions, a group of international experts was assembled to present this information in a comprehensive, authoritative, concise, and readily accessible fashion. The chapters have been extensively revised and updated.

Nephrology is a discipline that combines the basic and clinical sciences. Successful integration of this knowledge is the goal of this Ninth Edition. The 11 sections of the two-volume book are actually individual texts that can stand on their own.

The first section presents an overall view of the structural, physiologic, and biochemical aspects of the kidney. This section incorporates the latest developments in cellular and molecular biology, emphasizing the most current information and concepts on cell signaling, receptors, and ion channels. The subsequent 10 sections are disease oriented, with each section beginning with a pathophysiology chapter. The goal of Schrier's Diseases of the Kidney is to publish the most comprehensive material for practicing and academic physicians caring for patients with kidney disease and hypertension. The 11 sections of the book cover 86 chapters and are summarized as follows:

I **Structural and Functional Correlations in the Kidney** includes structural, hemodynamic, hormonal, ion transport, and metabolic functions in eight chapters.

II **Clinical Evaluation** is covered in five chapters on urinalysis, laboratory evaluation, urography, tomography, angiography, with indications and interpretations for renal biopsy.

III **Cystic and Tubular Disorders** in seven chapters covers genetic mechanisms, medullary cystic and sponge disorders, polycystic kidney disease, Alport syndrome, Fabry disease, and nail-patella syndrome, as well as isolated renal tubular disorders.

IV **Infections of the Urinary Tract and the Kidney** are contained in seven chapters, including host-defense mechanisms; urinary bacterial infections, including tuberculosis as well as fungal infections; renal abscesses; and cystitis.

V **Acute Kidney Injury** is described in 11 chapters, including the pathophysiology of renal cell ischemia and nephrotoxic injury, acute tubular necrosis, acute interstitial nephritis, and acute nephrotoxic renal disease.

VI **Hypertension** and its renal manifestations are covered in six chapters, which include pathophysiology, renal vascular, and endocrine-related hypertension as well as hypertension in pregnancy and in diabetes.

VII **Glomerular, Interstitial, and Vascular Renal Diseases** are discussed in 13 chapters, including collagen vascular diseases, chronic interstitial nephritis, primary glomerulonephritides, and vasculitides.

VIII **Systemic Diseases of the Kidney** are covered in eight chapters, including diabetes, hepatorenal syndrome, sickle cell disease, gout, myeloma/amyloidosis, and tropical diseases.

IX **Disorders of Electrolyte, Water, and Acid Base** are covered in nine chapters, including syndrome of inappropriate antidiuretic hormone secretion, central and nephrogenic diabetes insipidus, cardiac failure, cirrhosis, and the nephrotic syndrome.

X **Chronic Kidney Disease,** a section of six chapters, covers pathophysiology, anemia, osteodystrophy, the nervous system, cardiovascular complications, and metabolic and endocrine dysfunctions.

XI **Management of End-Stage Renal Disease** by transplantation, peritoneal dialysis and hemodialysis, including complications, outcomes, and ethical considerations, is discussed in six chapters.

As editors, we have substantial expertise in most of these areas of nephrology and are very pleased with the content of the Ninth Edition. Out of 86 chapters, 44 have new authors. We would like to thank our authoritative and remarkably talented contributing authors, whose dedication to nephrology is unmatched.

Thomas Coffman, MD
Ronald Falk, MD
Bruce Molitoris, MD
Eric Neilson, MD
Robert W. Schrier, MD

CHAPTER 1

Structural–Functional Relationships in the Kidney

Wilhelm Kriz • Astrid Weins • Ruth Ellen Bulger

STRUCTURE–FUNCTION CORRELATIONS ALONG THE RENAL STRUCTURE

The kidney functions as it does, in large part, because of its architecture. In no instance is this more evident than in the urinary concentrating mechanism, where the complex nephron and vascular interrelationships permit the coordinated function of different nephron and vascular elements into countercurrent multiplication and exchange processes. A recent proliferation of detailed structural, biochemical, and functional information has led to an appreciation of other structural–functional relationships that are relevant to solute and water handling by the kidney. Moreover, it has become evident that an understanding of the pathologic developments in kidney diseases is only possible on the basis of a thorough knowledge of kidney structure.

The purpose of this chapter is to review some of the recent findings, with special emphasis on structural–functional relationships, to enhance our understanding of overall renal function; therefore, this chapter is divided into two parts. The first part considers the structural and functional interrelationships of each morphologic segment of the urinary tubule, stressing the unique characteristics of each segment. The second part discusses structure and function in terms of more general mechanisms used by several segments of the renal tubule to accomplish specific functions, such as ion or water transport.

FORM OF THE HUMAN KIDNEY

Human kidneys are paired, bean-shaped organs situated in a retroperitoneal position on the posterior aspect of the abdominal cavity, on either side of the vertebral column against the psoas major muscle. A fibrous capsule located within the perirenal adipose tissue and surrounded by perirenal fascia surrounds each kidney. The lateral border of each kidney is convex. The kidneys of an adult man weigh approximately 120 to 170 g each and measure roughly 11 × 6 × 2.5 cm; those of an adult woman weigh slightly less and are somewhat smaller. In both men and women, total kidney mass best correlates with body surface area.

The concave medial margin has a slitlike aperture, called the renal hilum. Branches of the renal artery, vein, nerves, lymphatics, and the expanded pelvis of the ureter pass through the hilum. The hilum communicates with a flattened space within the kidney called the renal sinus. Within this space, the renal pelvis branches into major and minor calyces.

Sections through the kidney reveal the cortex and medulla (Fig. 1.1). The human kidney is a multilobar organ containing 4 to 18 (average, 8) pyramids of medullary substance[1] and is situated so that their bases are adjacent to the cortex. The darker red cortical substance covers the base of each medullary pyramid like the cap of an acorn. During fetal life, the kidney surface is demarcated by clefts that gradually disappear in the normal adult kidney. The apex of each medullary pyramid (called the papilla) extends into the renal sinus and is capped by a funnel-shaped, minor calyx. The minor calyces receive the urine that is released from the kidney into the extrarenal collecting system. A lobe of the kidney is composed of the conical medullary pyramid and the surrounding cortical substance. During fetal development, some lobes may fuse and calyces are remodeled so that the mature kidney has fewer calyces and papillae than the original number of papilla anlagen[1]; one calyx may drain a fused papilla developed from up to four anlagen, predominantly at the kidney poles. Striated elements called medullary rays extend peripherally at intervals from the bases of the medullary pyramids and penetrate into the cortex. These rays resemble the medulla in structure and although they extend deeply into the cortex, they are part of the cortex. The rest of the cortex is called the cortical labyrinth.

The medulla can be subdivided further either grossly or microscopically (Fig. 1.2). The medulla has an outer zone that is adjacent to the cortex and an inner zone that includes the papilla. The outer zone is subdivided into an inner and outer stripe. This zonation is important because it represents the location and orientation of the various segments of the renal tubules within the kidney.

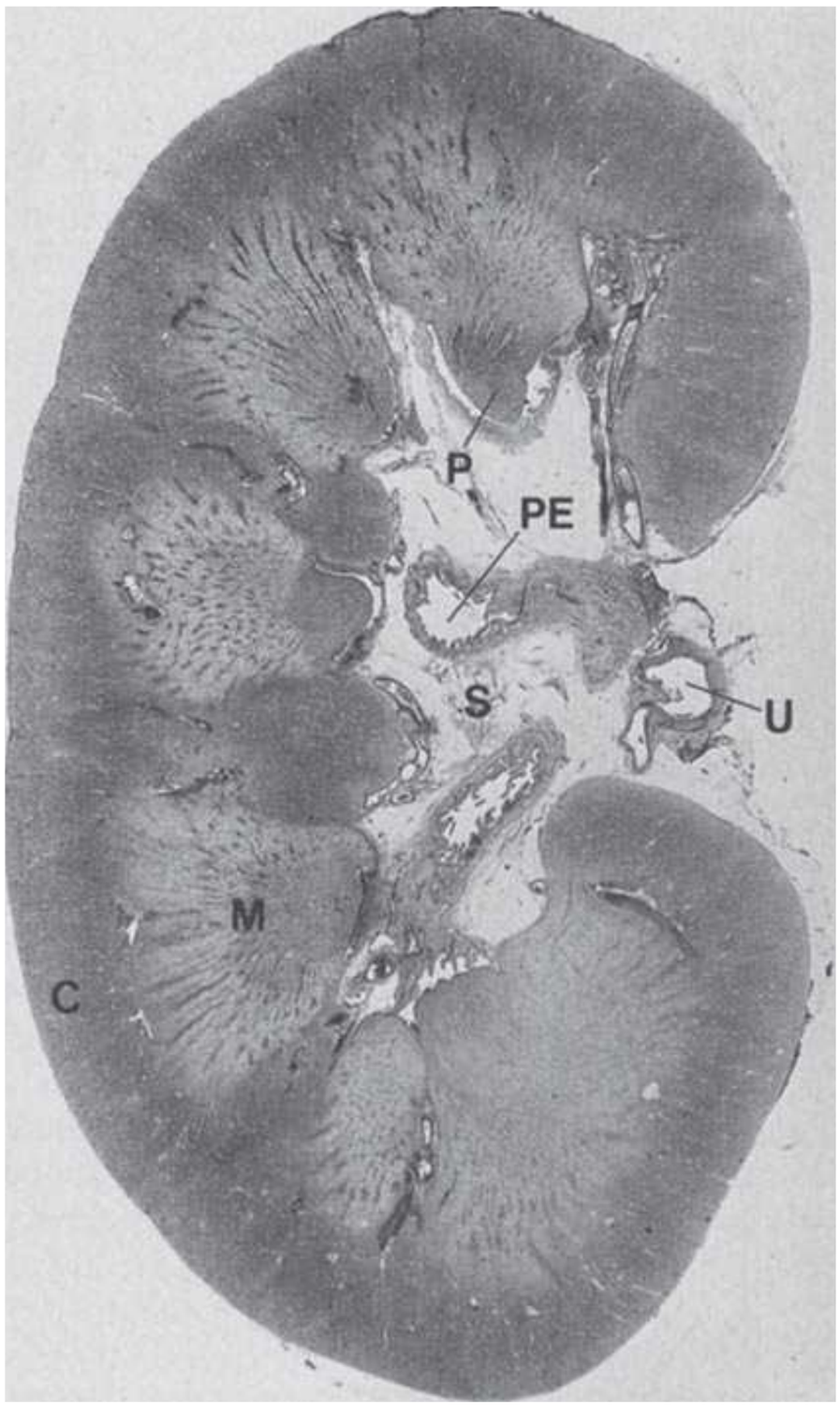


FIGURE 1.1 Gross anatomic appearance of a human kidney. A paraffin section through the whole kidney shows elements of the internal structure: *C*, cortex; *M*, medulla; *P*, papilla projecting into a minor renal calyx; *PE*, pelvis; *S*, sinus; *U*, ureter.

The relative volumes occupied by the cortex, outer medulla, and inner medulla are 70%, 27%, and 3%, respectively,[2] in humans. The relative thicknesses vary considerably among mammalian species.

RENAL (URINIFEROUS) TUBULES

Human renal morphology resulted from a long evolutionary process in which animals adapted to many changing environmental conditions. The three sequential types of kidneys that evolved were the pronephros, mesonephros, and metanephros. The urogenital system of each human embryo repeats this evolutionary process. The pronephros develops first, but degenerates before attaining any functional capacity.

The mesonephric kidney functions for a short period in utero, but it also degenerates, with the notable exception of the part of the mesonephric tubules that form a portion of the excurrent duct system of the male reproductive tract. The metanephric kidney forms last and eventually becomes the functional kidney of the human. The metanephric kidney is well suited to the human condition because of its efficient filtering device and its complex tubule, which allows for the production of not only dilute urine but also concentrated urine. This process occurs only in mammals and birds. Although it is well suited for maintaining homeostasis, the mammalian kidney is an inefficient organ for the elimination of salt and water. In humans, 180 L of fluid are filtered into the tubular lumen every 24 hours, of which approximately 178 L must be returned to the blood.

Each human kidney contains approximately 1 million functional units, called nephrons (with considerable interindividual variation).[3] Each nephron is made up of a renal corpuscle (glomerulus) and a complex tubular portion, which drain into a unifying tubular system called the collecting duct system. Both kinds of tubules represent the renal (or uriniferous) tubules.

The nephrons are derived from the metanephric blastema, the collecting ducts from the urethral bud. A connecting tubule lies between the nephron and collecting ducts. At present, there is controversy as to whether the connecting tubule is derived from the metanephric blastema[4–6] or the ureteric bud.[7] As is discussed in the next section, the connecting tubule has marked morphologic similarities to the cortical collecting duct (CCD).

The segmentation of the renal tubule then includes the following regions.[8]

The Nephron

I. Renal corpuscle (most of which is called glomerulus)
- A. Bowman's capsule
- B. Glomerular tuft

II. Proximal tubule
- A. Convoluted part (pars convoluta) consists of P_1 and the first part of P_2 (PCT)
- B. Straight part (pars recta) consists of the last part of P_2 and all of P_3 (PST)

III. Thin limbs of the loop of Henle (intermediate tubule)
- A. Thin descending part of short-looped nephrons (SDTL)
- B. Upper thin descending part of long-looped nephrons (LDTL up)
- C. Lower thin descending part of long-looped nephrons (LDTL lp)
- D. Ascending thin part of long-looped nephrons (ATL)

IV. Distal tubule
- A. Straight part (pars recta)
  1. Medullary thick ascending limb (MTAL), which includes regions located within the inner stripe and outer stripe of the medulla
  2. Cortical thick ascending limb (CTAL), which includes the part ascending through the cortex, the macula densa (MD), and the post macula densa segment
- B. Convoluted part (pars convoluta) (DCT)

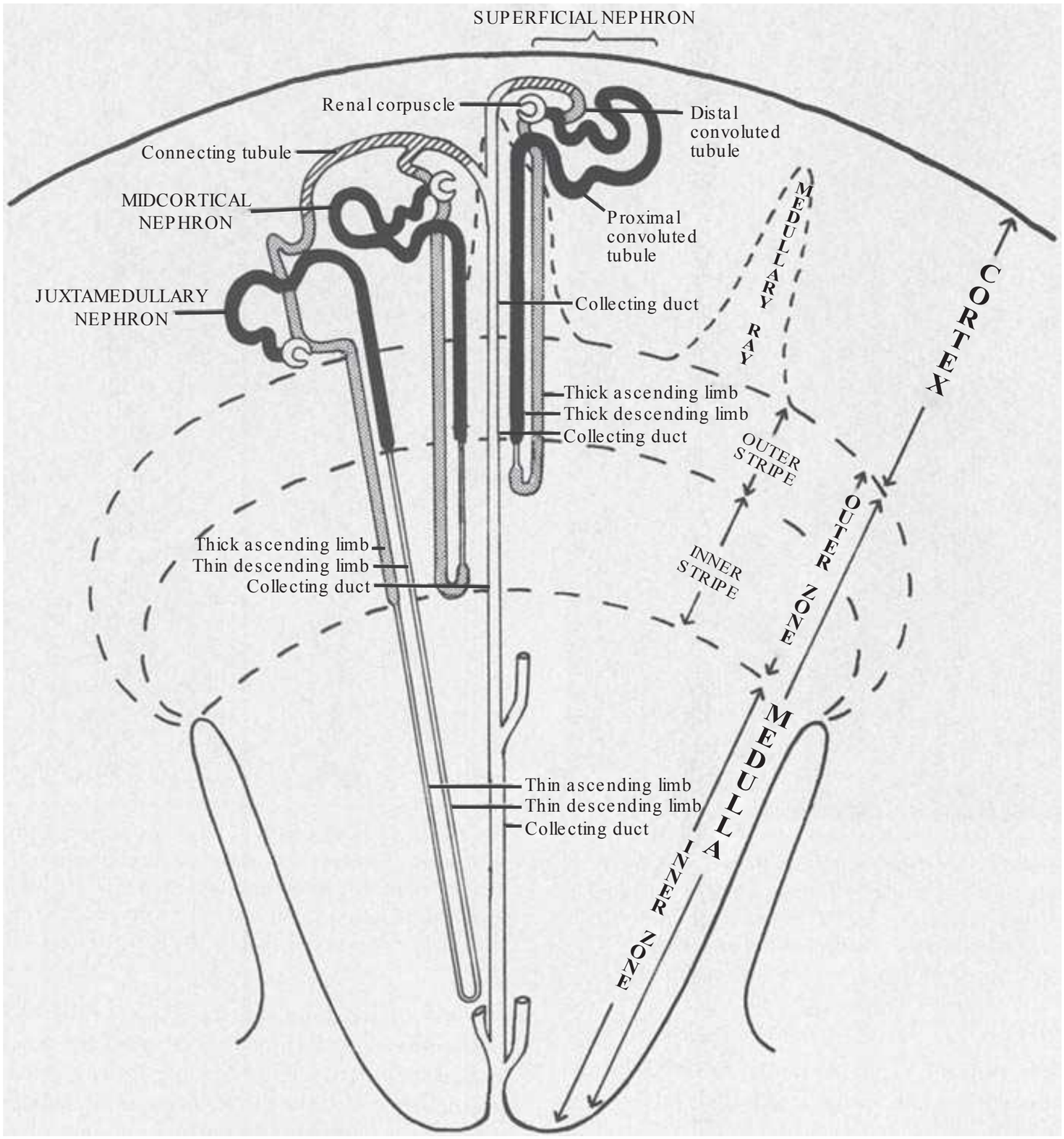


FIGURE 1.2 The relationship of nephron segments to zones of the kidney.

The Collecting Duct System

I. Connecting tubule (CNT)
II. Cortical collecting duct (or tubule) (CCD)
III. Outer medullary-collecting duct (or tubule) (OMCD)
IV. Inner medullary-collecting duct (or tubule) including the papillary collecting ducts (also called the ducts of Bellini; IMCD)

Nephrons lie in characteristic positions (Fig. 1.2), with the renal corpuscles and proximal convoluted segments in the cortex (Figs. 1.3 and 1.4). The straight part of the proximal tubule, the thin limb segments, and the straight part of the distal tubule form the loop of Henle, which enters a medullary ray of the cortex and extends into the medulla, where it bends, returning to the cortex by means of the same medullary ray. The loops of juxtamedullary nephrons directly connect the outer stripe of the medulla without ever being contained in a medullary ray. As the straight part of the distal tubule returns to the cortex, it passes by the renal corpuscle from which the nephron originated, forming the macula densa; then, after a short postmacula densa segment, it continues as the distal convoluted tubule within the cortex.

The morphology of the nephron varies with the position of the renal corpuscle in the cortex. Each nephron is classified as superficial, midcortical, or juxtamedullary, according to the position of its renal corpuscle within the respective regions of the cortex (Fig. 1.2) and the pattern of efferent vessel formation.[9–11] A given segment tends to occupy a specific region of the kidney, which gives rise to the gross zonation referred to in the preceding text. In the human kidney, superficial nephrons empty singly into a terminal collecting duct, whereas several juxtamedullary nephrons empty into an arched tubular portion (arcade) that courses peripherally in the cortex before it turns to enter a medullary ray. Most midcortical nephrons from humans empty individually as well.[5,12] As known from the study of several species (rats and rabbits), an arcade is established by the connecting tubule epithelium (data from studies in humans are not available).

Nephrons also are classified as short or long looped according to the location of the position where their loops of Henle turn within the kidney. Short-looped nephrons arise from renal corpuscles located in superficial and midcortical regions and have loops of Henle that turn within the outer medulla. In humans, some superficial nephrons may have loops within the cortex itself. Short-looped nephrons have

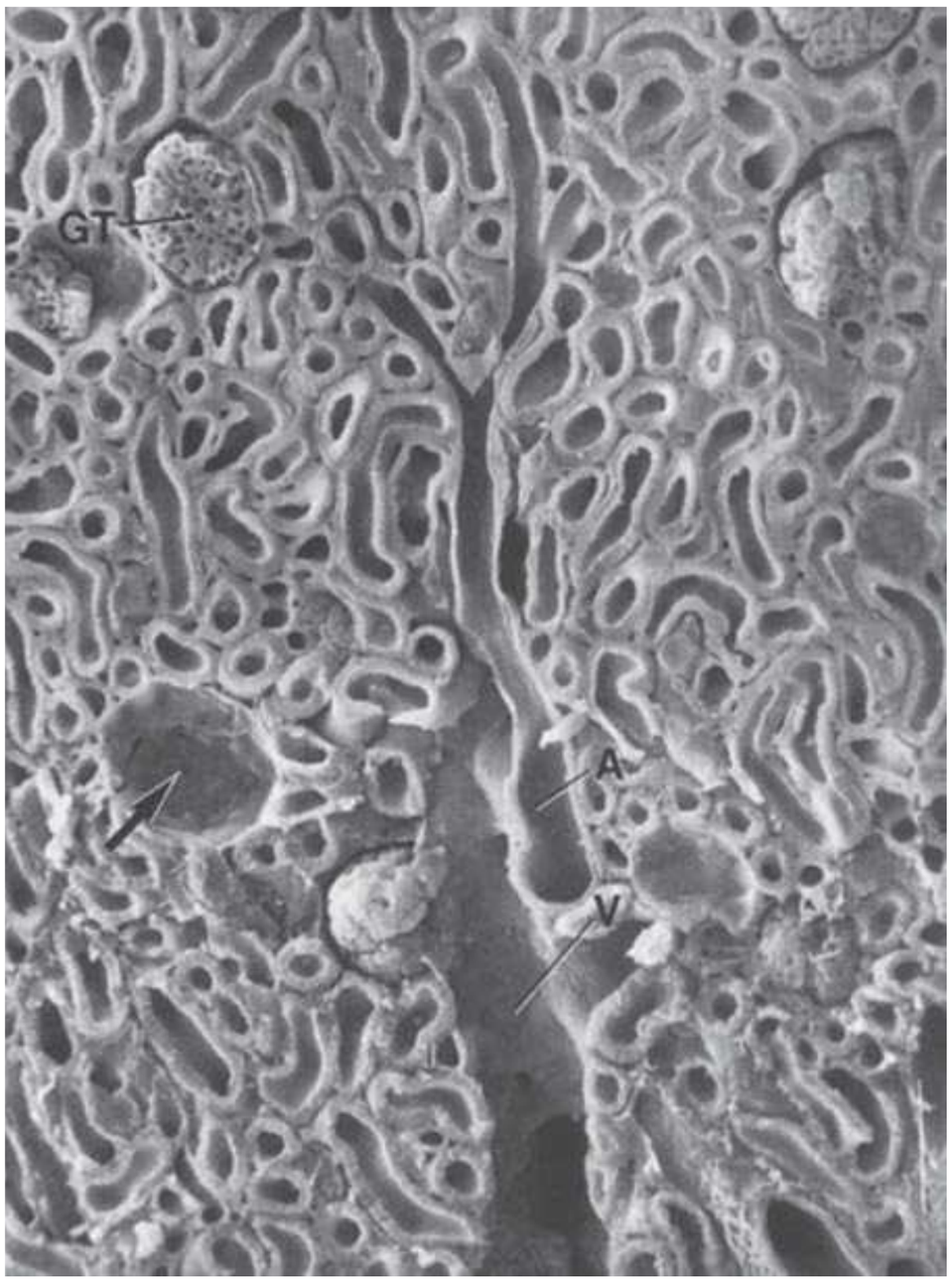


FIGURE 1.3 Scanning electron micrograph of the cortex. Convoluted tubules are shown, along with renal corpuscles, some of which contain a glomerular tuft (*GT*) and some from which the tuft is removed (*arrow*). A cortical radial artery (*A*) and vein (*V*) are also apparent. Note the thin wall of the vein. (Magnification ×140.)

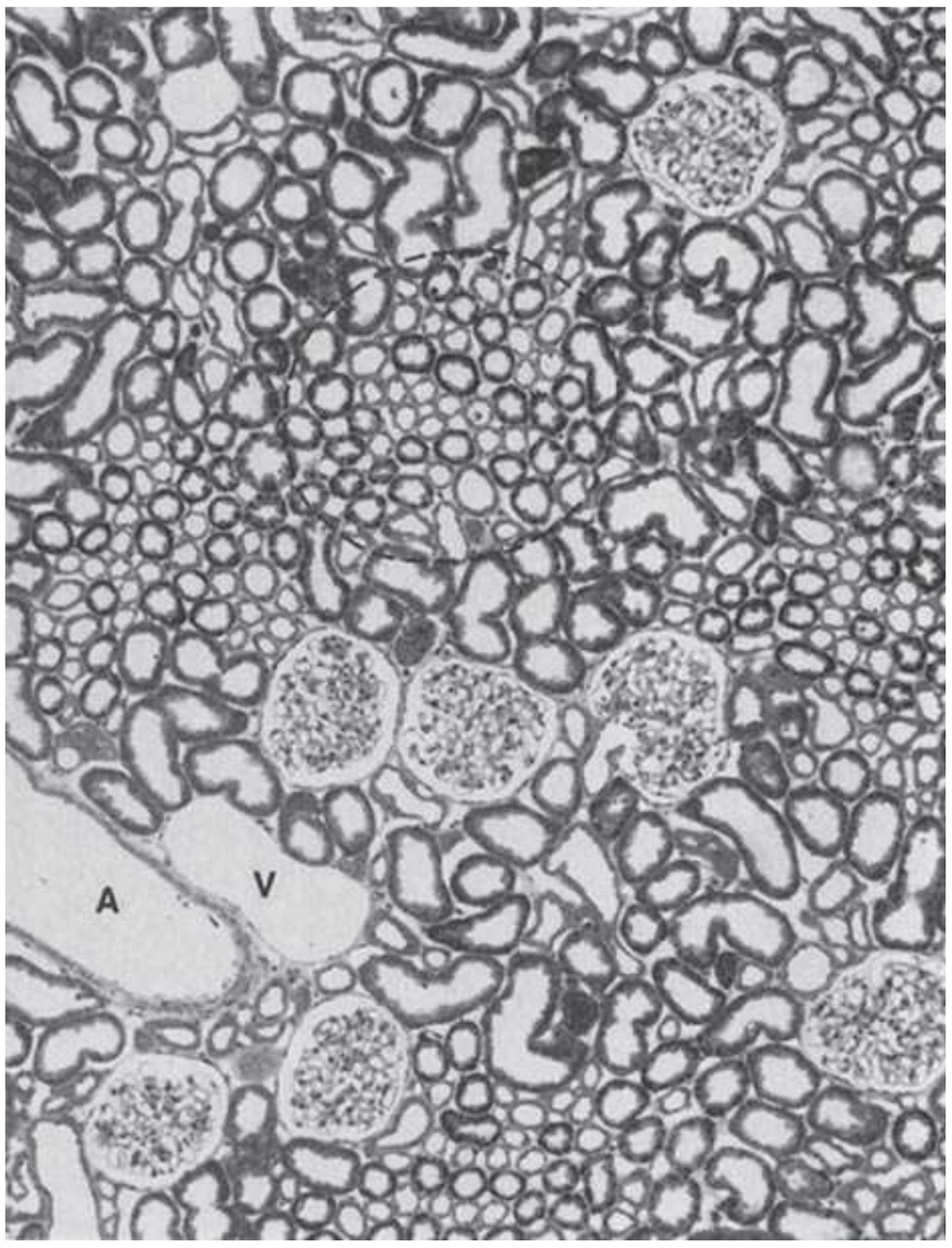


FIGURE 1.4 Light micrograph of renal cortex (rat). Cortical radial vessels (*A*, artery; *V*, vein), glomeruli, and convoluted tubules make up the cortical labyrinth. The straight tubular portions are found in the medullary rays of the cortex (one medullary ray is delineated by the *hatched line*). (Magnification ×80.)

short, thin limb segments that occur only along the descending limb. Long-looped nephrons have loops of Henle that turn within the inner medulla and have thin limb segments in both descending and ascending limbs. Although most species have both long- and short-looped nephrons, some species, such as dogs and cats, have only long ones,[13] whereas other species, such as beavers, have only short ones.[14,15] In human kidneys, the ratio of short- to long-looped nephrons is 6:1 to 7:1.

THE RENAL CORPUSCLE

The renal corpuscle (first segment of the nephron) is the site at which an ultrafiltrate of the blood is produced (Fig. 1.5). The filtrate moves from the capillary lumen into Bowman's space. This flow is influenced by the following factors: renal blood flow; the oncotic and hydrostatic pressures in the capillaries and in Bowman's space; the size, shape, and charge of plasma molecules; and the various morphologic components of the wall separating the capillary lumen from Bowman's space. The filtrate contains only barely detectable quantities of plasma proteins.[16] The filtration barrier increasingly restricts the passage of larger molecules, with very little filtration of molecules larger than albumin (70 kDa).[17]

The renal corpuscle consists of Bowman's capsule and the glomerular tuft. The latter is made up of capillaries, derived from the afferent arteriole, their supporting cells, and an envelope consisting of the glomerular basement membrane (GBM) and the visceral (podocyte) layer of Bowman's capsule (Fig. 1.5). At the vascular pole, the visceral epithelium becomes the parietal epithelium, which then transforms into the proximal tubule epithelium at the urinary pole (Fig. 1.5). The space between both layers is the urinary space (Bowman's space).

The human renal corpuscle is roughly ovoid and approximately 150 to 240 μm in diameter. The term glomerulus is widely used to refer to the entire renal corpuscle. The renal corpuscle without the parietal epithelial cells is referred to as the glomerular tuft. The afferent arteriole enters the renal corpuscle at the vascular pole, where it divides into several primary branches that each ramify to form a network of anastomosing capillaries, called a lobule. The lobule has a supporting region called the mesangium (Figs. 1.6 and 1.7). All lobules together establish the tuft; the converging mesangial regions are called the glomerular stalk, by which the tuft is connected to the extraglomerular mesangium (see Juxtaglomerular Apparatus). The capillaries coalesce toward the center of the capillary tuft to form

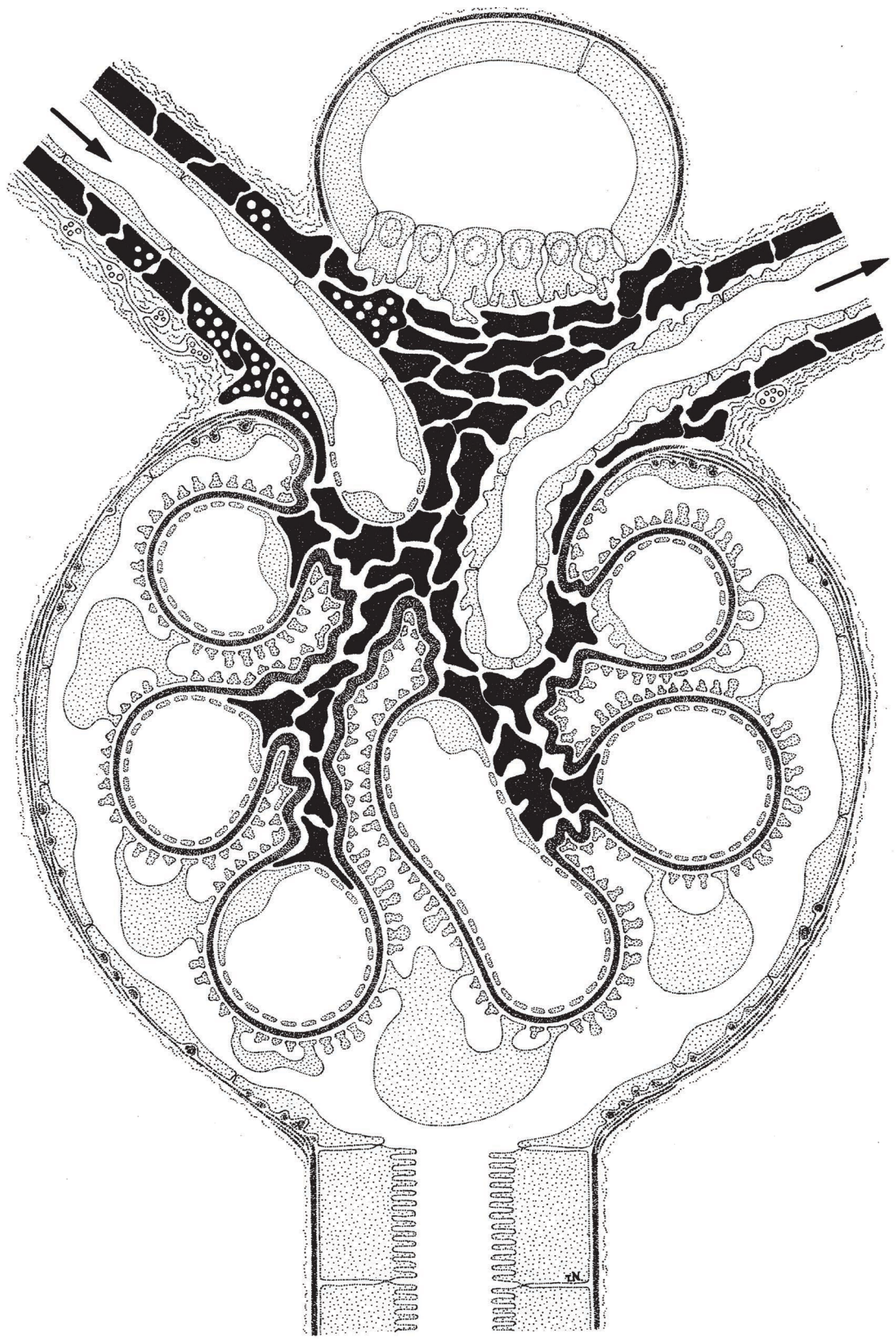


FIGURE 1.5 Schematic of a longitudinal section through a glomerulus and juxtaglomerular apparatus (*JGA*). The direction of blood flow in the glomerular arterioles is indicated by arrows. The capillary network, together with the mesangium, is enclosed in a common compartment bounded by the glomerular basement membrane (*GBM*) (shown in *dark gray*). The outer aspect of the GBM is covered by the glomerular visceral epithelium (podocytes). Note that there is no basement membrane at the interface between the capillary endothelium and the mesangium. At the vascular pole, the visceral epithelium, together with the GBM, is reflected into the parietal epithelium of Bowman's capsule, which, at the urinary pole, passes over into the epithelium of the proximal tubule. The JGA consists of the macula densa of the distal tubule, the extraglomerular mesangium (which is continuous between both arterioles and continues via the glomerular stalk into the intraglomerular mesangium), and the granular cells within the afferent arterioles. All cells that have been suggested to be of smooth muscle origin are shown in black. Note the sympathetic nerve terminals at the afferent arteriole. (From Kriz W, Sakai T, Hosser H. Morphological aspects of glomerular function. In: Davison AM, ed. *Nephrology*, Vol. 1. Proceedings of X International Congress of Nephrology, London, 1987. London: Bailliere Tindall; 1988:3, with permission.)

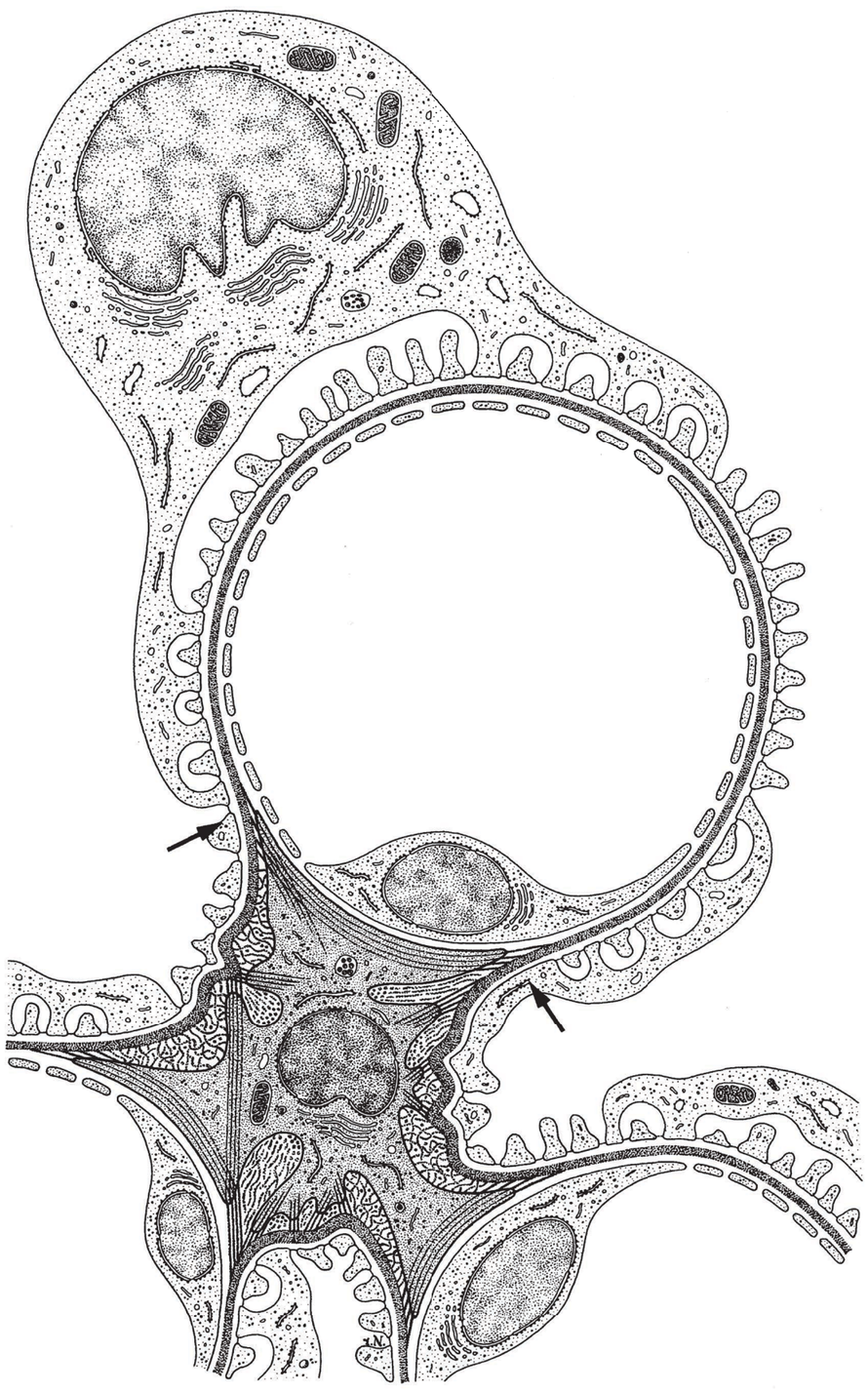


FIGURE 1.6 Schematic of a cross-section of a glomerular capillary and its relationships to the mesangium. The capillary is made up of a fenestrated epithelium. The peripheral part of the endothelium tubule is surrounded by the glomerular basement membrane (GBM; shown in *dark gray*), which, at mesangial angles (*arrows*), deviates from a pericapillary course and covers the mesangium. The outer aspect of the GBM is covered by the interdigitating pattern of podocyte foot processes. In the center, a mesangial cell is shown; its many processes contain microfilament bundles and extend toward the GBM, to which they are connected. The mesangial matrix contains an interwoven network of microfibrils. (From Venkatachalam MA, Kriz W. Anatomy of the kidney. In: Heptinstall R, ed. *Pathology of the Kidney*, 4th ed. Boston: Little, Brown; 1991, with permission.)

the efferent arteriole, which runs through the stalk and exits from the vascular pole. The efferent arteriole again breaks up to form a second capillary network, which surrounds the tubules and is called the peritubular capillary network.

The renal corpuscle, therefore, consists of the following parts: (a) the parietal epithelium, (b) the visceral epithelium (podocytes), (c) the endothelial cells lining the capillaries, (d) the glomerular basement membrane (GBM), and (e) the intraglomerular mesangial cells and matrix. In the rat, the ratio of the number of endothelial cells to mesangial cells to visceral epithelial cells is 3:2:1.[18]

The Visceral Epithelium of Bowman's Capsule

The visceral epithelial cells (nowadays generally called podocytes) are octopus-shaped cells that reside in Bowman's space and attach to the GBM only by way of their processes (see below). This shape was well described by Zimmermann[19] and is seen to advantage in scanning electron micrographs (SEM) (see later, Fig. 1.10). The exact details of cell shape differ depending

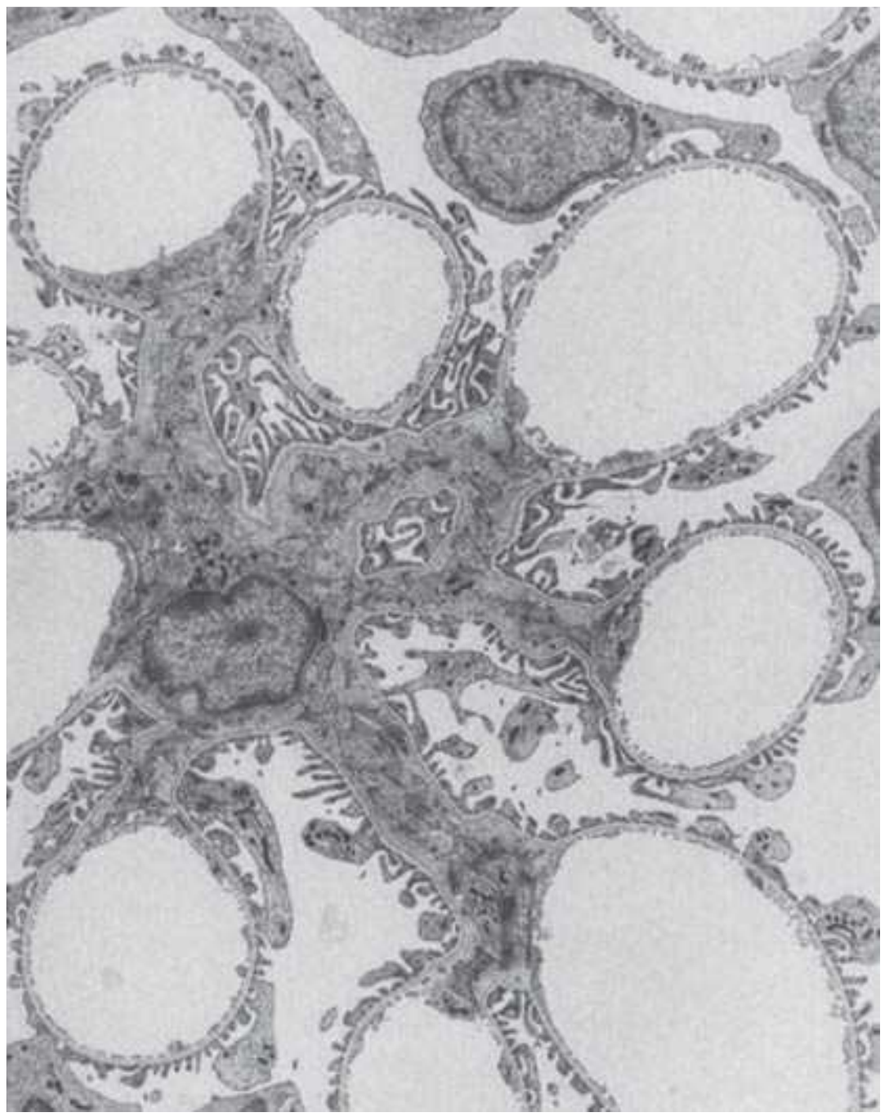
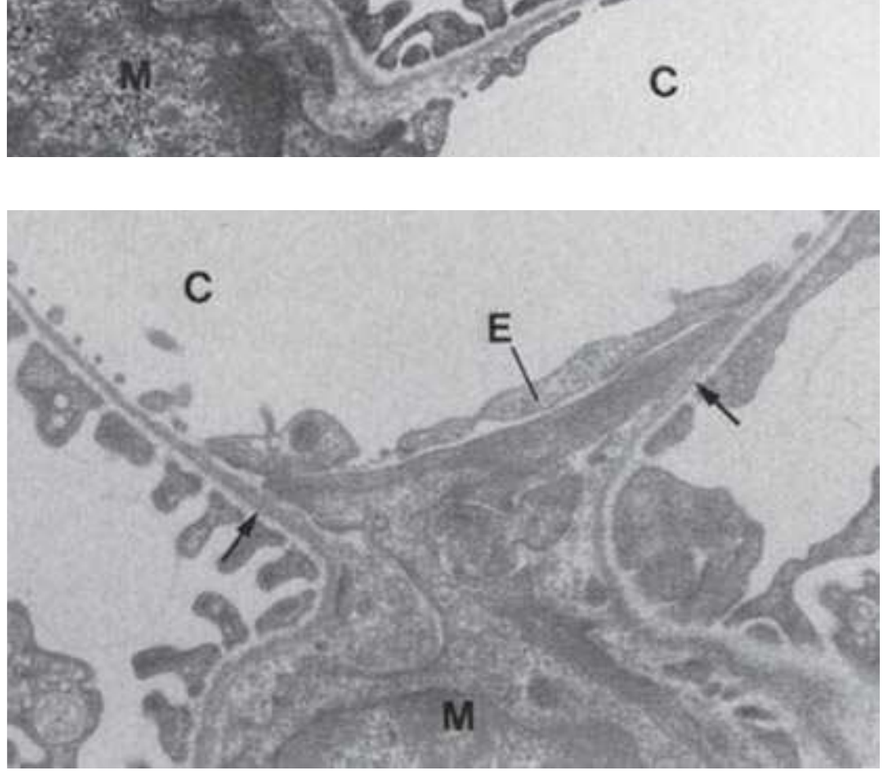


FIGURE 1.7 Transmission electron micrograph of a rat glomerular lobule. Glomerular capillaries and the glomerular mesangium occupy a common compartment enclosed by the glomerular basement membrane (GBM). The mesangial cell body (*in the center*) gives rise to many processes that fill (together with the mesangial matrix) radial arms that extend to the peripherally located capillaries. The outer aspect of the GBM is covered by podocytes. (Magnification ×3,500.)

on the species being studied.[20,21] As a consequence of the high degree of differentiation, podocytes, like neurons, are incapable of regenerative cell replication in the adult. Thus, podocytes that were lost, for any reason, cannot be replaced by new ones.[22–24] On the other hand, in glomerular diseases based on dedifferentiation of podocytes (collapsing glomerulopathy), a vivid proliferation of the dedifferentiated cells accompanies the disease.[25]

Recent observations in parietal epithelial cells question this view. First, it has been proposed that a niche of glomerular epithelial stem cells resides within the parietal epithelium at the transition to the proximal tubule.[26,27] It is an intriguing hypothesis that proliferating stem cells from this locus may transform into podocytes and may reach the tuft via the transitions of the epithelia at the glomerular vascular pole. Migration of parietal cells onto the the vascular pole and subsequent transition into podocytes have been shown to occur in the newborn mouse.[28] However, evidence that such a process may be of any relevance in the adult has so far not been presented.[28] Moreover in the adult mouse, it has been shown that proliferating parietal epithelial cells may reach the glomerular tuft via tuft–capsule bridges; there they replace podocytes causing the collapse of the concerned tuft area obviously by bringing the local cross talk between podocytes and the endocapillary compartment to an end.[29]

The cell bodies give rise to large primary processes that may branch another time, finally splitting apart into terminal processes, called foot processes (as seen from SEM pictures, "finger processes" would be a better word) or pedicels (Figs. 1.6, 1.8 to 1.11, and see Fig. 1.13). The foot processes are anchored within the GBM to a depth of about



A

B

FIGURE 1.8 Transmission electron micrographs of glomerular capillaries (*C*) and associated mesangium. **A:** A mesangial cell body (*M*) gives rise to cell processes that extend to peripherally located capillaries. Note that there is no basement membrane at the interface between the capillary endothelium and the mesangium. (Magnification × 13,000.) **B:** Capillary mesangium interface. Beneath the endothelium (*E*), tonguelike mesangial cell processes run toward both opposing turning points of the GBM (*arrows*). They contain microfilament bundles that obviously interconnect the GBM of both mesangial angles. (Magnification ×24,000.)

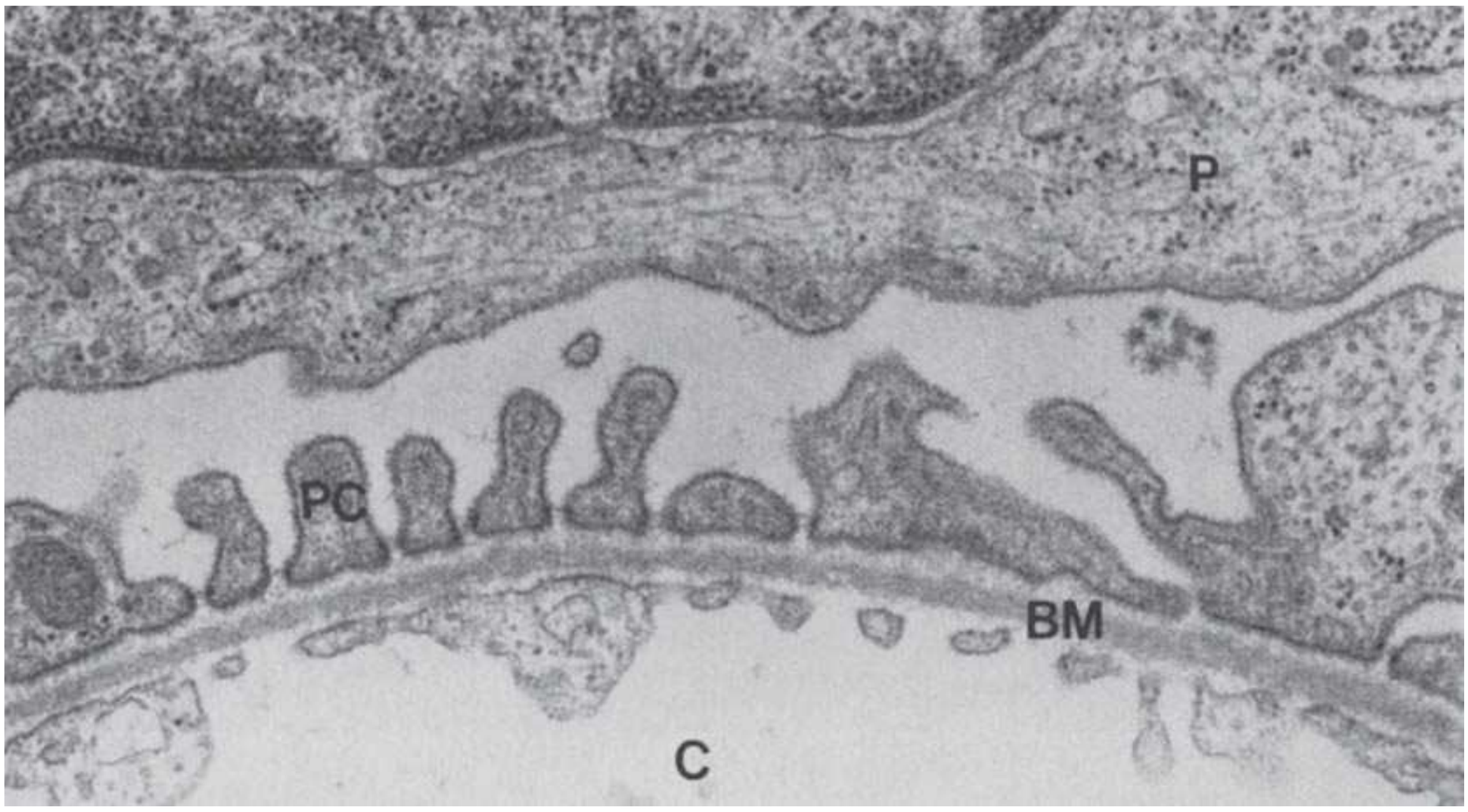


FIGURE 1.9 Transmission electron micrograph showing the podocyte (*P*), pedicels (*PC*) near the basement membrane (*BM*), and the endothelial cells lining the capillary (*C*). (Magnification ×34,000.)

40 to 60 nm. The foot processes interdigitate in a complicated manner with those from adjacent cells to form an elaborate layer of small processes along the glomerular basement membrane. This interdigitation results in the formation of an extensive series of narrow slits between the foot processes, which provide a long extracellular path for filtration of water and solutes (Fig. 1.10). In transmission electron micrographs, these slits are bridged by a layer of extracellular material (4 to 6 nm thick) called the *filtration-slit membrane* (see Figs. 1.9 and 1.13). If tannic acid is added to the fixative solution, a highly ordered isoporous substructure is revealed in en face views of the filtration-slit membrane.[30] Staggered rodlike units project from the podocyte plasmalemma and connect to a central linear bar. These rodlike units delineate rectangular pores 4 × 14 nm within the slit membrane (i.e., which approximate the size of an albumin molecule).

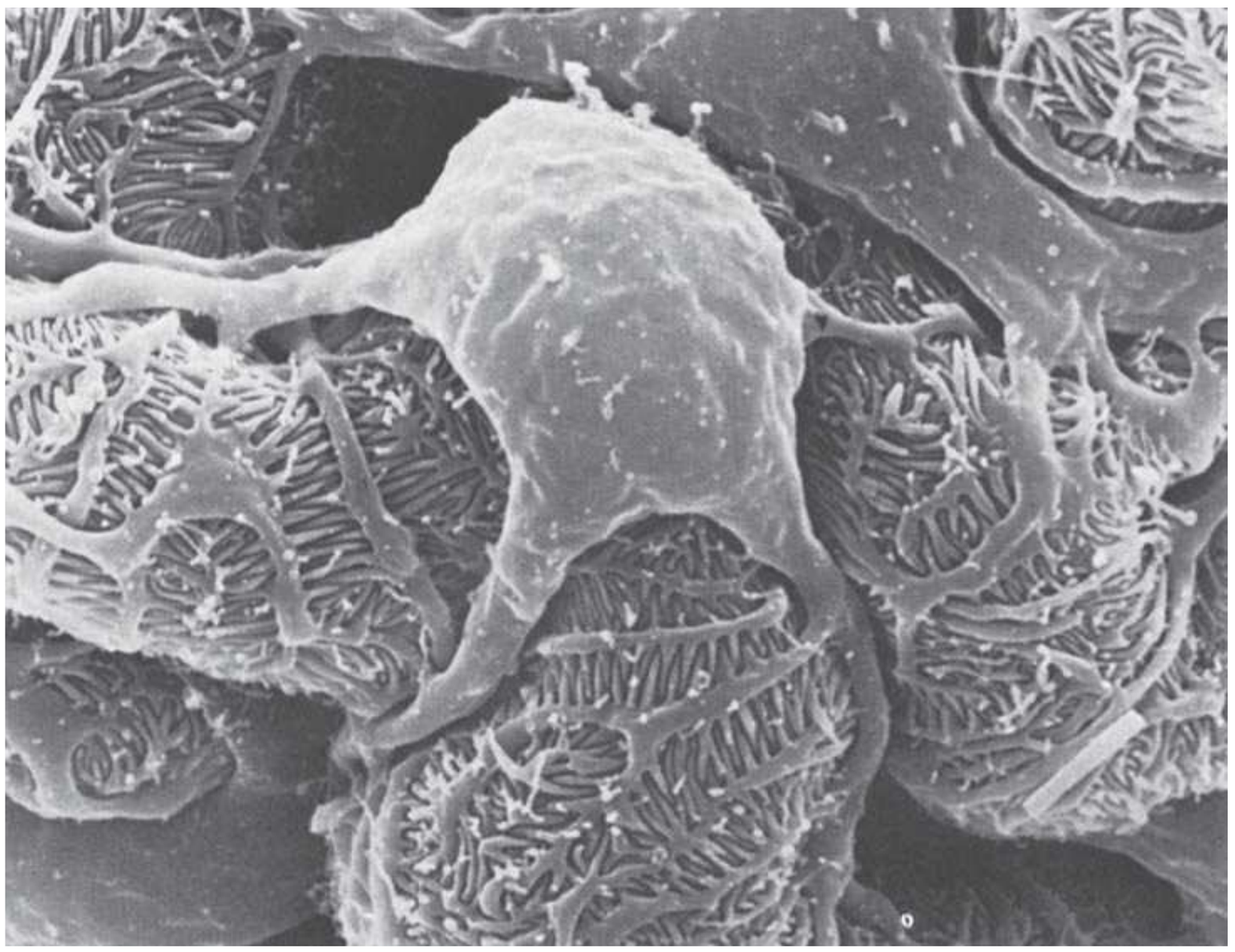


FIGURE 1.10 Scanning electron micrograph showing the elaborate cell shape of rat podocytes. (Magnification ×5,900.)

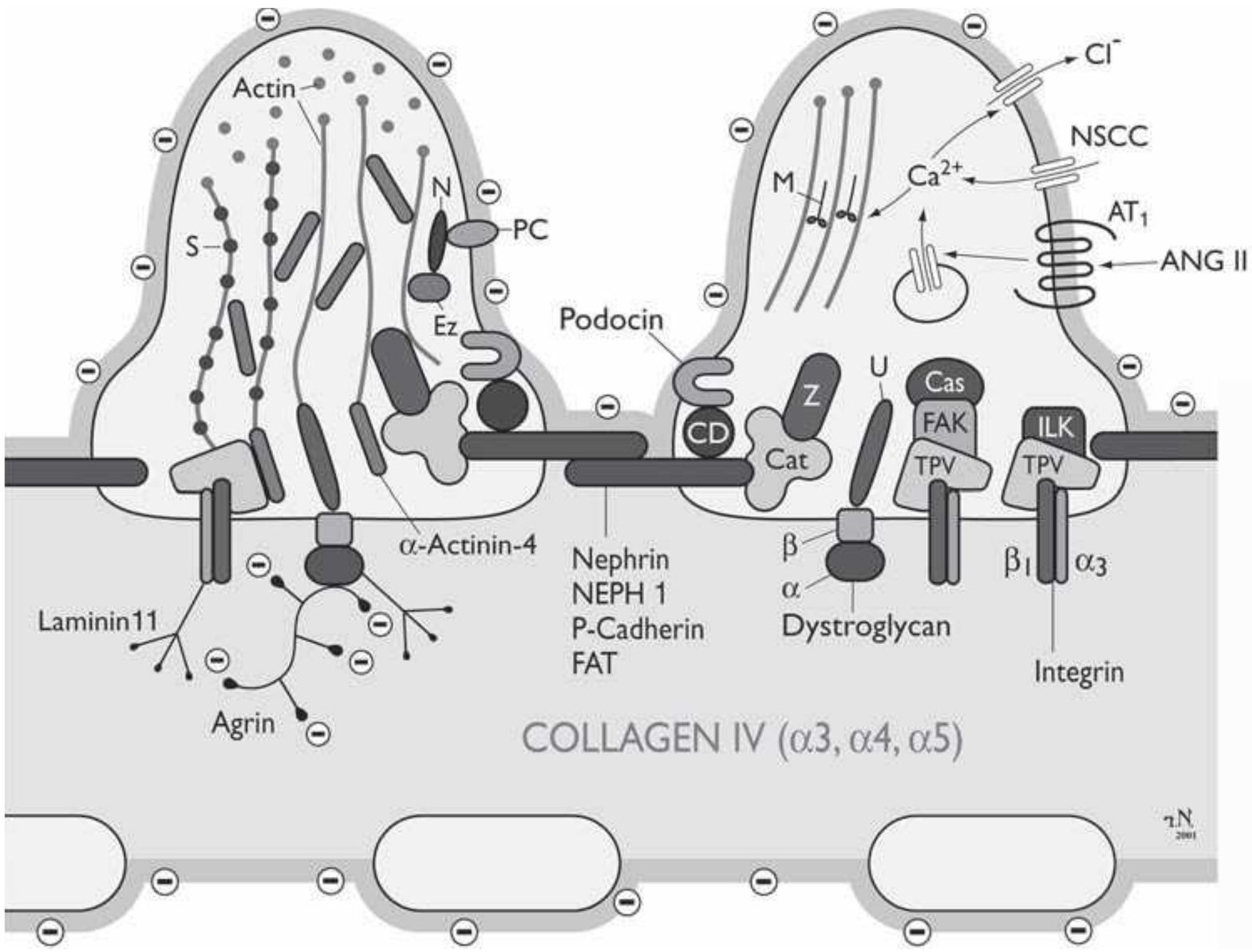


FIGURE 1.11 Schematic drawing of the molecular equipment of podocyte foot processes. *Cas*, p130Cas; *Cat*, catenins; *CD*, CD2-associated protein; *Ez*, ezrin; *FAK*, focal adhesion kinase; *ILK*, integrin-linked kinase; *M*, myosin; *N*, NHERF2; *NSCC*, nonselective cation channel; *PC*, podocalyxin; *S*, synaptopodin; *TPV*, talin-paxilinvinculin; *U*, utrophin; *Z*, ZO-1. See text for further explanations. (From Endlich K, Kriz W, Witzgall R. Update in podocyte biology. *Curr Opin Nephrol Hypertens*. 2001;10:331, with permission.)

Generally, the slit diaphragm is considered as an adherenslike intercellular junction.[31] Intensive research in recent years has uncovered several transmembrane proteins that participate in the formation of the slit membrane (Fig. 1.11)—including P-cadherin,[31] nephrin,[32] Neph1,[33] and FAT.[34] Other molecules, such as ZO1,[35] Podocin,[36] CD2AP,[37] and catenins mediate the connection to the actin cytoskeleton (see below). Nephrin is a member of the immunoglobin superfamily (IgCAM); its gene, NPHS1, has been identified as the gene whose mutations cause congenital nephrotic syndrome of the Finnish type.[32] In addition to its role as a structural component, nephrin acts as a signaling molecule that can activate MAP kinase cascades.[38] Neph1 is considered as a ligand for nephrin. Podocin belongs to the raft-associated stomatin family, whose gene, NPHS2, is mutated in a subgroup of patients with autosomal recessive steroid-resistent nephrotic syndrome.[36] These patients show disease onset in early childhood and rapid progression to end-stage renal failure. Podocin interacts with nephrin and CD2AP.[39] FAT is a novel member of the cadherin superfamily with 34 tandem cadherinlike extracellular repeats and a molecular weight of 516 KDa.[40] Because FAT has a huge extracellular domain, it is speculated that it dominates the molecular structure of the slit membrane[34]; the FAT mutant mouse fails to develop a slit membrane.[41] P-cadherin[31] is thought to mediate with its intracellular domain the linkage to β- and γ-catenin, a complex which then connects to the actin cytoskeleton via α-catenin and α-actinin. Taken together, many components of the slit membrane are known, but an integrative model of its substructure including all components is thus far lacking.

The cell body of podocytes contains a large nucleus that tends to be indented in the region of the large Golgi apparatus. Furthermore, it houses abundant rough-surface endoplasmic reticulum; individual cisternae, generally arranged in one complex per cell, are widened and filled with fine granular material of varying electrondensity—their relevance is unknown. In addition to the synthesis of membrane proteins necessary to supply the huge surface of their processes, podocytes in the adult synthesize and secrete all components of the GBM[42] (see below). On the other hand, abundant multivesicular bodies (predominantly found in the large cell processes) demonstrate strong catabolic activity in podocytes.

Podocytes contain a well-developed cytoskeleton that accounts for the unique shape of the cells and the maintenance of the processes. In the cell body and the primary processes, microtubules and intermediate filaments, such as vimentin and desmin, dominate, whereas microfilaments are densely accumulated in the foot processes. Here, they are part of a complex contractile apparatus.[43] The microfilaments form loop-shaped bundles, with their limbs running along the longitudinal axis of the foot processes. The bends of these loops are located centrally at the transition to the primary processes and are probably connected to the microtubules by the microtubule-associated protein τ.[44] Peripherally, the actin bundles appear to be anchored in the dense cytoplasm associated with the cell membrane of the soles of foot processes, and are dynamically linked to the slit diaphragm complex (discussed earlier). The importance of the podocyte actin cytoskeleton is emphasized by the discovery of inherited forms of focal segmental glomerulosclerosis (FSGS) caused by

mutations in actin-binding proteins: α-actinin-4 is a widely expressed homodimeric protein that bundles and crosslinks actin, and is highly expressed within podocyte foot processes. It interacts with a variety of other adhesion and signalling molecules. Several mutations in the ACTN4 gene encoding this protein cause a late-onset, autosomal dominant form of kidney failure.[45,46] More recently, nine independent missense mutations in INF2, which encodes a member of the formin family of actin-regulating proteins, were shown to segregate with FSGS in 11 unrelated families.[47]

Specific transmembrane matrix receptors anchor the podocyte foot processes to the GBM. Two systems are so far known. The first is a specific integrin heterodimer consisting of $\alpha_3\beta_1$ integrins. Within the GBM, these integrins bind to collagen type IV, fibronectin, and laminin 11.[48,49] Second, a dystroglycan complex connects the intracellular molecule utrophin to laminin 11, agrin, and perlecan in the GBM.[50,51] Both integrins and dystroglycans are coupled via adapter molecules (paxillin, vinculin, α-actinin, etc.) to the podocyte cytoskeleton, allowing outside-in and inside-out signaling as well as transmission of mechanical force in both directions.

In addition to the actin cytoskeleton within the foot processes, a subplasmalemmal actin system is found in podocytes.[43] This actin network is connected to the transmembrane sialoprotein podocalyxin, which represents the major protein of the negatively charged surface coat of podocytes.[52,53] The cell coat has the characteristics of a glycocalyx that contains sialic acid. A decrease in the content of sialic acid is associated with a podocyte foot process effacement and results in protein leakage through the filter, which has been shown under a great variety of circumstances.[54–58]

A huge body of data has been accumulated in recent years concerning the inventory of receptors and signaling processes starting from them in podocytes. cGMP signaling (stimulated by atrial natriuretic peptide [ANP], brain natriuretic peptide [BNP], and C-type natriuretic peptide [CNP], as well as by nitric oxide [NO]), cAMP signaling (stimulated by prostaglandin E_2, dopamine, isoproterenol, parathyroid hormone [PTH]/PTH-related peptide [PTHrP]), and Ca^{2+} signaling (stimulated by a huge number of ligands, including angiotensin II, acetylcholine, prostaglandin F_2 (PGF_2), arginine vasopressin [AVP], adenosine triophosphate [ATP], endothelin, histamine, etc.) have been identified.[59] An example of an ion channel of particular importance in podocytes is transient receptor potential canonical 6 (TRPC6), a nonselective cation channel, which is activated by diacylglycerol in a protein kinase C-dependent manner.[60] Mutations in TRPC6 were found to cause autosomal dominant, late adult-onset proteinuria, with a similar clinical phenotype as seen in ACTN4-mediated FSGS.[37] The major target of this signaling orchestra is the cytoskeleton, the concrete effects of which, however, are poorly understood. Other receptors, such as for C3b,[61] Heymann's antigen,[62] transforming growth factor β (TGFβ),[63,64] fibroblast growth factor 2 (FGF2),[65] and various other cytokines and chemokines have been shown to be involved in the development of podocyte diseases.[59]

Endothelium

The endothelium consists of a simple squamous layer of fenestrated (porous) cells with the cell nuclei generally located near the axial region of the capillary loop (Fig. 1.6). The fenestrated regions (Fig. 1.12), which compose roughly 55% of the surface area,[66] have a thin layer of cytoplasm (about 50 nm thick) penetrated by numerous fenestrae of round, oval, or irregular shape and varying sizes. The total area occupied by fenestrae accounts for 13% of the capillary surface. The fenestrated regions outline the pericapillary portions of the glomerular basement membrane, but also may be found adjacent to the mesangium (Fig. 1.8). The fenestrae have a diameter of 50 to 100 nm (thus they are larger than endothelial fenestrae elsewhere in the body) and are not bridged by diaphragms. Thus, this kind of a porous endothelium is unique for glomerular capillaries. Fenestrae with diaphragms are found only in the outflow segment of the efferent arteriole.[67] Nonfenestrated regions are generally seen over nuclei and mesangial cell regions. Human glomerular endothelial cells also are fenestrated.[68,69] A cell coat that is rich with polyanionic glycoproteins, including podocalyxin, covers the endothelial surface[70–72] and appears to fill the fenstraelike "sieve plugs."[73] In addition, above this "classic" glycocalix a 200-nm thick endothelial surface layer has been revealed consisting of loosely attached plasma compounds.[74]

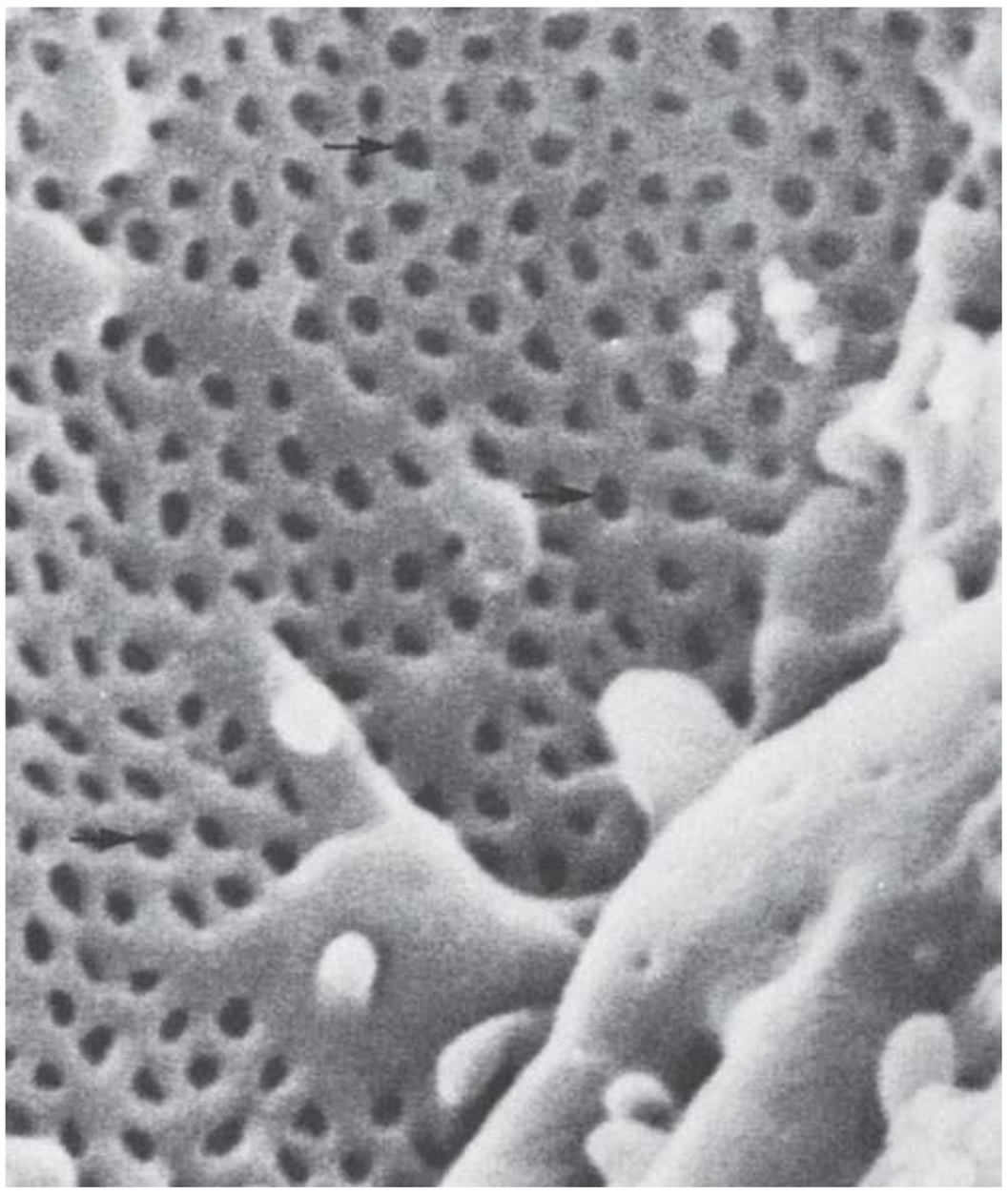


FIGURE 1.12 Scanning electron micrograph of a sectioned capillary loop showing the pores (*arrows*) of the endothelium. (Magnification ×50,400.)

As elsewhere in the body, glomerular endothelial cells are active participants in processes controlling coagulation, inflammation, and immune processes. Renal endothelial cells express surface antigens of the class 2 histocompatibility complex. Like platelets, glomerular endothelial cells contain components of the coagulation pathway and are capable of binding factors IXa and Xa and synthesize, release, and bind von Willebrand factor (factor VIII).[69] Glomerular endothelial cells synthesize and release endothelin-1 and endothelium-derived relaxing factor (EDRF).[75] Glomerular endothelial cells have receptors for vascular endothelial growth factor (VEGF) and angiopoetin I that are produced by podocytes.[76,77] The signaling axis via VEGF appears to have a major relevance for the maintenance of the glomerular tuft. Glomerular endothelial cells, in turn, synthesize and secrete platelet-derived growth factor β (PDGFβ) that acts on adjacent mesangial cells.[78]

Glomerular Basement Membrane

The GBM covers the capillary loops except in the axial region, where it is reflected over the mesangium to the next capillary loop, accompanied by the layer of foot processes (Figs. 1.6 to 1.8). The endothelial cells do not have a separate basement membrane; thus, the endothelial cells directly abut the mesangium toward axial regions. In adult humans, the basement membrane has a mean diameter of 320 to 340 μm.[79] It is thinner in young children and most laboratory animals.[80]

The basement membrane is composed of three layers: an outer, less dense subepithelial layer, the lamina rara externa; a central, electron-dense layer, the lamina densa; and an inner subendothelial layer, the lamina rara interna, which is continuous with the mesangial matrix (see Figs. 1.9 and 1.13).

During glomerulogenesis, the GBM is generated as two separate layers produced by glomerular endothelial and epithelial cells. The two sheets are fused together to form the mature GBM.[81–84] In the adult, the GBM is subject to a continuous turnover,[85–88] but few details are known so far about these processes. Podocytes appear to be the dominant cell type to synthesize and probably to degrade the GBM. Podocytes are alone capable to synthesize all the components of the GBM[42,89,90]; glomerular endothelial cells and also mesangial cells may contribute to the formation of the GBM.[91] It is less clear how the GBM degrades. In recent years, several extracellular matrix degrading enzymes have been described being produced by podocytes and mesangial cells.[92–94] The relevance of these enzymes for the turnover of the GBM remains to be established.

The GBM is generally considered as a hydrated meshwork consisting of collagen type IV, laminin, entactin/nidogen, and sulfated proteoglycans including agrin and perlecan.[42,95–99] Models of the ultramicroscopic structure of the basement membrane picture the GBM as a mat of collagen type IV. Monomers of type IV collagen consist of a 400-μm triple helix that, at its carboxy-terminal end, has a large noncollagenous globular domain called NC 1. At the amino-terminus the helix possesses a 60-μm triple helical rod, the 7S domain. Interactions between the 7S domain and the NC1 domain allow collagen type IV monomers to form tetramers that, by lateral association of triple helical strands, assemble into a three-dimensional network.[100,101] Laminin forms a second network that is superimposed to the collagenous network. Laminin is a noncollagenous glycoprotein consisting of three polypeptide chains, two of which are glycosylated and cross-linked by disulfide bridges.[95,102,103] Laminin binds to specific sites on the polymerized network of type IV collagen as well as the basal endothelial and epithelial integrins (see the preceding). The α-5-, β-2-, γ-1-laminin chains are assembled to form the GBM-specific heterotrimeric laminin 11.[48] This combined network of collagen type IV and laminin provides mechanical stability to the basement membrane and serves as a basic structure on which other matrix components attach.

The proteoglycans of the basement membrane consist of core proteins and covalently bound glycosaminoglycans, which are concentrated in the laminae rarae internae and externae, where they have been referred to as anionic sites and can be localized with cationic probes.[104] The major proteoglycans of the GBM are of the heparan sulfate type—the most prominent is agrin,[96] but perlecan also has been shown to occur in the GBM.[42] Digestion of these molecules with heparinase leads to a dramatic increase in the permeability of the basement membrane to anionic native ferritin used as a probe.[70]

Mesangium

The mesangium consists of mesangial cells that are embedded in a mesangial matrix. The term mesangium was introduced by Zimmermann in 1929[19] to describe the cells that form the stalk of the glomerulus and the axes of its lobules. Glomerular capillaries pursue a tortuous, highly anastomosing course around the mesangial axes. Together with the capillaries, the mesangium occupies the space inside the GBM, frequently termed the "endocapillary region." Topographically, the mesangium can be subdivided into a juxtacapillary region, where it abuts the capillary endothelium, and more centrally located axial regions, which are bound by the perimesangial GBM (Figs. 1.5 to 1.7).[105] The glomerular mesangium is continuous with the extraglomerular mesangium (Polkissen or lacis cells) along the glomerular stalk (Fig. 1.5). Both intraglomerular and extraglomerular mesangial cells have many similarities.

Mesangial cells are quite irregular in shape, with many cytoplasmic processes extending from the cell body toward the GBM. They have structural characteristics similar to those of smooth muscle cells in that they contain many bundles of microfilaments (especially within the cell processes) and peripheral dense bodies. Actin, myosin, and α-actin have been shown by immunocytochemistry to be contained in mesangial cells.[106,107] The relevance of mesangial cell contractility is discussed in the following text.

The processes of mesangial cells extend toward the GBM, to which they are attached either directly or by the interposition of extracellular bundles of microfibrils (Figs. 1.6 and 1.9). The GBM has to be considered as the effector structure of mesangial contractility.[105,108] Connections between mesangial cells and the GBM are especially prominent in the juxtacapillary region. At this site, tonguelike mesangial cell processes (packed with microfilament bundles) run underneath the capillary endothelium toward the turning points (mesangial angles) of the GBM, where they are anchored. Generally, two of these processes interconnect the GBM from two opposing mesangial angles (Fig. 1.8). In the axial mesangial region, contractile filament bundles are predominantly found within the numerous fingerlike projections of mesangial cells. These microprojections also run toward the GBM and are anchored to it. As in the juxtacapillary region, these microfilament bundles interconnect opposing parts of the GBM.[105]

The mesangial matrix fills the highly irregular spaces between mesangial cells and the perimesangial part of the GBM. In immunocytochemical studies, collagen types IV and V, heparan sulfate proteoglycan, fibronectin, laminin, and entactin have been localized within the mesangial matrix.[99,109,110] Among these components, fibronectin is the most abundant and has been shown to be associated with microfibrils.[109,111] Fibrillin 1 and other specific elastic fiber proteins have been detected in the glomerular mesangium and have been shown to be produced by mesangial cells.[112,113]

In specimens prepared for transmission electron microscopy according to routine methods, the mesangial matrix appears as basement membranelike material, albeit more fibrillar in character than the basement membrane proper.[114] In specimens prepared by a technique that avoids osmium tetroxide and uses tannic acid for staining, the mesangial matrix is seen to contain a dense network of microfibrils.[105,115] Microfibrils are noncollagenous, nonbranching, hollow structures of indeterminate length that are about 15 μm thick.[116] Within the mesangium, microfibrils form a three-dimensional network that establishes a solid base of contact between mesangial cells and the GBM, fettering the GBM to mesangial cells. Distinct bundles of microfibrils may be regarded as "microtendons" that allow the transmission of contractile force of mesangial cells to specific sites of the GBM.[105,115] The functional relevance of this system is discussed later.

The relevance of mesangial cells in phagocytosis is well documented. Mesangial cells are able to ingest particular tracers as well as macromolecules, such as thorotrast,[117] ferritin,[114] and aggregated proteins, as well as immune complexes.[118] An increased uptake of such materials by mesangial cells has been noted in proteinuric states.[114,119] It appears, however, that mesangial cells proper (i.e., mesangial cells that have contractile properties) are not primarily phagocytotic. A small subpopulation (3% to 7%) of cells in this region has been recognized as bone marrow-derived—they represent macrophages that have taken up residence in the mesangium.[120]

Supportive Functions of the Mesangium and Podocytes

The glomerular tuft is constantly exposed to comparably high intraglomerular pressures within glomerular capillaries and mesangium. The high intraglomerular pressures challenge not only the glomerular capillaries themselves but also the folding pattern of the glomerular tuft. Increased pressures lead to the loss of the folding pattern and to dilation of the glomerular capillaries. Therefore, we have to ask: what are the specific structures and mechanisms that counteract the expansile forces in the glomerular tuft? To answer this question we have to distinguish between the structures and mechanisms maintaining (1) the folding pattern of the glomerular tuft and those maintaining (2) the width of glomerular capillaries.

The folding pattern of the glomerular tuft is primarily sustained by the mesangium.[105,108,121] Mesangial cells are connected to the GBM by their contractile processes—they maintain the infoldings of the GBM by centripetal contractions, thereby allowing for the capillaries to arrange in the peripheral expansion of the GBM. This supporting role of mesangial cells is best illustrated under circumstances with loss of mesangial cells, such as Thy-1 nephritis.[122] Under those circumstances the folding pattern of the GBM is progressively lost, finally resulting in mesangial aneurysms. Podocytes clearly contribute to maintenance of the folding pattern by specific cell processes that interconnect opposing parts of the GBM from outside within the niches of the infoldings. This function is also best illustrated under circumstances with loss of mesangial support: podocytes are capable of maintaining a high degree of the GBM folding pattern for 2 to 4 days after which they obviously fail, and mesangial aneurysms become prominent.[122]

The width of glomerular capillaries, in the long run, is probably controlled by growth processes accounting for differently sized capillaries. The width of a given capillary, in an acute situation being exposed to changes in blood pressure, appears to be stabilized by the GBM, which is a strong elastic structure[123] and, together with the mesangial cell bridges (see previous text), capable of developing wall tension.[121,124] In addition, the tensile strength of the GBM is reinforced by podocytes. Podocytes are a kind of pericyte; their foot processes represent a unique type of pericyte process which, like elsewhere in the body, counteract the dilation of the vessel. Podocyte processes are firmly attached to the underlying GBM (see previous text); their cytoskeletal tonus counteracts the elastic extension of the GBM. In this function, podocytes cannot be replaced by any other cell—failure in this function will lead to capillary dilation.

Glomerular Filtration Barrier

The essential components of the glomerular filtration barrier are the endothelium (Fig. 1.13), which is perforated by large open pores, the extracellular matrix feltwork of the GBM membrane, and the slit diaphragms between the podocyte

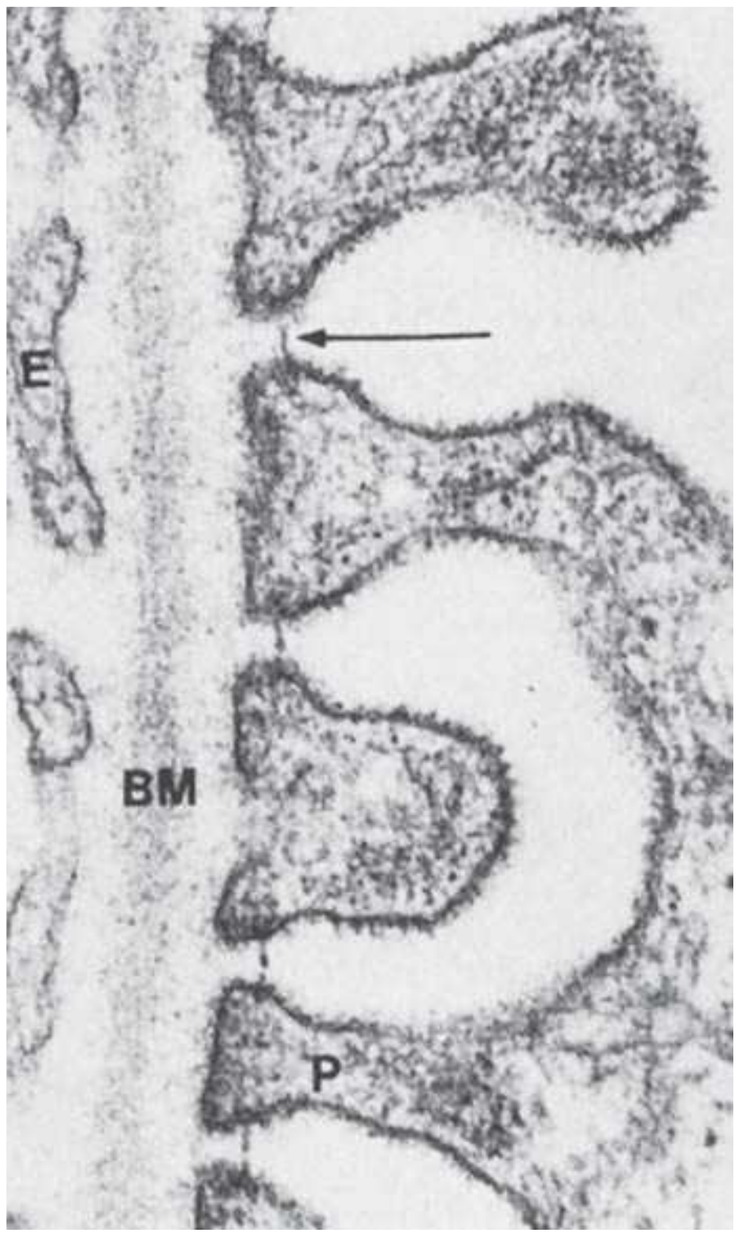


FIGURE 1.13 Transmission electron micrograph from a rat renal corpuscle showing the endothelial lining (*E*), the basement membrane (*BM*), and the pedicels (*P*). The filtration-slit membrane (*arrow*) bridges the pedicels. (Magnification ×23,600.)

foot processes. When compared with the barrier established in capillaries elsewhere in the body, a glomerular filtration barrier is quite different in two respects: its permeability to water, small solutes, and ions is extremely high, whereas its permeability to plasma proteins the size of albumin and larger is very low.

The high hydraulic permeability is rooted in the fact that filtration occurs along extracellular routes. All components of this route—the endothelial pores, the highly hydrated GBM, and the slit membrane—can be expected to be quite permeable to water and small solutes. Drummond and Deen[125] have calculated the hydraulic conductance of the individual layers. According to this calculation, the hydraulic resistance of the endothelium is negligible. The GBM and the filtration slits each make up roughly one-half of the total hydraulic resistance of the filtration barrier.

Any decrease in the length of the filtration slit, and thus in slit area, as in experimental and clinical glomerulopathies along with footprocess effacement, correlates with the decrease in the ultrafiltration coefficient K_f.[126,127] A model simulating those conditions showed, along with a decrease in slit area, its relevance in determining increases in flow resistance. The decrease in filtration slit area caused the average path length for the filtrate, through the basement membrane, to increase, thereby explaining the overall decreased hydraulic permeability.[128]

On the basis of evidence of contractility of mesangial cells exposed to vasoconstrictor stimuli in culture,[129,130] of dimensional changes observed in intact glomeruli ex vivo,[131,132] and of changes in ultrafiltration coefficient K_f found in vivo in response to vasoactive substances,[133,134] some researchers have concluded that mesangial cells contract in situ and that this contraction alters glomerular filtration dynamics by decreasing filtration surface area.

Other considerations speak against this possibility. The geometric arrangement of the mesangial contractile apparatus (Fig. 1.6), however, does not seem to be compatible with the previously mentioned sequence of actions. Shortening of the mesangial cell processes connecting opposing angles would only bring the angles closer together, compressing the mesangial capillary interface, but leaving peripheral capillary wall area (filtration area) unaltered. In addition, with regard to the contractility of mesangial cells ex vivo (in culture as well as preparations of whole glomeruli), it should be remembered that mesangial contraction in these cases is not opposed by intercapillary hydrostatic pressure as it is in situ. These considerations, together with the absence of measurable changes in glomerular tuft dimensions in morphometric studies,[135,136] as well as in response to vasoactive substances in vivo,[137] have led to the suggestion[121] that the mechanical action of the mesangial cell contraction is essentially static in nature, developing tension that serves to counteract expansile forces on the tuft without inducing significant changes in capillary dimension. If mesangial contractility acutely alters the glomerular ultrafiltration coefficient K_f, it is, therefore, probably not because of an acute change in filtration surface area.

The low permeability of the glomerular filtration barrier to plasma proteins is still poorly understood. Several points seem to be relevant. First, there is no vesicular transport of proteins through this barrier as in most other capillaries elsewhere in the body. The barrier function of the glomerular filter for macromolecules is quite specific and is selective for size, shape, and charge.[138–140]

In early extensive studies, Farquhar and associates,[138,141,142] as well as Rennke and associates,[143] used tracers, such as ferritin and dextrans of different sizes and charge, to elucidate the role of the various layers in determining the selectivity of this filtration barrier. When their results are summarized, it appears that the basement membrane may be the major barrier to anionic substances, whereas the most restrictive part for uncharged and cationic substances may be the slit diaphragm. Uncharged macromolecules up to an effective radius of 1.8 μm pass freely through the filter. Larger compounds are more and more restricted (indicated by their fractional clearances, which progressively decrease) and are totally restricted at effective radii of more than 4.0 μm. The effective radius is an empiric value, measured in artificial membranes, that takes into account the shape of micromolecules and also attributes a radius to nonspherical molecules. Plasma albumin has an effective radius of 3.6 μm; without the repulsion because

of the negative charge, plasma albumin would pass through the filter in considerable amounts.[144]

Thus, since these early studies, the glomerular barrier has been generally suggested to contain a size- and a charge-restrictive element. Despite more than 40 years of intensive research, the principles of the glomerular barrier function are still controversial and poorly understood—a situation that gives room even to hypotheses claiming that the glomerular barrier itself does not have any restrictive properties to macromolecules, such as albumin.[145–147] A more realistic and elegant recent barrier hypothesis is based on the observation that filtration—the flow of filtrate through the barrier—creates a potential difference of 0.02 to 0.05 mV negative in Bowman's space and that this potential difference is sufficient to hinder the negatively charged macromolecules like albumin from passing the filter.[148]

Summarizing the present status of knowledge, accumulating evidence suggest that the GBM has little relevance in size restriction, but the slit membrane is the decisive size-restrictive element within the glomerular barrier.[149] The current strong engagement in elucidating the molecular architecture of the slit membrane promises to enlighten the porous pattern of the slit membrane and, hopefully, explain its size-restrictive property. Regarding the charge selectivity, it appears that the proximal portion of the barrier, above all the endothelium, are most important.[73,150–152] Furthermore, prevention of albumin from entering the filter is also dependant on normal hemodynamic conditions in glomerular capillaries, as first shown by Ryan and Karnovsky.[153]

PROXIMAL TUBULE

At the urinary pole of the glomerulus, the flat parietal epithelium of Bowman's capsule transforms abruptly into the high epithelium of the proximal tubule. In some species (rabbits),[154] a neck segment is found interposed between the glomerulus and the proximal tubule—short neck segments also may be seen in humans.[80] In contrast, in mice, the proximal tubule epithelium generally begins deep within the Bowman's capsule.

The proximal tubule is composed of segments that have differing morphology, functional relevance, and vulnerability to toxins (Figs. 1.14 to 1.18). The two segments most frequently identified are the proximal convoluted portion (pars convoluta) occupying the cortical labyrinth (Figs. 1.14 to 1.17), and the straight portion (pars recta) in the medullary rays of the cortex and the outer stripe of the outer medulla (Fig. 1.18). Further subdivision on structural criteria results in the three segments of P_1, P_2, and P_3 according to Jacobsen and Jorgensen,[155] and S_1, S_2, and S_3 according to Maunsbach.[156,157] S_1, or P_1, corresponds to the first segment of the pars convoluta and lies exclusively in the cortical labyrinth; S_2, or P_2, corresponds to the remainder of the convoluted segment and the beginning of the pars recta, with the first part occupying the cortical labyrinth and the remainder

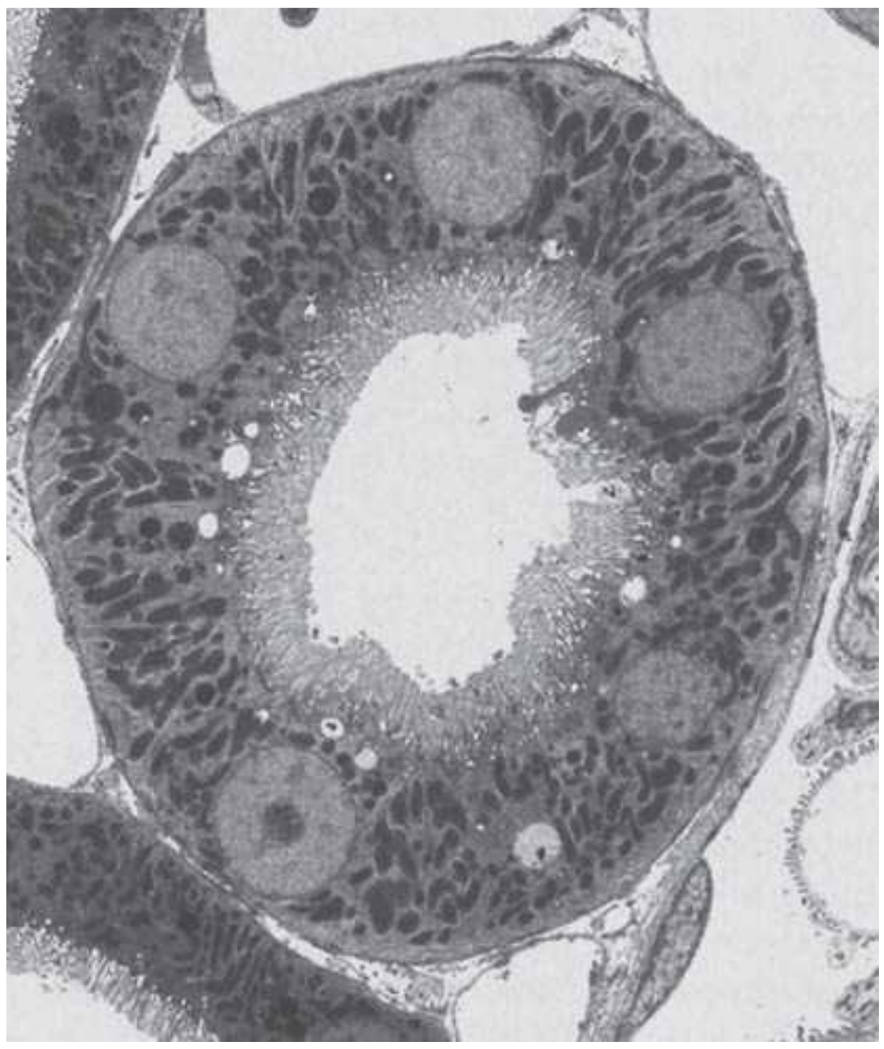


FIGURE 1.14 Transmission electron micrograph of a proximal tubule (P1 segment) of the rat. Note the apical brush border, the vesicular zone of the apical cytoplasm, and the basal zone of interdigitating cell processes filled with mitochondria. (Magnification ×2,300.)

occupying the medullary ray; and S_3, or P_3, corresponds to the remaining part of the pars recta located primarily in the outer stripe of the outer zone of the renal medulla. The transition from P_1 to P_2 is gradual, whereas the transition from P_2 to P_3 is generally abrupt, except in rabbits.[158]

To confuse matters further, the morphology of the subdivisions differs not only from each other but also among species, including mice,[159] rats,[156] rhesus monkeys,[160] rabbits,[158] and dogs.[161] For a detailed description of a particular species, the reader should consult the original studies. Segmentation of human proximal tubules has not been studied as recently or thoroughly because of a lack of availability of well-fixed normal human kidney and, therefore, has been divided only into convoluted and straight regions.[162]

In general, cells of the P_1 region are tall, have a well-developed apical microvillus border, an elaborate cell shape with well-developed lateral interdigitating processes containing abundant, large mitochondria, and a well-developed endocytic apparatus (Fig. 1.16). P_2 cells decrease in cell height from those seen in P_1, have a shorter microvillus border, and are less elaborately shaped cells with smaller mitochondria (Fig. 1.17). The P_3 cells are more cuboidal in shape (less elaborate) and their microvillus border is generally less elaborate. The cells of rats are an exception and have a well-developed brush border of very long microvilli (Fig. 1.18).[156] This cytologic segmentation of the proximal tubule is maintained in superficial, midcortical, and juxtamedullary nephrons.

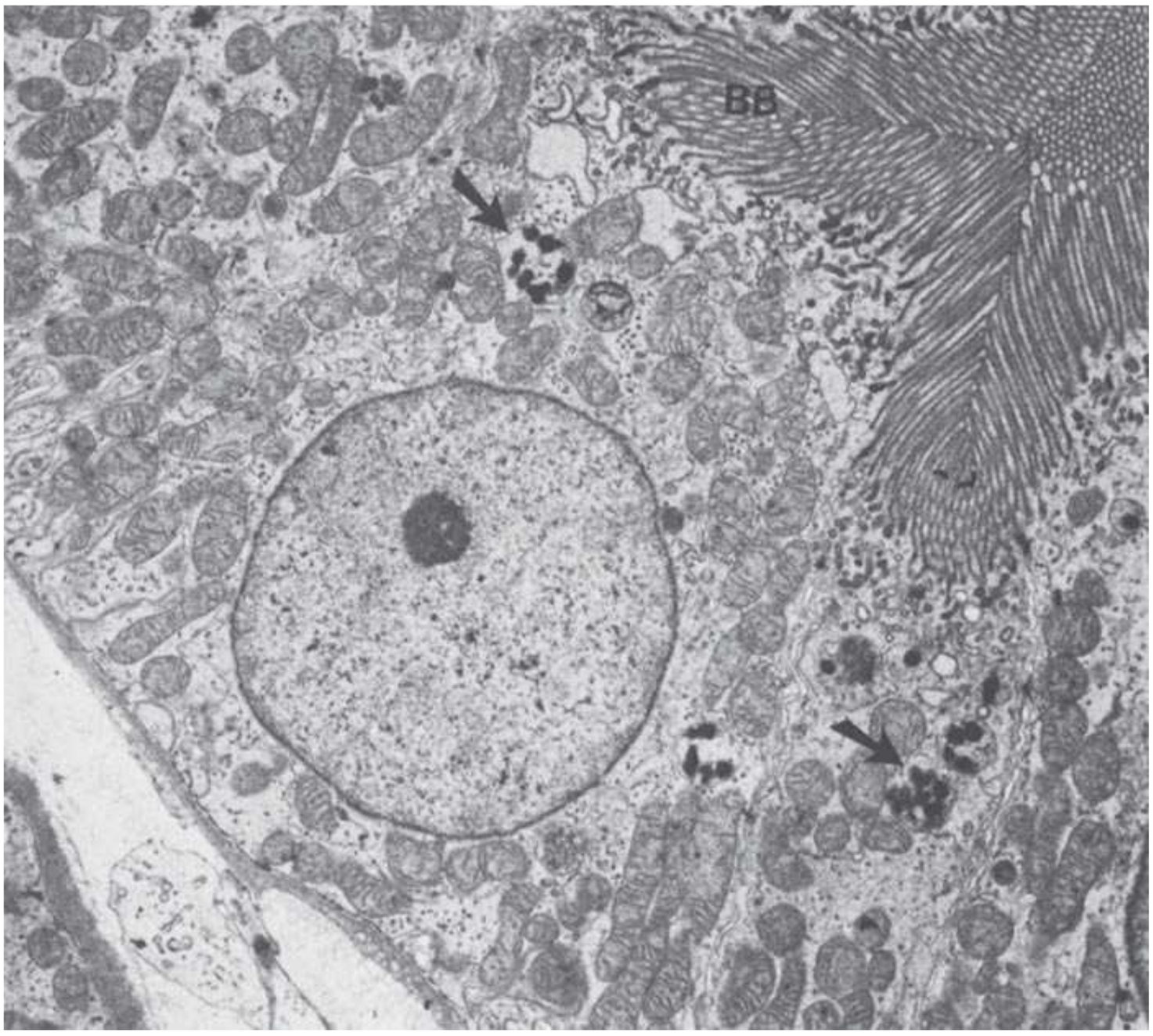


FIGURE 1.15 Transmission electron micrograph of a human proximal convoluted tubule showing the brush border (*BB*) and apical condensing vacuoles containing small dense bodies (*arrows*). (Magnification ×7,800.)

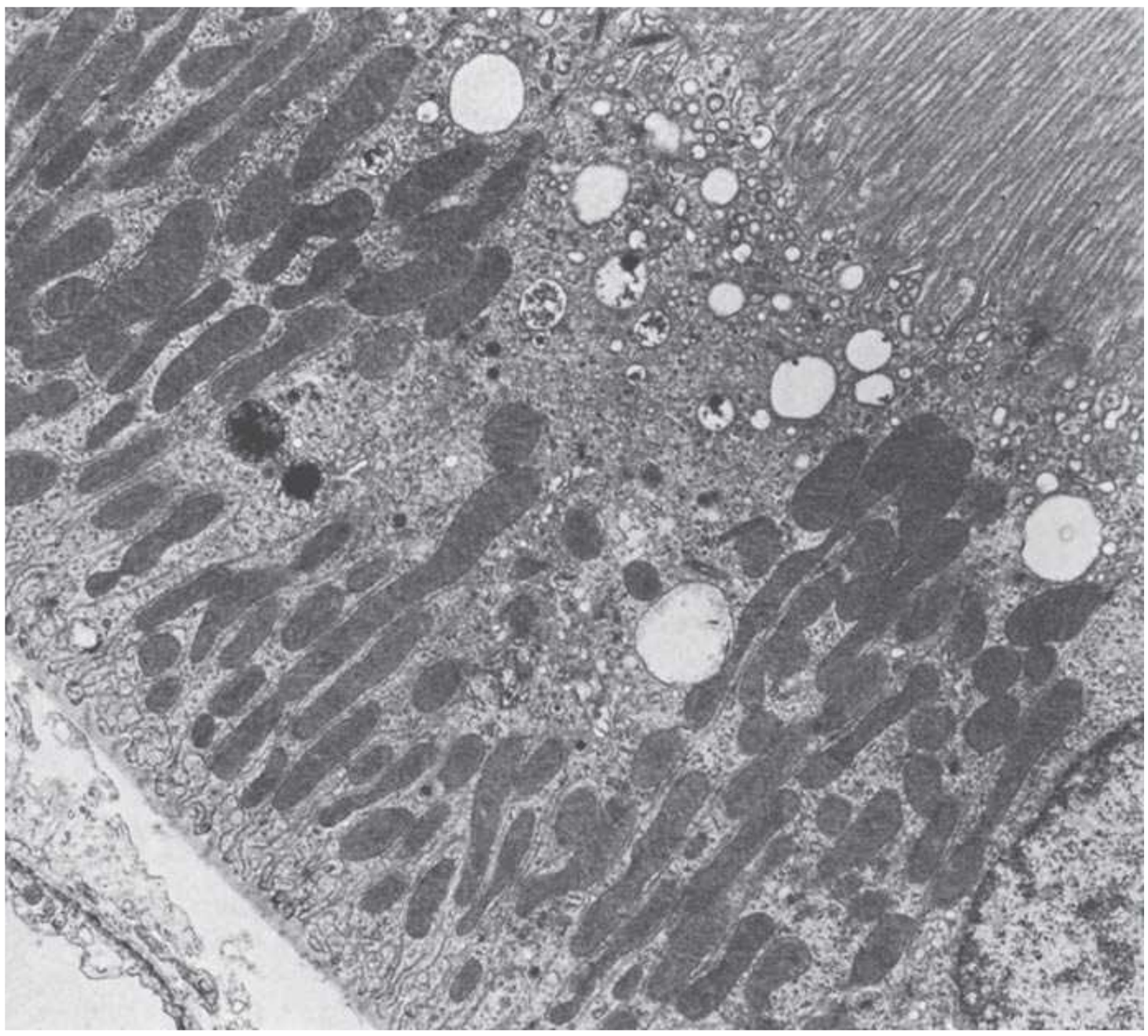


FIGURE 1.16 Transmission electron micrograph of a P1 segment from a rat kidney. (Magnification ×8,000.)

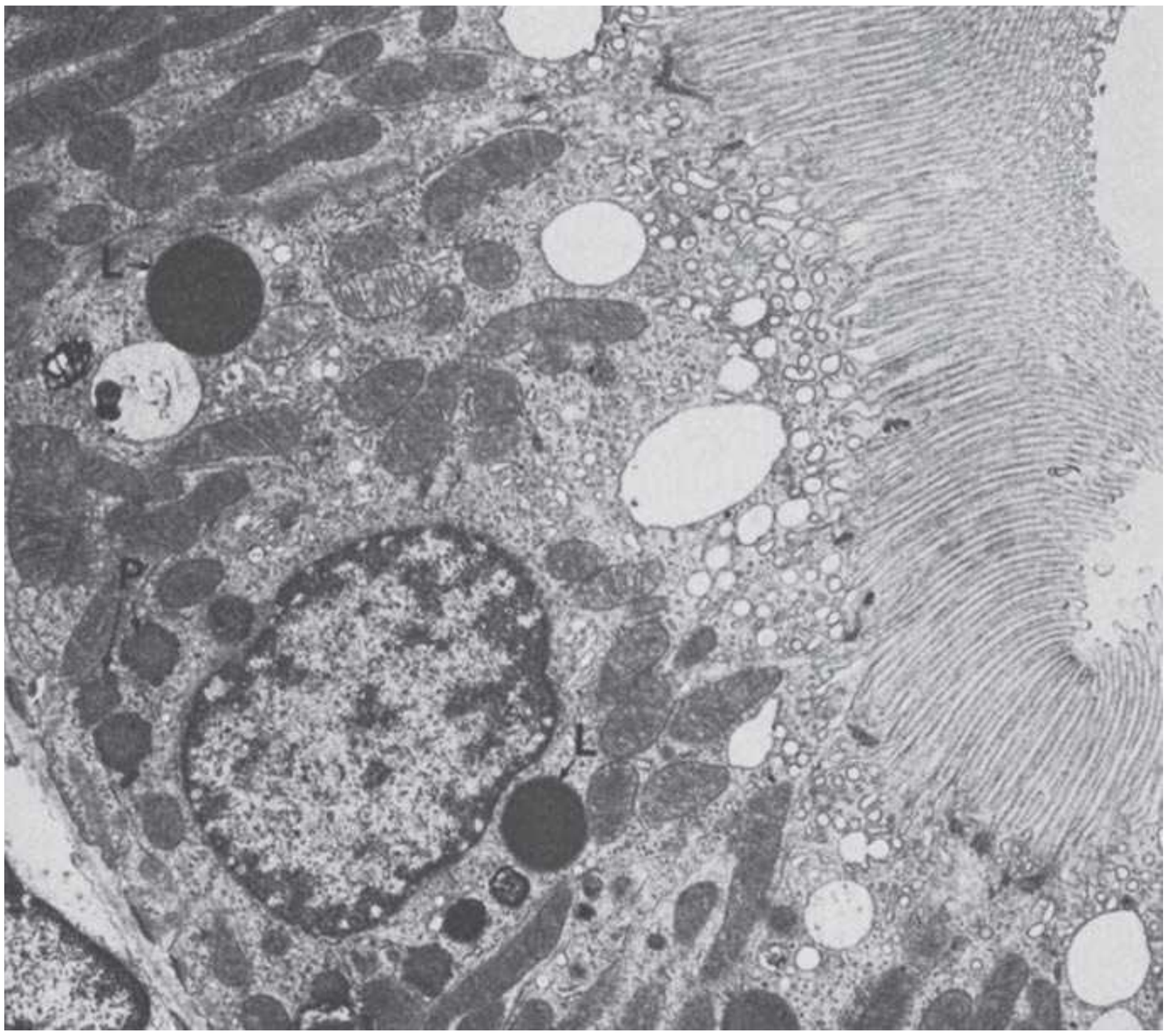


FIGURE 1.17 Transmission electron micrograph of a P2 segment from a rat kidney. Note the large dense lysosomes (*L*) and peroxisomes (*P*). (Magnification ×10,000.)

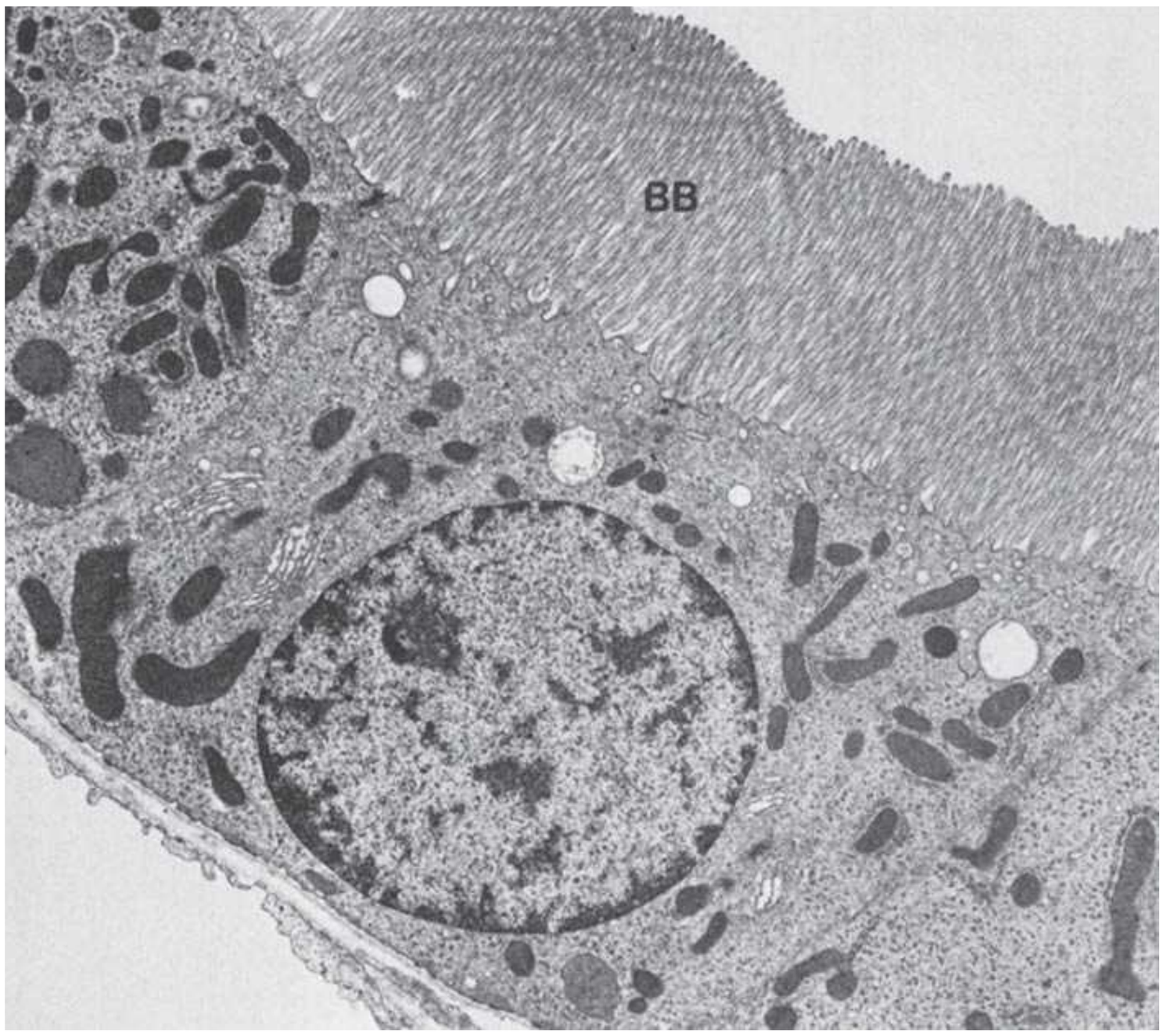


FIGURE 1.18 Transmission electron micrograph of a P3 segment from a rat kidney showing the extensive microvillus border (*BB*) and more simple cell shape of this segment. (Magnification ×8,000.)

Proximal Convoluted Tubule (P_1 and Part of P_2)

The proximal convoluted tubule is the longest and largest segment of the mammalian nephron. The tubule is lined by cells that have an elaborate cell shape based on extensive basolateral interdigitations (Figs. 1.19 to 1.21), a well-developed microvillus border, a prominent intracellular digestive tract (endocytic apparatus and lysosomes (Fig. 1.15), and numerous large peroxisomes (microbodies). The single ovoid nucleus lies in the middle to basal region of the cytoplasm.

Cell Shape and Mitochondria

The cells are characterized by an extensive system of lateral cell processes that interdigitate with lateral processes from adjacent cells (Figs. 1.19 to 1.21). These lateral processes can extend the entire height of the epithelium, especially in the P_1 segment, but become more elaborate toward the basal regions of the lateral surface (Fig. 1.16). The complex shape of these cells serves to increase the lateral cell surface area manyfold, which provides a greater area for transporters, above all of the Na^+-K^+-ATPase. These lateral extensions usually contain one or two layers of mitochondria (Fig. 1.20). The mitochondria are long, narrow rods that branch and double back on themselves. They are oriented perpendicular to the basement membrane and lie adjacent to the cell membrane, to which they presumably supply energy for transport processes.

The lateral extensions and their mitochondria cause the pattern of basal striations that are typical for numerous transporting cells. The lateral processes of proximal convoluted tubule cells in humans are not as elaborate as those seen in rats (compare Figs. 1.15 and 1.16). In rabbits, the lateral cell surface is increased 20 times over that of the basal cell surface.[163] The lateral extensions also establish a complex and extensive surface labyrinth of lateral intercellular spaces ("basal labyrinth").

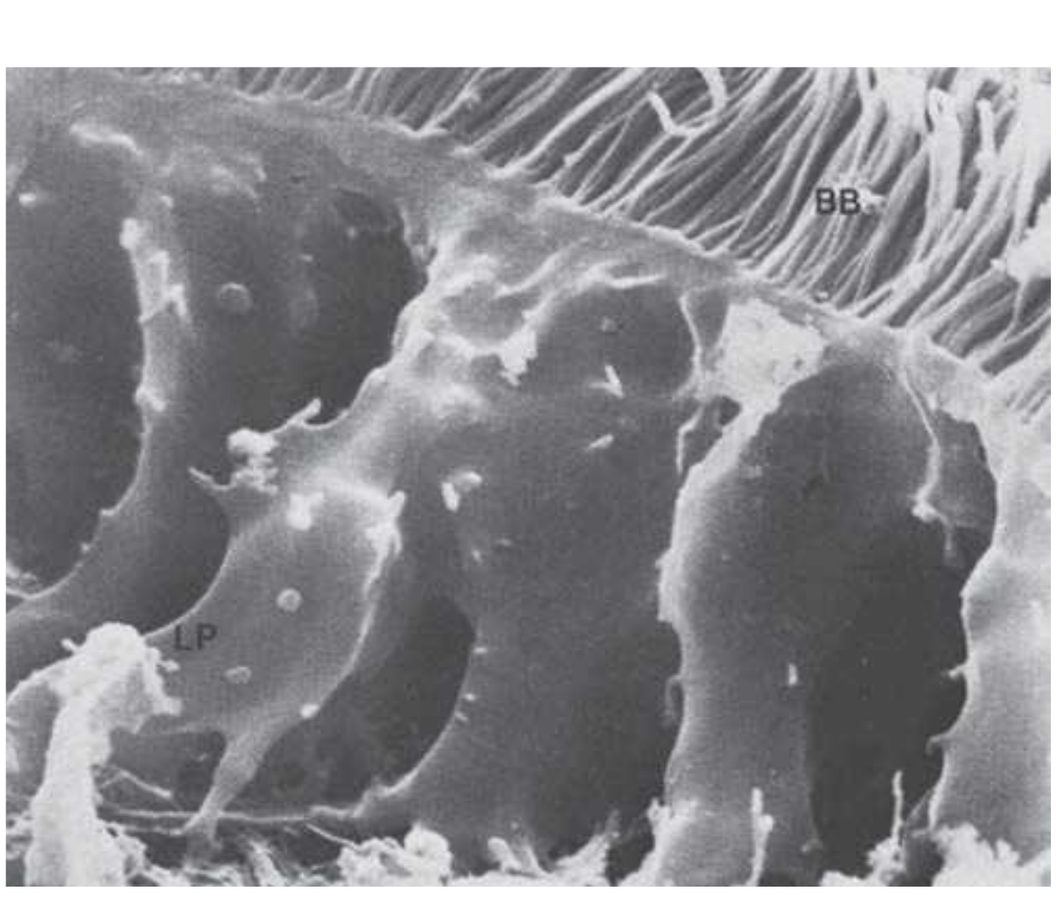


FIGURE 1.19 Scanning electron micrograph of a rat proximal tubule epithelial cell showing the apical brush border (*BB*) and the lateral processes (*LP*). (Magnification ×14,700.)

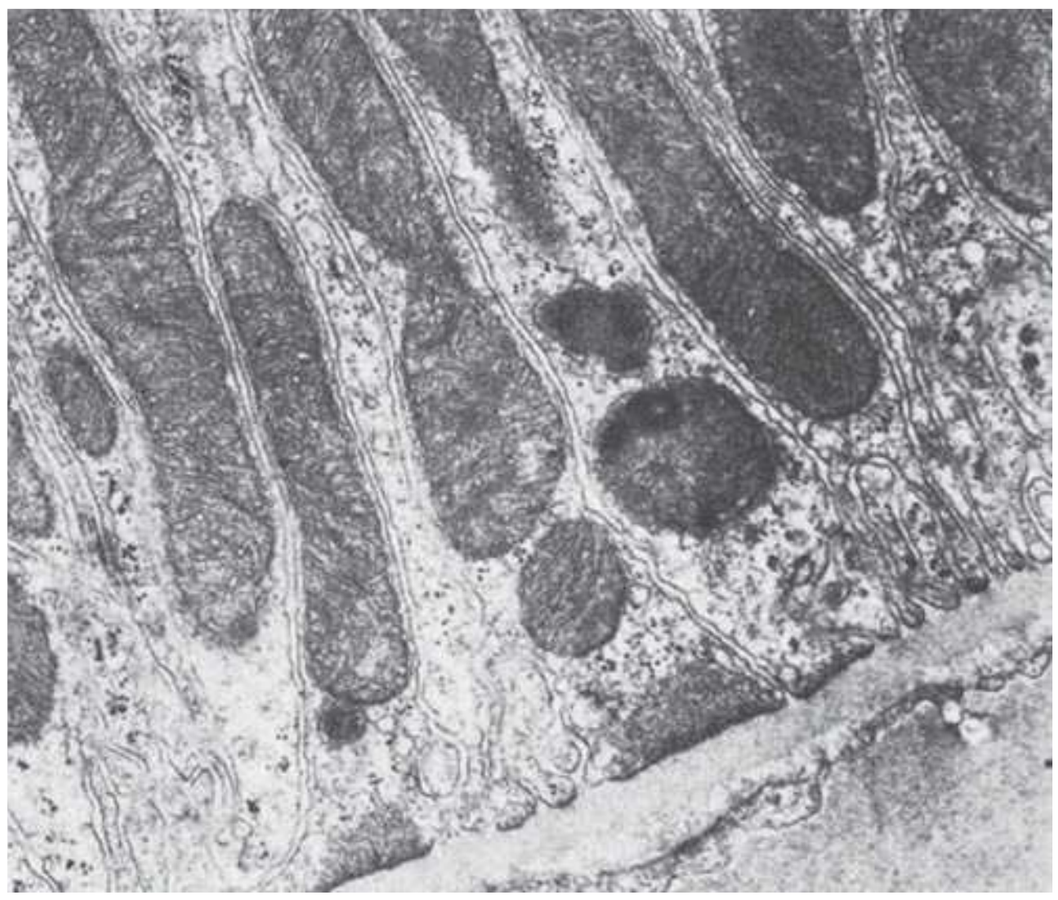


FIGURE 1.20 Transmission electron micrograph of the basal cytoplasm from a rat proximal tubular cell. (Magnification ×20,600.)

Cell Junctions

The proximal convoluted tubular cells have an apical junctional complex that consists of a shallow, beltlike, tight junction next to the tubular lumen; a deeper, beltlike intermediate junction (the zonula adherens); and only small and infrequently seen desmosomes. The proximal tubular tight junctions are shallow and consist of only one or two lines of fusion of the outer leaflet of the cell membrane (Fig. 1.22). Freeze-fracture studies of normal proximal tight junctions, however, reveal focal discontinuities. During volume expansion, striking increases in the length of discontinuities were found.[164] Transmission micrographs also show multiple areas of nonfusion of tight junctions associated with renal venous constriction and increased ureteral pressure.[165] These sites of nonfusion may explain the increased permeability seen in these situations. Proximal tubular cells are electrically coupled by gap junctions (Fig. 1.22).[166]

Microvilli

A layer of slender (approximately 80 to 90 μm), finger-shaped processes extends into the tubular lumen, forming the microvillus, or brush border. The length and number of processes vary with the segment and species. The luminal surface of the pars convoluta is increased 40 times in rats.[157] The luminal surface is increased 36 times in the pars convoluta and 15 times in the pars recta in rabbits.[163] Each microvillus has a core of microfilaments that extend into the apical cytoplasm connecting them to the cytoskeleton.

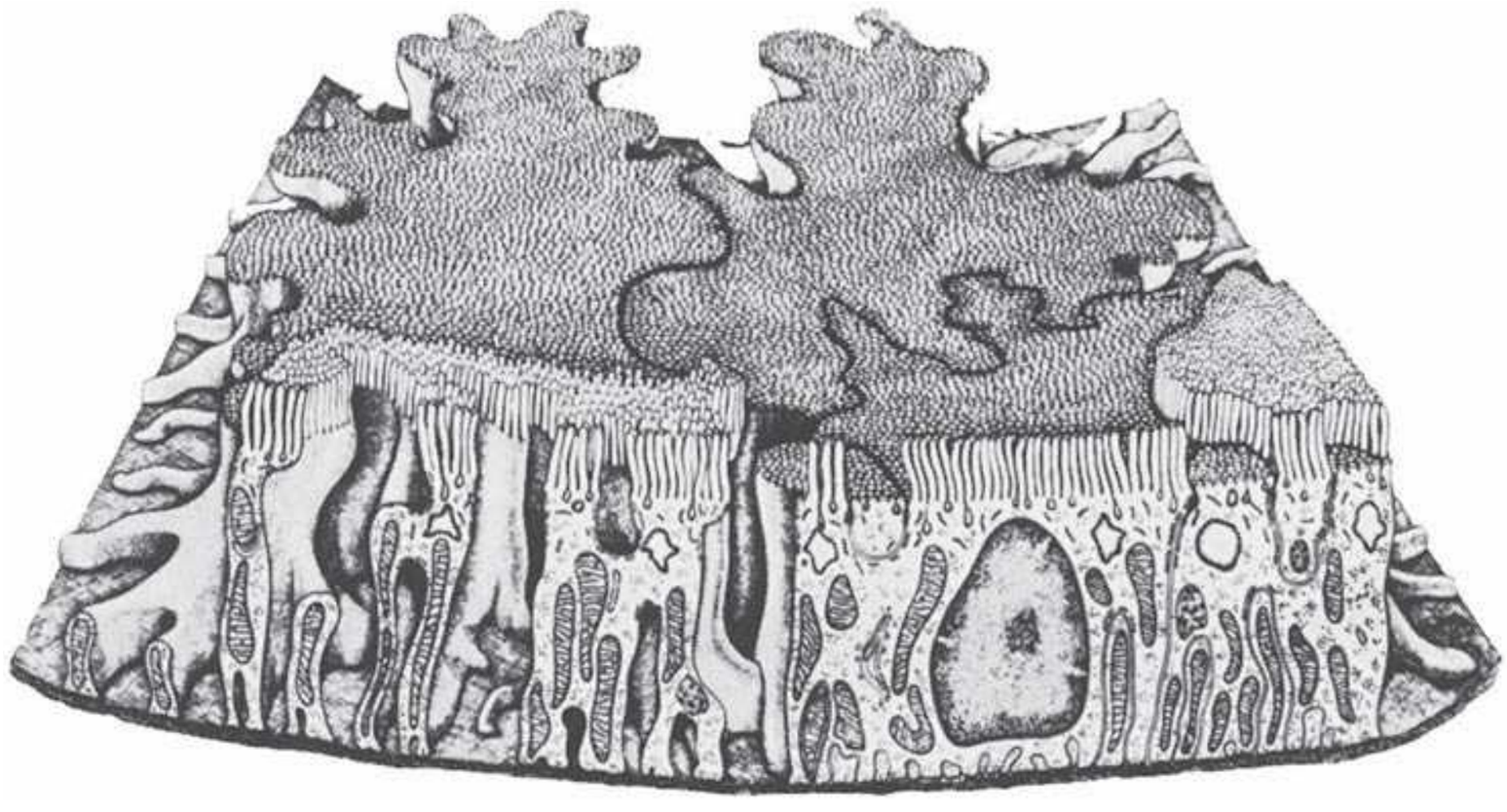


FIGURE 1.21 Diagram of a proximal convoluted tubular cell showing the elaborate shape of these cells. Some interdigitating processes extend the full height of the cells. The apical and basal cytoplasmic regions also have smaller, more elaborate interdigitating processes. (From Bulger RE. The shape of rat kidney tubular cells. *Am J Anal.* 1965;116:237, with permission.)

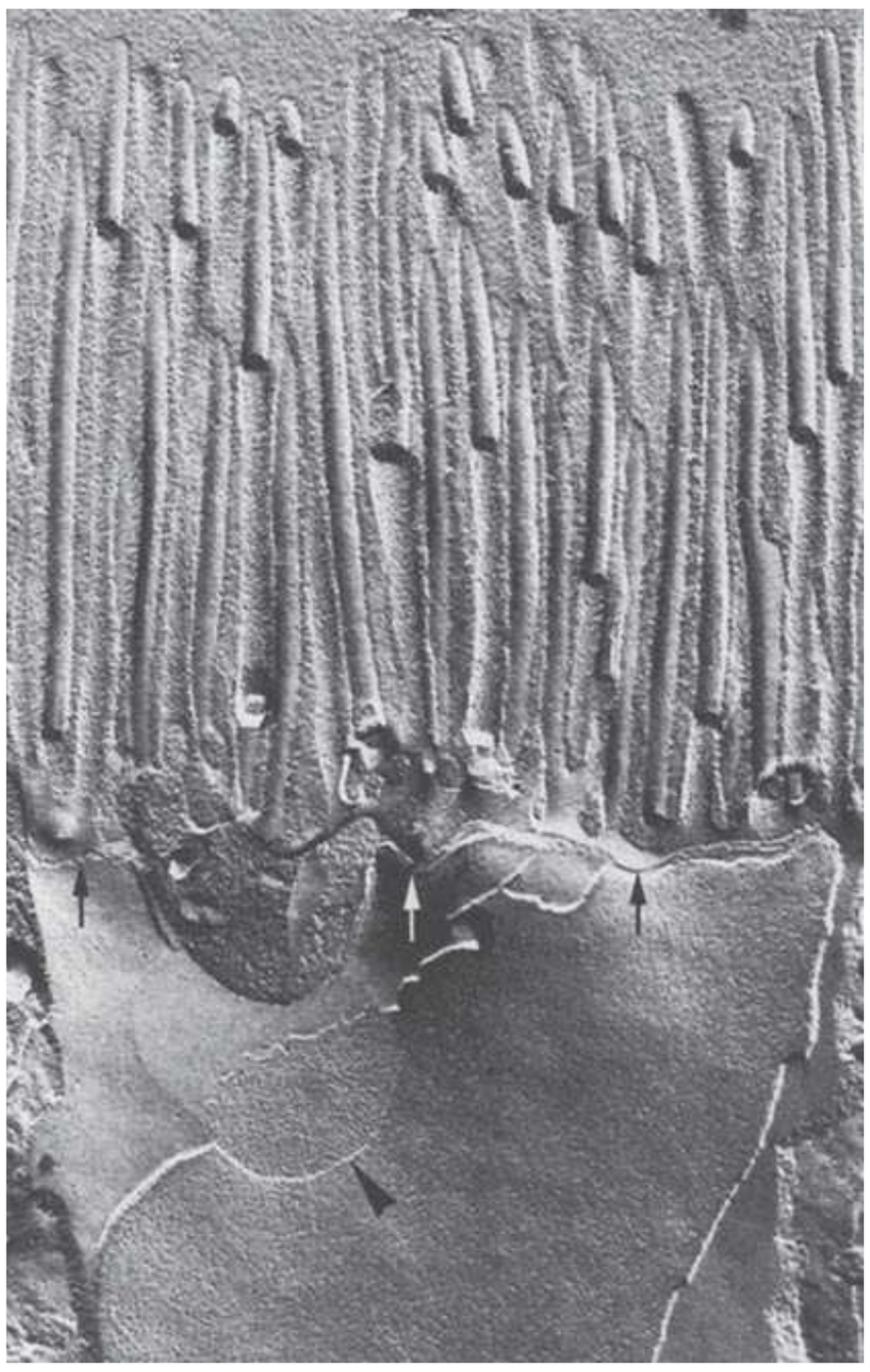


FIGURE 1.22 Freeze-fracture electron micrograph of the apical part of proximal tubule cells (rabbit) showing the shallow tight junction (*arrows*) and a gap junction (*arrowhead*). (Magnification ×37,000.) (From Kriz W, Kaissling B, Schiller A, et al. Morphologische merkmale transportie-render epithelien. *Klin Wochenschr.* 1979;57:967, with permission.)

Cytoplasm

The cells contain a very prominent vacuolar apparatus (see later text), a Golgi apparatus, free ribosomes, and cisternae of rough and smooth surface endoplasmic reticulum. The latter forms specialized cisternae, called the perimembranous cisternal system, near the lateral cell membranes.[80] In addition, the cells contain many peroxisomes that frequently have eyecatching shapes.[167] Peroxisomes are limited by a single membrane and contain a dense matrix, frequently exhibiting crystalloid nucleoids (Fig. 1.17); platelike inclusions at the peroxisomal edge, which are called marginal plates, are also frequently encountered. Peroxisomes are invariably wrapped by elements of the smooth-surfaced endoplasmic reticulum. In the kidney, large peroxisomes are exclusively found in the proximal tubule, most frequently in the P_3 segments[167]—all other nephron portions contain only microperoxisomes. Peroxisomes contain enzymes from a primitive respiratory chain in which oxidases produce hydrogen peroxide, which is, in turn, destroyed by the catalase, hence the name peroxisome.[168] Peroxisomes participate in the breakdown of very-long-chain fatty acids by lipid β-oxidation; in addition, they may play a protective role by destroying hydrogen peroxide produced by free radicals.

Straight Part of the Proximal Tubule (Last Part of P_2 and All of P_3)

The straight part of the proximal tubule (pars recta) begins in the medullary ray and penetrates into the outer stripe of the outer zone of the medulla (Fig. 1.18). The proximal pars recta converts into the thin limb near the junction of the inner and outer stripe. In rats, the pars recta includes the final region of both P_2 and P_3,[169] and the transition

between the two segments occurs at various levels in the medullary ray. This region is marked by a sudden increase in microvillar length, a decrease in endocytic apparatus, and a decrease in interdigitation between adjacent cells. The microvilli of the pars recta cells decrease in height and number in rabbits[158] and humans[162] but remain high in rats (Fig. 1.18).

In general, the P_3 pars recta cells have been described as lower in height with a less elaborate shape. In humans, the pars recta cells have a convex apical surface, and some lipid droplets are found in the basal cytoplasm. The mitochondria are fewer and no longer closely applied to the cell membrane. The lysosomes are smaller and the Golgi and endocytic apparatuses are less well developed. Peroxisomes are more numerous in the pars recta.[80,162] The tight junctions of P_3 can be more complex in shape, consisting of several junctional strands in rats, dogs, and cats.[170]

Structure–Function Correlations

The functional relevance of the proximal tubule is manifold and quantitatively enormous. It reabsorbs about 70% of filtered Na^+, Cl^-, and water; 95% of bicarbonate; 60% of Ca^{2+}; and sodium phosphate. Reabsorption of the filtered glucose is complete, whereas reabsorption of amino acids is almost complete. This occurs either by transcellular transport via specific channels and transporters in both membranes (water, sodium, phosphate, glucose, amino acids, and bicarbonate in a specific enzyme-mediated mode) or predominantly by paracellular transport through the leaky tight junctions (Ca^{2+}, Cl^-). Furthermore, filtered macromolecules undergo reuptake by a prominently developed endocytotic apparatus. In addition, the proximal tubule (predominantly S_3 segments) secretes organic cations and anions, which constitute an extraordinarily diverse array of compounds of physiologic, pharmacologic, and toxicologic importance. The transepithelial transport involves separate entry steps at the basolateral membrane and exit steps at the luminal membrane with specific transporters at both sites.[171–174] Other potentially toxic xenobiotic compounds are metabolized within the well-developed smooth endoplasmic reticulum of S_3 segments.[175]

Unique to the proximal tubule is the reabsorption and degradation of filtered protein and peptides (Figs. 1.15 and 1.16). As discussed previously, all proteins smaller than albumin leak through the glomerular filter; even albumin, in small amounts, is always contained in the filtrate. All these diverse macromolecules are taken up by proximal tubule cells via receptor-mediated endocytosis and degraded in lysosomes. In proteinuric states, this function is greatly enhanced. The uptake and digestion include the following steps.[176,177]

1. Binding of the filtered protein to two multiligand receptors, megalin and cubulin, which are densely accumulated within clathrin-coated pits in the intermicrovillar areas of the apical membrane.

   Megalin is a 600-kDa transmembrane protein belonging to the LDL-receptor family (see reviews).[176,177] The extracellular domain contains four clusters of cysteine-rich, complement-type repeats, constituting the ligand-binding regions. Cubulin is a 416-kDa peripheral membrane protein identical to the intrinsic factor vitamin B_{12} receptor, known from the small intestine. It contains 27 CUB domains, which are responsible for the ability to interact with a great variety of ligands.
2. Small apical vesicals pinch off from the tubular invagination at the intermicrovillar areas to ferry the protein to the next component.
3. Large apical vacuoles located in the apical cytoplasm are formed by fusion of small vesicles representing the early endocytotic compartment.
4. Condensing vacuoles form in which the protein is condensed. These vacuoles move basally in the cell and acquire hydrolytic enzymes by fusion either with primary or secondary lysosomes. The proteins sequestered within lysosomes appear to be broken down into amino acids that are reused by the cell.
5. Already sequestered in the early endosomal compartment, the receptors are concentrated in dense, apical tubules by which they are returned to the apical plasma membrane.

This process of megalin-mediated internalization appears to be of even much wider relevance. As mentioned previously, the proximal tubule reabsorbs phosphate, representing a key element in phosphate homeostasis. Depending on the amount of sodium phosphate cotransporters present in the brush border cell membrane, the reabsorption varies. Internalization of the phosphate transporter type II (e.g., PTH induced to downregulate reabsorption) occurs via megalin-mediated endocytosis.[178,179] The proximal tubule has also endocrine relevance. In response to stimulation by PTH, it produces, by adding a second hydroxyl group, the active vitamin D_3, calcitriol.

Thin Limbs of the Loop of Henle (Intermediate Tubule)

Thin limbs can be short, occurring only along the descending limb, or they can be long, reaching varying distances into the inner medulla. In the long-looped variety, thin limb segments compose part of both the descending and ascending limbs. Four types of thin limb segments are routinely identified: (1) descending thin limbs of short-looped nephrons (SDTL) (Figs. 1.23 to 1.25), (2) upper portions of descending thin limbs of long-looped nephrons (LDTL up), (3) lower portions of descending thin limbs of long-looped nephrons (LDTL lp), and (4) ascending thin limbs of long-looped nephrons (ATL) (Figs. 1.24 and 1.25). This pattern has been observed in rats,[180,181] mice,[182] syric hamsters,[183] the desert rodent Psammomys obesus,[184,185] and the rabbit.[158] Thin limbs have not been studied as carefully in humans as

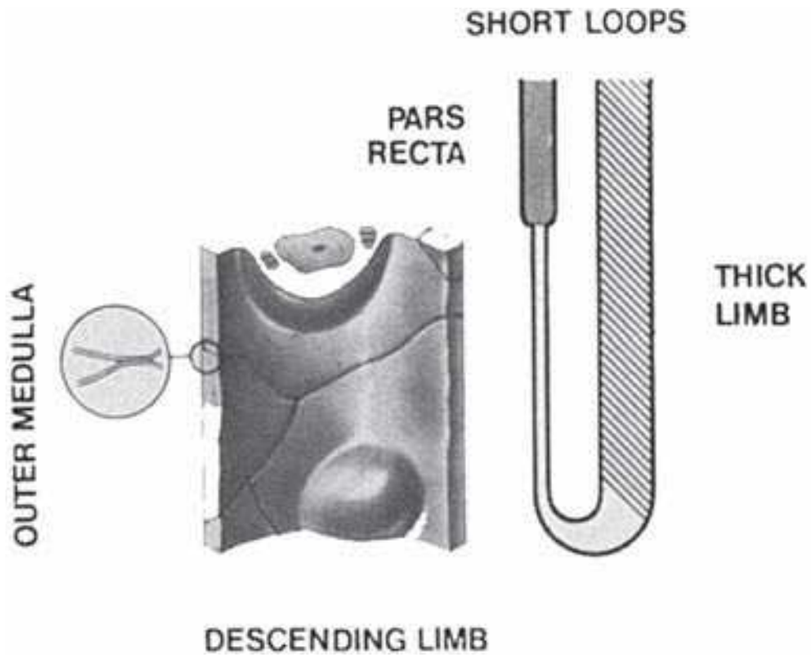


FIGURE 1.23 Diagrammatic representation of thin limb structure in short loops of Henle showing the shapes of constituent cells and the morphology of the occluding junctions (*inset*). (From Schwartz MM, Venkatachalam MA. Structural differences in thin limbs of Henle: physiologic implications. *Kidney Int*. 1974;6:193, with permission.)

in several other animal species.[13,186,187] An additional subsegment of thin limbs has been identified in chinchillas.[188]

Surprisingly, as seen by light microscopy, these simple looking epithelia are strikingly different from each other—not only the ascending from the descending limbs but, most remarkably, the descending limbs of short from those of long loops. Furthermore, within the descending segments, the proximal portion, although structurally not different from the distal portion, express a different pattern of transporters than the distal portion. Even islets or interposed limb pieces of functionally different cells are encountered within an otherwise homogenous thin limb segment.[189,190] Beyond all of these heterogeneities, there are prominent differences among species. This situation may explain the doggedly persistent discussion about the integrated function of thin limbs in the urine-concentrating process; a generally accepted concept of how the final concentration of the urine in the inner medulla occurs is lacking.

The type-1 epithelium lining (SDTL) is composed of flat, noninterdigitating cells, joined by tight junctions that consist of several anastomosing strands. Cell organelles are exceedingly sparse. Functionally, this segment contains aquaporin 1 (AQP1) and the urea transporter UT-A2 in its membranes.[191–194] Thus, it is water and urea permeable. However, these properties are unequally distributed; within the proximal part, the water permeability is high, whereas, within the distal part, the urea permeability is high.[191,193,195] In species with complex vascular bundles (rat, mouse, etc.), the SDTLs lie within vascular bundles[187]; in these surroundings, the thin limbs are in an ideal position to recycle urea from the ascending vasa recta into the short-loop nephrons.

The descending limbs of long loops (LDTL) are generally much larger in diameter and have a thicker epithelium than those of short loops (Fig. 1.25). Moreover, these thin limbs segments are heterogeneous; those of the longest long loops begin in the inner stripe as a much thicker tubule than those of shorter long loops. The character of the epithelium gradually changes as the limbs descend toward and into the inner medulla. The subdivision of the long descending thin limbs into an upper part (type-2 epithelium) and a lower part (type-3 epithelium) is an approximation and reflects the gradual change

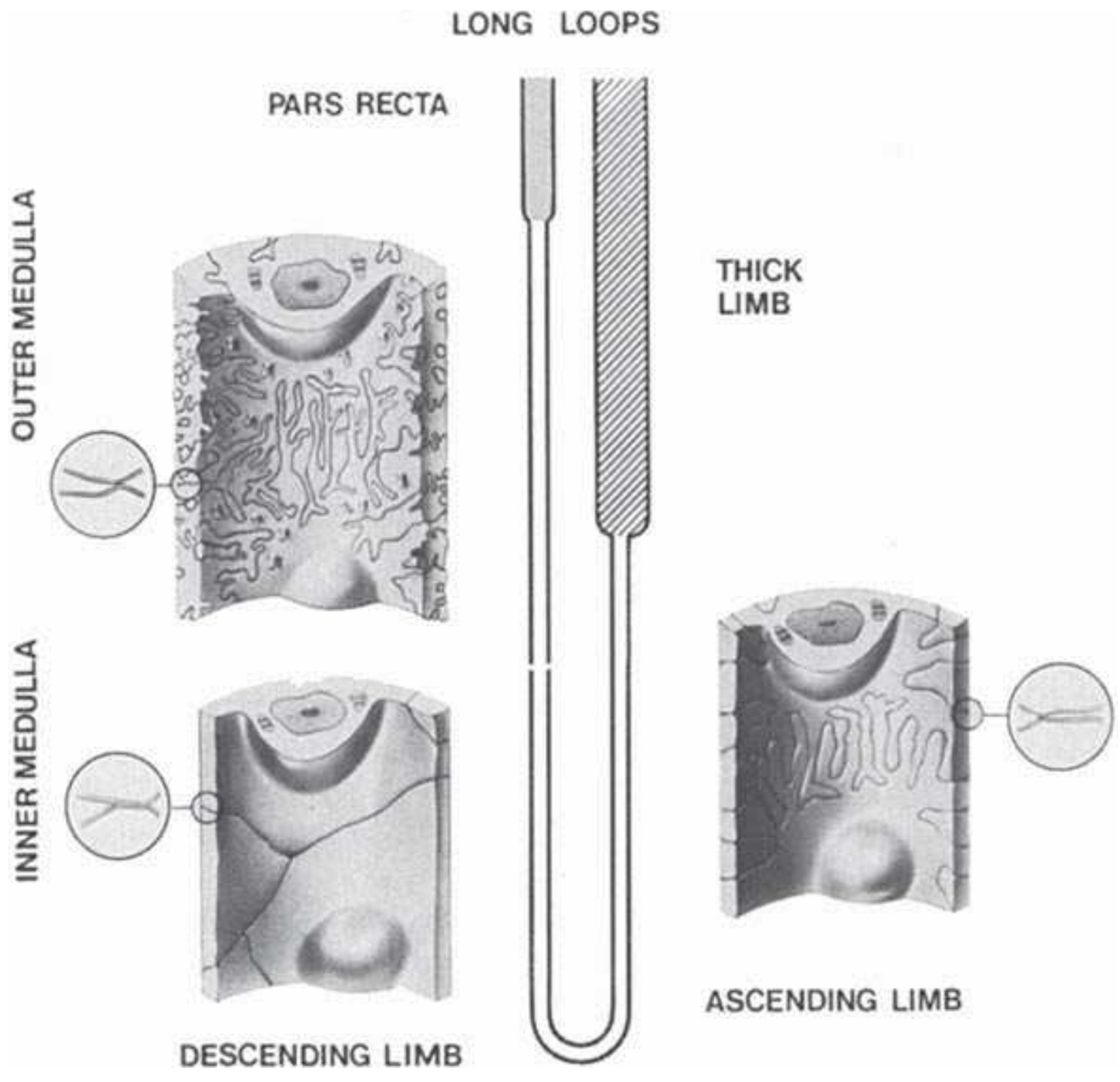


FIGURE 1.24 Diagrammatic representation of thin limb structure in long loops of Henle, showing the shapes of constituent cells and the morphology of the occluding junctions (*inset*). (From Schwartz MM, Venkatachalam MA. Structural differences in thin limbs of Henle: physiologic implications. *Kidney Int*. 1974;6:193, with permission.)

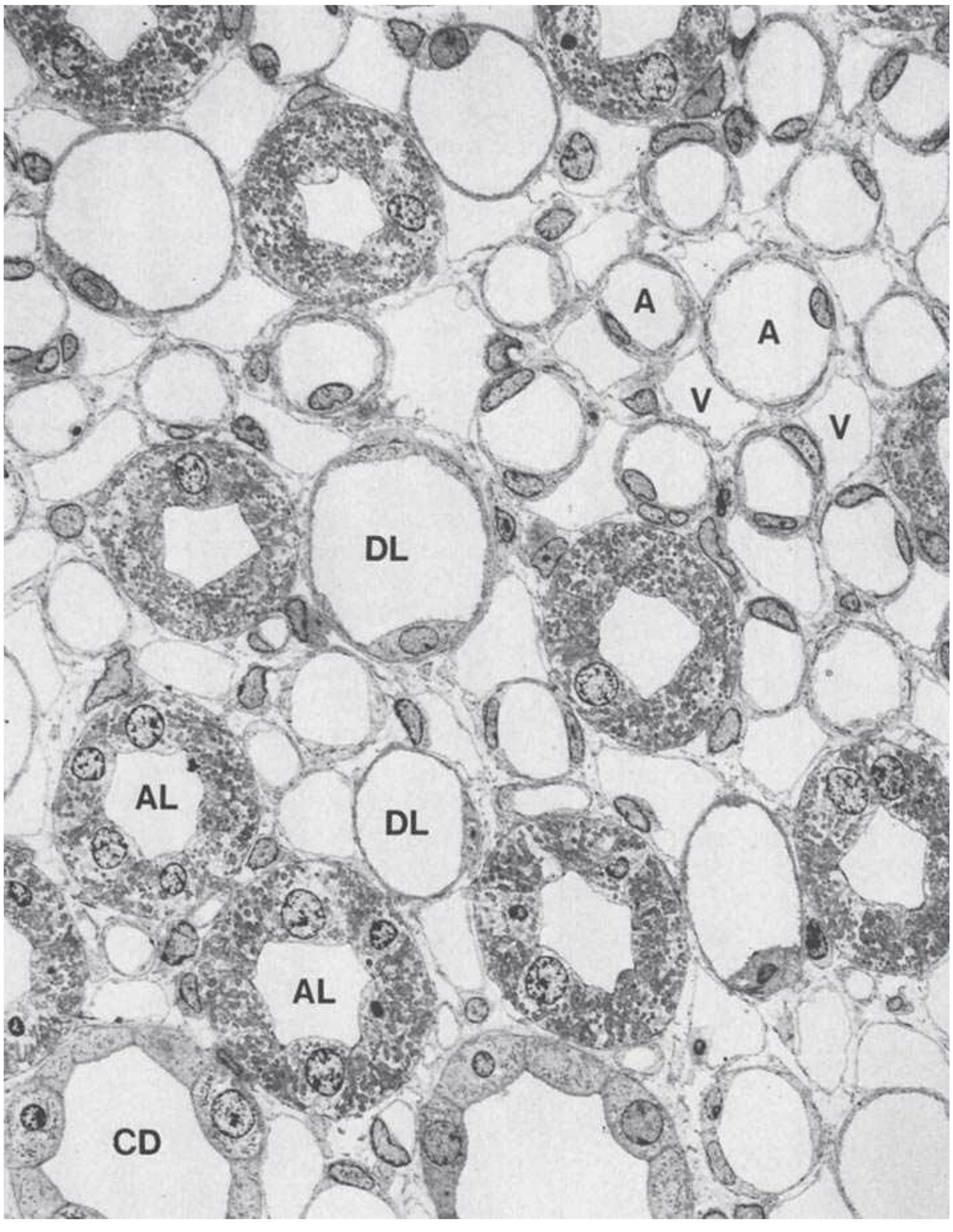


FIGURE 1.25 Low power electron micrograph of a cross-section through the inner stripe (rabbit). A vascular bundle with arterial (*A*) and venous (*V*) vasa recta is surrounded by descending thin limbs (*DL*; the small profiles belong to short loops and the large profiles to long loops), and thick ascending limbs (*AL*) and collecting ducts (*CD*); note the dense pattern of capillaries between the tubules. (Magnification ×900.)

between the two epithelia. Moreover, this process of epithelial transformation appears to be related to the length of each loop. It occurs earlier and more quickly in short long loops and is delayed in the longest long loops.[158,159,181,182,187,189,196]

Furthermore, considerable interspecies differences, particularly prominent in type 2 epithelia, complicate the situation. Two patterns of type-2 epithelium may be distinguished.[187,197] In one group of species (mouse, rat, Psamomys, etc.),[159,184,185,198,199] the type-2 epithelium is characterized by an extremely high degree of cellular interdigitation and a shallow tight junction consisting of only one junctional strand pointing to prominent paracellular transports. In addition, the epithelium has numerous apical microvilli, considerable numbers of mitochondria, and exhibits a high expression of Na^+-K^+-ATPase.[200,201] In a second group of species (rabbit, minipig, and, possibly, man),[202,203] the type-2 epithelium is much more simply organized. The prominent paracellular pathway is lacking; the cells do not interdigitate and are joined by deep tight junctions. In other respects, however, the epithelia are similar in the two groups: numerous luminal microvilli, many mitochondria, and a dense assembly of intramembrane particles in luminal and basolateral membranes are present in both groups. The high density of intramembrane particles may, at least, be partially due to the high density of AQP1 channels in both membranes—corresponding to the decrease of particle density along its descending course. The density of AQP1 channels also decreases and finally terminates completely.[195,204,205] Because structurally a clear cut border between the upper and the lower segment of the LDTL cannot be defined, it may be reasonable to discriminate both segments by the absence of AQP1 in the lower segments.[189,195,204]

Type-3 epithelium (found in LDTL lp) is comparably simple; interspecies differences are no longer prominent. The epithelium consists of flat, noninterdigitating cells, joined by tight junctions of intermediate apico-basal depth[181]; it lacks AQP1 and may, accordingly, have a very low water permeability. Regarding the permeability to urea and the distribution of the urea transporter, UT-A, conflicting data are published, especially when comparing data from different species.[190,191,204,206–208]

The ascending thin limb (ATL) is present only in long-loop nephrons and is uniformly organized among mammals. Generally the transition from the type-3 epithelium of the DTL to the type-4 epithelium of the ATL occurs in a short, but fairly constant, distance before the bend ("prebend segment").[180,185,189] Therefore, functionally, the entire bend should be regarded as part of the ATL. The type-4 epithelium is characterized by very flat but heavily interdigitating cells joined by shallow tight junctions, consisting of only one, but prominent, junctional strand. This leaky organization of the paracellular pathways correspond with functional studies,[209,210] which all demonstrate that the ATLs are highly permeable for ions.

The change from the type-3 epithelium to the type-4 epithelium coincides with the full disappearance of the urea transporter UT-A and the abrupt beginning of the expression of the chloride channel ClC-K1[189,204]; aquaporins are also lacking. Thus, the ATL is water and urea impermeable, but highly permeable for Cl^- and also Na^+.

In humans,[211,212] dogs,[161] and minipigs,[203] a gradual transition is seen from the thin limb to the ascending thick limb; however, an abrupt transition is seen in most other species.[158,213,214]

THE DISTAL TUBULE OVERVIEW

The distal tubule of mammalian kidneys has been defined in many and conflicting ways. For morphologists, the distal tubule is divided into several serial segments with differing locations in the kidney and of varying ultrastructural patterns[215] (Fig. 1.2). The first morphologic segment of the distal tubule for long-looped nephrons begins at the boundary between the inner zone of the medulla and the inner stripe of the outer zone of the medulla. At this boundary, the cells lining the ascending thin limbs of long-looped nephrons increase in height, forming the MTAL. The MTALs of short-looped nephrons are encompassed within the inner stripe of the outer zone in which the descending thin limb of short-looped nephrons converts into a descending thick limb, which then makes a hairpin turn and ascends as an MTAL. The MTAL traverses the inner stripe of the outer zone of the medulla and continues toward the cortex through the outer stripe of the outer zone of the medulla. The MTAL then enters the cortex, ascending within the medullary ray as the CTAL. These two segments, the MTAL and CTAL, are also called the straight part of the distal tubule. The CTAL then leaves the medullary ray and enters the pars convoluta of the cortex, running between the afferent and efferent arteriole of the renal corpuscle from which that tubule was derived. A plaque of taller, but narrower, cells is found in the wall of the CTAL in this region; this plaque of cells forms the macula densa (JGA). In humans, the cells of the MTAL are not as elaborately shaped as those described in the majority of laboratory animals that have been studied.[212]

After a short post macula densa region of the CTAL, the distal tubule becomes more convoluted and it is now lined by epithelial cells that increase in height from the CTAL, which forms the distal convoluted tubule (DCT) (see Fig. 1.27).[214,216] In rabbits, the DCT contains one type of cell known as the distal convoluted tubular cell.[158] In this species, the DCT is abruptly replaced by the connecting tubule (CNT). In other species, such as rats and mice,[217] humans,[218] or minipigs,[203] the transition is more gradual with intermixing of cells so that in the late DCT of these species connecting tubule cells and intercalated (IC) cells begin to dominate, although DCT cells and principal cells of the collecting duct also can be identified. This segment is then defined as the CNT. The CNT of superficial nephrons begin as unbranched segments, each emptying into a collecting duct (some investigators call the first part of the collecting duct the initial collecting duct). In midcortical and juxtamedullary nephrons, the change from the DCT to the CNT occurs a few cells before the fusion of the two tubules.[158] These CNT segments join to form branching connecting tubule segments called arcades, which arch upward in the cortex and then empty into a CCD.[7] The number of each type of CNT architecture varies with the species.

Medullary Thick Ascending Limb

The MTAL is lined by cells of one type that has extensive basal interdigitating processes filling the basal three-fourths of the cytoplasm (Fig. 1.26). The larger cell processes contain large mitochondria that are elongated on an axis perpendicular to the tubular basement membrane. In addition to their normal contents, the mitochondria contain prominent intramitochondrial granules and occasional filamentous bodies.[219] The epithelium lining the MTAL is approximately 7 μm in height.[213,220]

The apical surface of the MTAL cells does not have an elaborate shape in rabbits[158]; it is more elaborate in rats.[221] Tisher and associates,[213,216,220] using scanning electron microscopy, have described two surface configurations of the MTAL cells, with variations in apical cell outlines and in the number of apical microplications. Some cells have smooth apical surfaces with a few microprojections, whereas others have a rough surface with numerous microprojections. The MTAL cells have both extensive invaginations of the basolateral plasma membrane, as well as a large lateral interdigitating process of adjacent cells.[220] Because a main function of the TAL is the reabsorption of sodium and chloride from the tubular lumen to the interstitium, these basolateral processes have Na^+-K^+-ATPase activity.[222] In addition, they have an apical bumetanide-sensitive sodium-potassium-2 chloride cotransporter called NKCC2 located in the apical

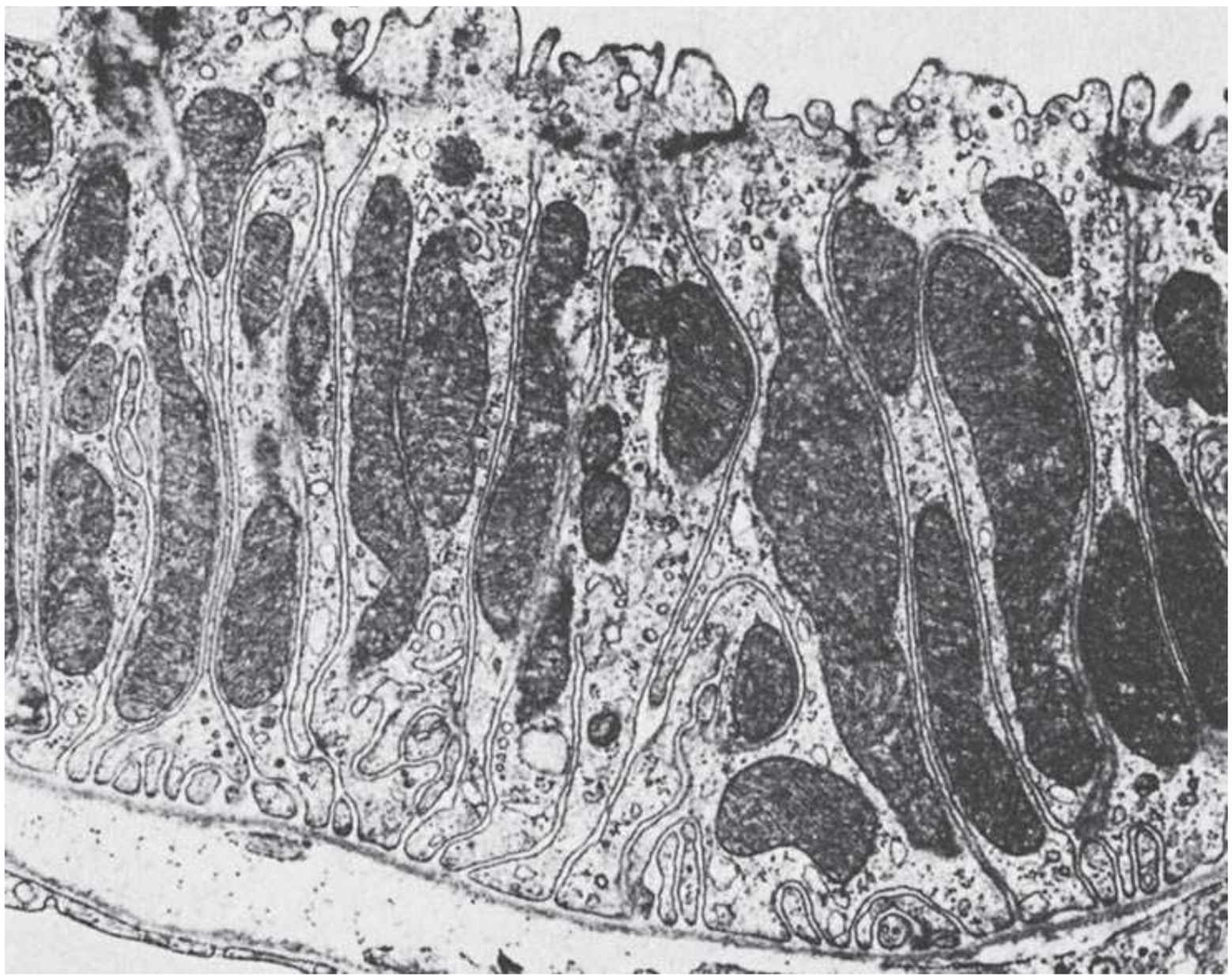


FIGURE 1.26 Transmission electron micrograph of the pars recta of the distal tubule from a rat showing the interdigitating cellular processes. (Magnification ×15,000.)

membrane (see the discussion of the membrane amplification principle later in this chapter). The MTAL demonstrates a low permeability for water so the active transport of the sodium ions out of the basolateral membranes of the cells in exchange for potassium ions participates in forming the hypertonicity of the medullary interstitium. The apical cytoplasm contains a variable number of vesicles and a prominent Golgi apparatus. Cisternae of rough-surfaced endoplasmic reticulum can be seen throughout the cytoplasm. The tight junction is of low to intermediate apical–basal depth, consisting of several parallel strands.[223] These tight junctions appear to be related to the relative impermeability to water (see discussion later in this chapter of tight junction structure and their role in transepithelial solute and water transport). As the MTAL traverses the outer stripe in rats, it decreases in cell height, but retains its prominent lateral interdigitations. In rabbits, the apical surface becomes more elaborate in this region.[158]

The administration of vasopressin (antidiuretic hormone) to rats that have hereditary diabetes insipidus causes hypertrophy of the MTAL in Brattleboro rats.[224] This effect can also be shown by water restriction in normal rats[225] and after high protein intake.[226,227]

Cortical Thick Ascending Limb

The cells of the CTAL are lower in height than those of the MTAL in the rat, measuring about 5 μm in height[221] and 2 μm in the rabbit.[2] The cells still have prominent basolateral interdigitating cell projections, which increase the basolateral cell membrane approximately tenfold over the apical or basal cell surface.[228] These interdigitating processes are more prominent in a circumferential direction.[228] Mitochondria are still prominent in the basolateral cell processes, but somewhat smaller than those of the MTAL. The apical cell border becomes more tortuous in this segment, having more prominent invaginations of the entire lateral cell margins, including the apical region of the cell,[158] and has more microvilli along its surface.[213] Tamm–Horsfall glycoprotein has been identified covering the plasma membrane in the TAL of rat.[229,230]

The MTAL and CTAL differ from each other physiologically, especially in hormone responsiveness.[231,232] The MTAL has a high density of Na^+-K^+-ATPase.[233] Hebert et al.[222] have shown in mice that antidiuretic hormone (ADH) increases the transepithelial voltage and net chloride reabsorption in the medullary, but not the cortical, region of the ascending thick segment. The ascending thick region functions in flow-dependent absorption of NaCl mediated by the furosemide (bumetanide)-sensitive cotransport NKCC2.[234,235] Nielsen et al.[236] and Obermuller et al.,[237] using immunohistochemistry, have demonstrated bumetanide-sensitive Na-K-2Cl labeling in the apical plasma membrane and the subapical intracellular vesicles of MTAL and CTAL in the rat and rabbit. The apical plasma membrane of the macula densa region also had distinct labeling consistent with a role in tubuloglomerular feedback.

The TAL in the inner stripe has a greater reabsorptive capacity for NaCl than the cortical segment,[238] but the cortical segment can maintain a higher concentration gradient[239] (see the discussion of Na^+-K^+-ATPase and the basolateral membrane area later in this chapter). The sodium chloride

reabsorbed by the TAL contributes to the hypertonicity of the medullary interstitium. As the CTAL ascends toward the renal corpuscle from which it was derived, there is an increase in apical plasma membrane surface area, both by an increase in apical microvilli and by an increase in lateral cell margins.[221]

The macula densa region is formed by a plaque of cells in the wall of the CTAL (see Fig. 1.35), where the ascending thick tubule runs between the afferent and efferent arterioles of the renal corpuscle from which the tubule is derived. A group of extraglomerular mesangial cells (lacis cells, Polkissen cells) fills the cone-shaped area formed by those three structures.[240] These four elements—the afferent and efferent arterioles, the extraglomerular mesangium, and the macula densa—constitute the juxtaglomerular apparatus, which will be discussed separately in more detail later in this chapter.

Distal Convoluted Tubule

The DCT (distal pars convoluta) is shorter than the proximal convolution, being about 1.2 mm in length in the rat.[217] Therefore, fewer profiles are seen in sections of the renal cortex. The DCT begins with a rather marked increase in the height of the lining cells from those of the TAL, although the cells are similar to those lining the TAL. In the rabbit, DCT cells are three to four times taller in the DCT than the cells lining the CTAL.[214] The DCT tubule has a variable diameter and contains one type of cell with more nuclear profiles than are seen in the proximal convoluted tubule (Fig. 1.27). The DCT extends from the CTAL to the CNT. The cells lining the DCT have short, bulbous luminal microvilli, but no regular brush border. The microvilli are more numerous than seen on the TAL cells, the CNT cells, or the principal cells of the CCD.[241] In humans, small lipid droplets are seen in the cytoplasm.[212] The endocytic apparatus is not well developed, but some vacuoles and numerous small vesicles are seen in the apical region. Dorup[241] describes four types of vesicles in these cells: intermediate vesicles (80 to 200 μm), which are generally uncoated; small vesicles with a mean diameter of 50 μm that are continuous with these intermediate vesicles; large vesicles (>200 μm); and tubular profiles (these being less frequent in DCT cells). Some rough-surfaced endoplasmic reticulum and lysosomes are also present in this segment. The cell nuclei occupy an apical position, because the basal two thirds of the cytoplasm is filled with extensive lateral interdigitating processes surrounded by basolateral cell membranes. These basolateral processes are filled with large elongated mitochondria that have their longitudinal axis perpendicular to the basement membrane. The presence of the large mitochondria lying adjacent to the basolateral cell membranes is consistent with the need for ATP for the continued active reabsorption of solute that occurs in this tubule (see Fig. 1.27). The volume of mitochondria in the DCT cells is larger than in the CNT cells or cortical collecting duct (CCT) cells.[241] An increased delivery of sodium to the DCT brings about an increase in the volume of the cell, in the mitochondrial volume, and in the proliferation of the basolateral membranes in the cells of the DCT, as well as in the CNT cells and the principal cells of the collecting ducts.[242–244]

The apical membrane of the DCT cells has numerous small microprojections. The tight junctions between the cells of the distal tubule are elaborate and are composed of multiple lines of membrane fusion,[245] a characteristic that correlates with the ability of the cells to maintain a large

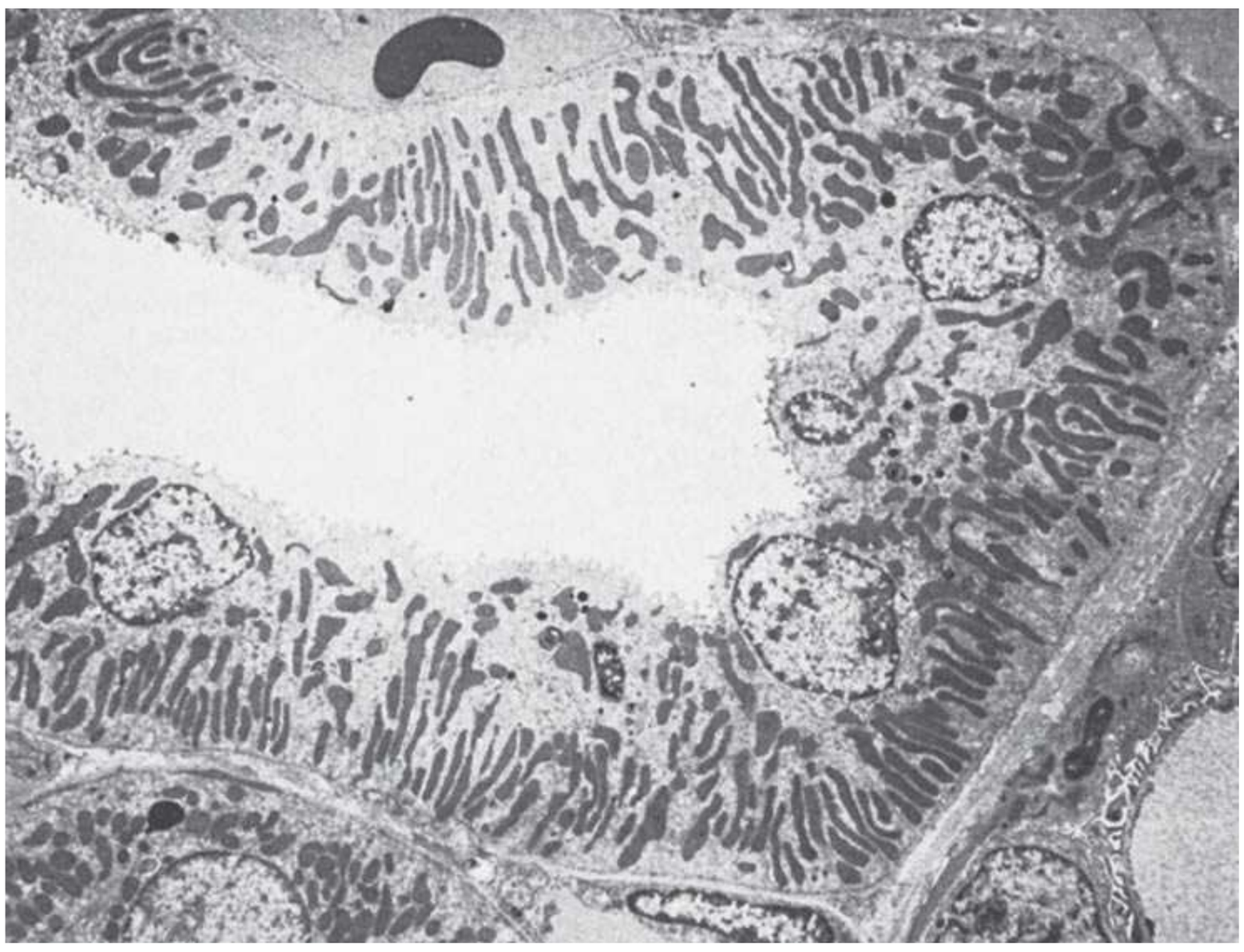


FIGURE 1.27 Transmission electron micrograph from a rat distal convoluted tubule showing the numerous mitochondria within interdigitating processes. (Magnification ×2,850.)

electrochemical gradient. The DCT reabsorbs NaCl against a steep chemical gradient and, as expected for such a sodium-absorbing epithelium, contains abundant Na^+-K^+-ATPase on its basal lateral cell membrane.[233,246,247] DCT proliferation and basolateral membrane amplification occur when active sodium reabsorption increases.[248,249] Kaissling et al.[250] increased the NaCl load to this segment by administering furosemide and demonstrated a marked adaptive increase in the basolateral membrane in the DCT.

In rabbits, an abrupt transition is seen from the distal tubule to the connecting piece of the nephrons.[158] The situation is less clear in other species, in which the transition does not appear to be either as abrupt or as completely studied. Recent studies by Biner et al.[251] used immunohistochemistry to localize the various transport systems along the human cortical distal nephron. The DCT demonstrates luminal thiazide-sensitive sodium-chloride cotransporter (NCC). The NCC overlaps with epithelial sodium chloride channels (ENaC) for a short region at the end of the DCT. IC cells were interspersed among the DCT cells near the end of the DCT. (For more detailed information, see recent reviews from the groups of Kaissling[251,252] and Bachmann.[253])

The Connecting Tubule

The CNT forms the next region of the distal nephron and lies between the DCT and the collecting duct system. At the present time, the CNT may be best classified as part of the DCT because it appears to be derived from the metanephric blastema.[4] Peter[7] believed that the arcades arise from the ureteric bud, whereas Oliver,[5] Potter,[254] and, more recently, Neiss,[4] all believe that the metanephric blastema is the correct source. However, Howie et al.,[255] using immunohistologic methods with substances related to the ABO blood groups, various cytokines, and Tamm–Horsfall protein, found that the ureteric bud and the connecting piece express the same antigens. The morphology of the CNT cells appears to have features of both the DCT cells, such as some degree of lateral interdigitations that contain mitochondria, but also has features of the CCD cells, such as the presence of more true basal infoldings (Fig. 1.28). Because of the intermixing of DCT cells, CNT cells, IC cells, and principal cells of the CCD in some species, such as rats and mice, there is a gradual transition from the DCT to the CNT in these species.[213,217] Neiss[4] demonstrated that IC cells (also called dark cells) in the CNT arose from the metanephric blastema, whereas the IC cells found in the collecting duct arose from the ureteric bud. Kim et al.,[256] using specific antibodies to carbonic anhydrase II, H^+-ATPase, and band-3 protein, demonstrated that IC appeared simultaneously in both the CNT and the medullary collecting duct. These IC cells differentiated from separate foci: one in the nephron (developing from the metanephric blastema) and one in the collecting duct (developing from the ureteric bud). When first identified, cells with distinct apical staining for H^+-ATPase (presumed to be type A intercalated cells), as well as cells with distinct basolateral H^+-ATPase labeling

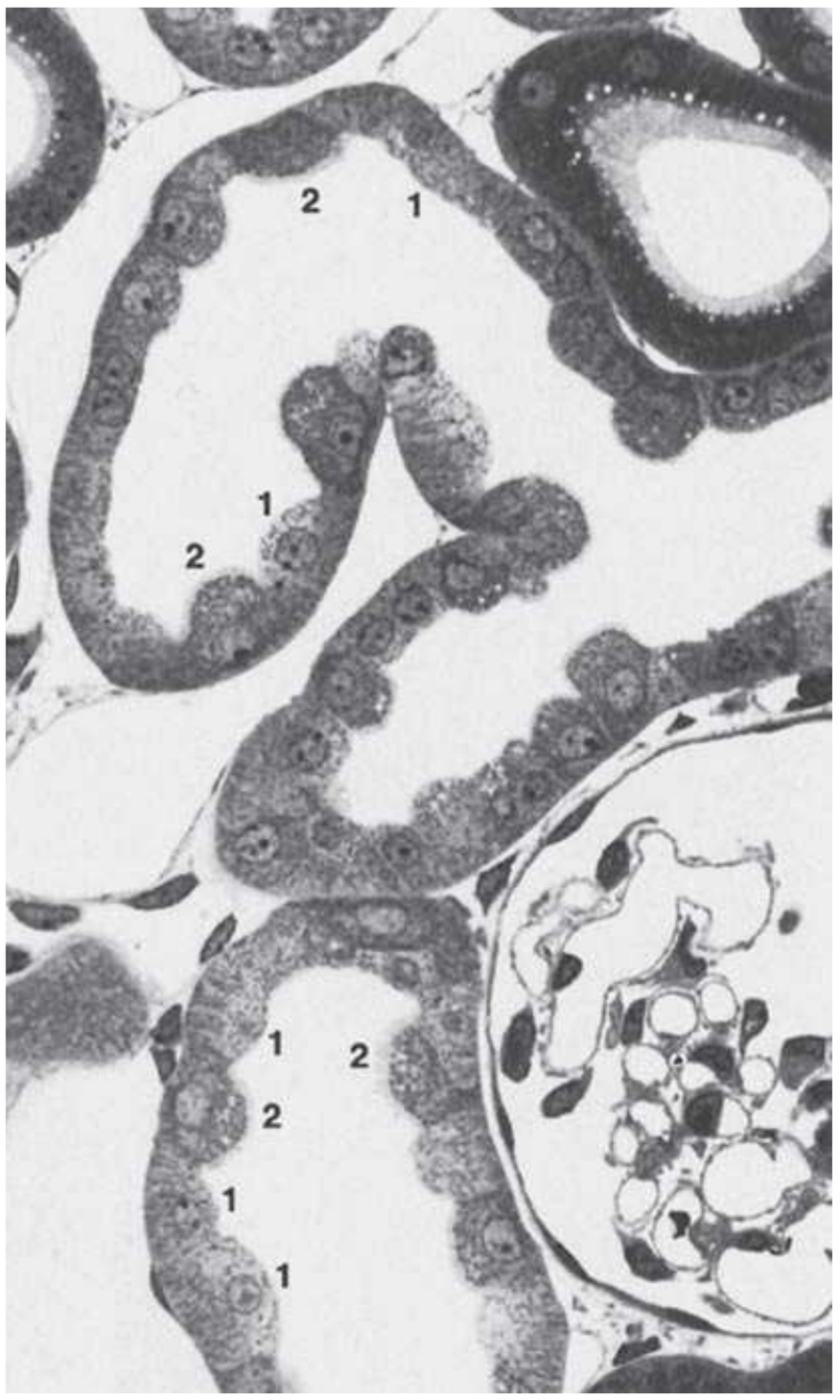


FIGURE 1.28 Light micrograph of the renal cortex (rabbit) showing a connecting tubule composed of connecting tubule cells (*1*) and intercalated cells (*2*). (Magnification ×900.) (From Kaissling B, Zurich, with permission.)

(presumed to be type B intercalated cells), were observed seemingly being developed from two embryologically distinct parts of the kidney. Some of these IC cells in certain locations subsequently disappeared.[256]

The CNT of superficial nephrons are short and drain individually into the collecting duct (some classify the point of junction as the initial collecting duct). CNTs of juxtamedullary and some midcortical nephrons generally form arched collecting ducts in many species. The arched duct starts deep within the cortex with the conversion and confluence of several DCT into the arcade and then ascends, while collecting parts of other nephrons, before the duct turns and enters a medullary ray. This arched duct results from an early embryonic type of nephron induction.[4,7] The number of nephrons that empty directly into the CCD, compared with the number that enter first into an arched tubule, varies with the species.[5] Both types occur in humans.

Two types of cells generally line the CNT, although intermixing of these two cell types with cells of other cell types, such as the DCT cells and the principal cells of the CD, are seen in some species. The two main types of cells

seen are CNT and IC cells (Fig. 1.28). The CNT cells appear to be characterized mainly by extensive true infoldings of the basal cell membrane, which can extend quite deeply into the cytoplasm. However, basolateral interdigitations have also been described, but are less pronounced than seen in DCT cells.[241] In rabbits, the plicated membranes can reach the apical cytoplasm.[158] Stanton et al.[244] demonstrated a striking increase in basolateral membranes in the rat CNT and the initial CCD of potassium-adapted animals, indicating that potassium is secreted by the CNT cells, as well as the principal cells of the (initial) collecting duct. In this study, no changes were seen in the IC cells. This suggests that potassium is secreted by the CNT cell and the principal cell of the initial collecting duct.[244,257]

The basolateral membranes of CNT cells are partially separated by mitochondria, which are smaller, more randomly distributed, and less numerous than those found in the DCT. The volume density of mitochondria in rat CNT cells was significantly lower than in the DCT cells.[241] This arrangement differs from principal cells of the collecting duct, in which the mitochondria are found mainly above the basal infoldings, not among the basolateral membranes. Microvilli on the apical surface tend to be slender and infrequent. Apical vesicles were about as frequent as seen in the DCT cells.[241] Mitochondria, the nucleus, and other cell organelles fill the apical cytoplasm. The CNT cells appear to exist in most species, including rats, mice,[212,217] and humans.[212]

Biner et al.[251] demonstrated an ENaC along the entire CNT in humans. The major part of the CNT also coexpresses aquaporin 2 with the ENaC. IC cells were identified interspersed among the CNT cells in the human. Loffing and Kaissling[258] reviewed the transport pathways along several mammalian distal nephrons and showed that ENaC was present along the CNT from the rabbit, rat, mouse, and human. Frindt et al.,[259] using patch clamp techniques in rat kidney tubule segments, demonstrated that the CNT could reabsorb sodium at a rate 10 times higher than that of the CCT. Using immunogold labeling and electron microscopy, aquaporin 2 (apical), 3, and 4 (basolateral) were all shown to be colocalized in CNT cells of rat.[260]

The CNT cell displays an amplification of basal cell membrane in rats[244] and rabbits[249] in situations in which there is a low Na^+ and high K^+ intake. This effect is axial along the tubule, being greatest at the early segment of the CNT and decreasing along its length.[249] The axial change in structure is paralleled by similar changes in Na^+-K^+-ATPase.[261]

The second cell type is the IC cells (or dark cells). Three types of IC cells have been described: type A involved with the secretion of protons into the lumen; type B involved with the secretion of bicarbonate into the tubular lumen; and non-A–non-B, which may be able to secrete both protons and bicarbonate into the tubular lumen. IC cells will be discussed under the collecting ducts, because these cells comprise such an important number of the cells lining the tubule in that region. Electrophysiologic studies in rabbits suggest that approximately 98% of the IC cells in the connecting tubule are the HCO_3^--secreting B type.[262]

THE COLLECTING DUCTS

The collecting ducts extend from the CNT through the medullary rays as CCDs. They then cross the outer and inner stripe of the outer medulla as well as the inner medulla, to empty their contents at the tip of the renal papillae. They include the CCDs (including the initial collecting ducts), the outer medullary collecting ducts (OMCDs), and the inner medullary collecting ducts (IMCDs).

The collecting ducts are the final regulators of fluid and electrolyte balance, playing roles in the handling of Na^+, Cl^-, K^+, and acid and base. Although there has been great emphasis on the role of the collecting ducts as the main controllers of urinary sodium and potassium excretion, Meneton et al.[263] stress the pivotal role played by both the late part of the DCT and the CNT, especially in situations that prevail in our current environment, in which the dietary sodium intake is high and the potassium intake is low. They propose that a large proportion of the aldosterone-regulated sodium reabsorption and potassium secretion occurs before the tubular fluid reaches the collecting duct. The difference between the function of the late DCT and the CNT compared to the collecting duct seems to be more quantitative than qualitative, with a large proportion of the aldosterone-regulated sodium reabsorption and potassium secretion being done in the late DCT and CNT. The collecting duct would function mainly when the requirement for sodium and water conservation is maximal and the upstream segments are overloaded by diet or some genetic defect.

The collecting ducts are lined by two types of cells: collecting duct principal (light) cells and intercalated (dark) cells (Figs. 1.29 and 1.30). About 30% of CCD cells are IC cells in rat.[264] Kaissling and Kriz[158] estimate that rabbits have 33% IC cells in the CCD and 50% in the outer medullary collecting duct. About one-third of the cells lining the outer medullary collecting duct in the rat are IC cells.[265] The number of IC cells decreases as the collecting duct descends into the medulla and are absent below the first portion of the IMCD. The principal cells of the collecting duct gradually change in morphology as they descend toward the papilla. They increase in cell height and have more complex tight junctions, whereas the amount of basal infoldings and the number of mitochondria decrease. Fusions of the collecting ducts occur in the inner renal medulla to form the large papillary collecting ducts (ducts of Bellini). These large collecting ducts exit at the papillary tip in an area known as the area cribrosa.

The principal cells of the collecting duct in the cortex are cuboidal in humans[218] and low cuboidal in rats (see Fig. 1.29). They form the most numerous cell types. They have a simple cell shape, with fairly straight lateral cell borders that have small interlocking projections. Their

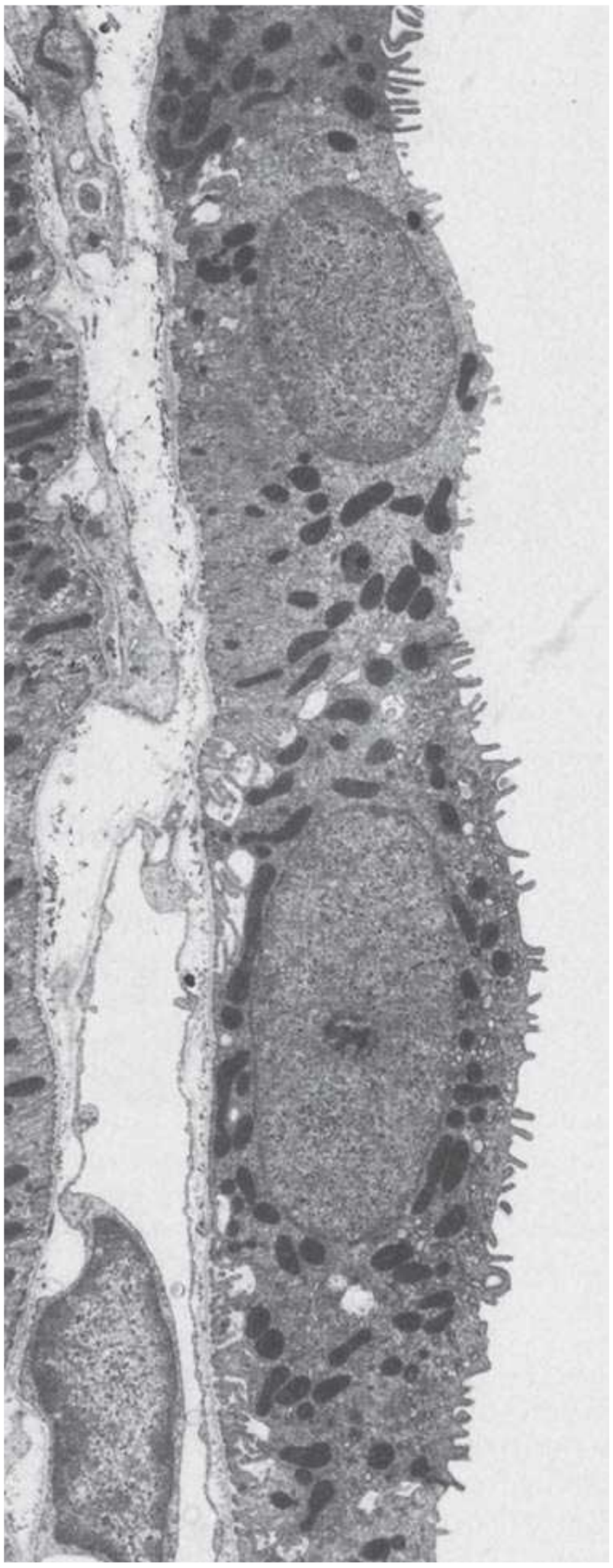


FIGURE 1.29 Transmission electron micrograph of the epithelium of a cortical collecting duct (rat) showing a collecting duct cell (principal cell) (*above*) and an intercalated cell (A-type) (*below*). Note the basal infoldings in the collecting duct cell and the apical vesicles in the intercalated cell. (Magnification ×5,000.)

pale-staining cytoplasm contains a few small, oval, randomly oriented mitochondria and other organelles, and the nucleus is situated in the middle to upper one-half of the cell in the cortex. The luminal surface is generally smooth with a few short microvilli and a single cilium. The basal surface is characterized by true basal infoldings, with few lateral interdigitations. The amplification factor of basolateral membranes was significantly lower than in the CNT cells.[241] Because these infoldings are short and closely spaced, they do not have mitochondria lying between them. The mitochondria are located mainly above the infoldings and in the apical cytoplasm, which contained few intermediate and small vesicles, tubular profiles, and large vesicles.[241] The tight junctions are deep,[223,266] and the apical surface has a prominent glycocalyx.[267]

Conditions that increase potassium secretion, such as potassium adaptation or high endogenous or exogenous mineralocorticoid levels, bring about dramatic increases in these basal cell membrane infoldings.[249,262,268,269] For example, striking increases in the basolateral membranes of the principal cells of the initial segment of the collecting duct have been demonstrated in potassium-adapted animals.[244] In addition, the principal cells in rats showed a 35% decrease in the basolateral membranes in adrenalectomized animals that was restored to control levels by the administration of physiologic amounts of aldosterone, but not of glucocorticoids.[270] In addition, increasing the dose of aldosterone over control levels caused an increase in the basolateral membranes by 111% compared with controls. They did not note changes in the luminal membranes of the principal cells.[270] These

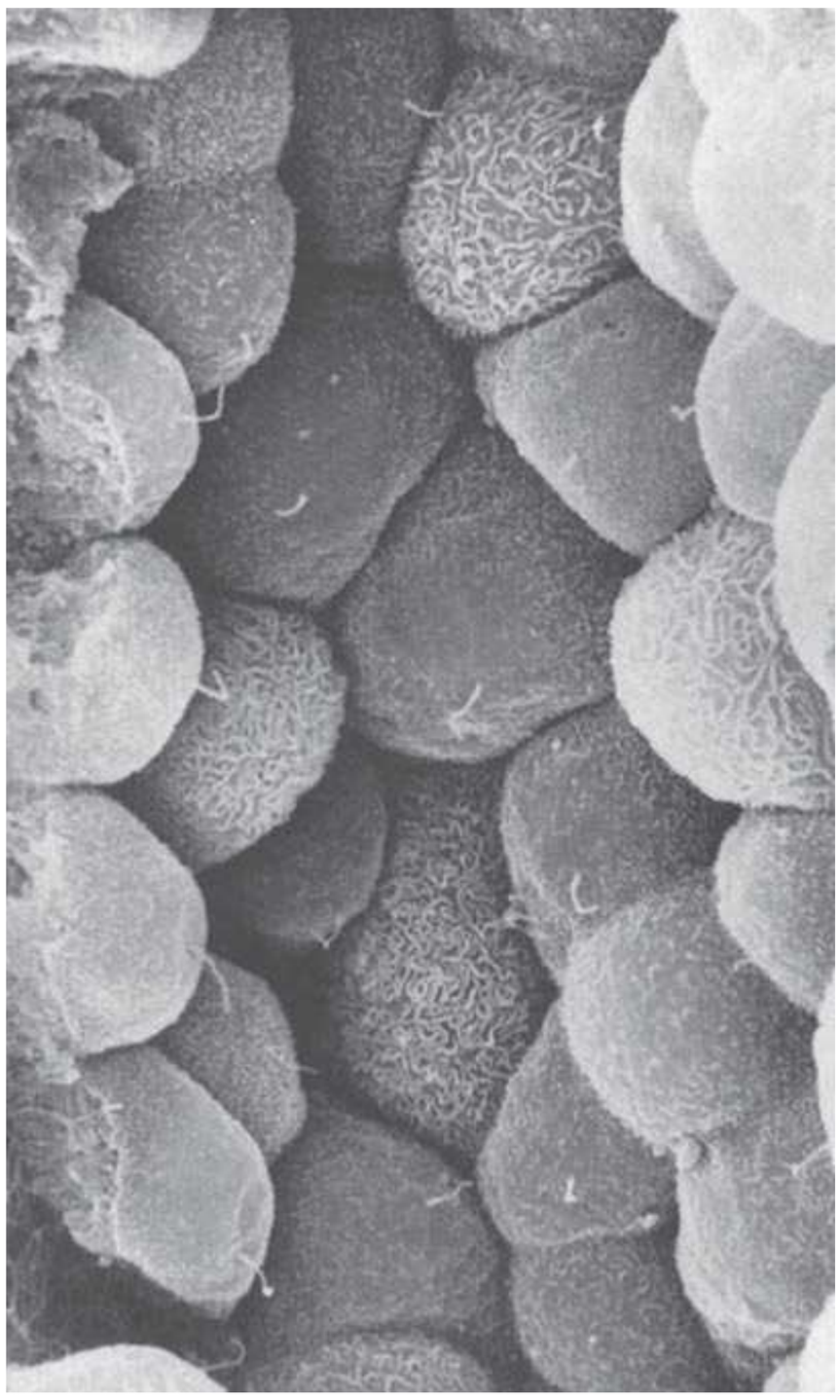


FIGURE 1.30 Scanning electron micrograph of a cortical collecting duct (rat) showing the apical aspect of collecting duct cells (with an apical cilium) and intercalated cells (with apical microfolds). (Magnification ×2,400.)

studies provide evidence that principal cells in the collecting duct are involved with potassium secretion.

The collecting duct responds to ADH by an increase in water permeability. The principal cells are vasopressin sensitive and show dramatic increases in water permeability of the apical membrane. Sun et al.[271] have demonstrated direct evidence that AQP2 on principal cells is located in clathrin-coated pits that recycle between the plasma membrane and intracellular vesicles in response to the availability of ADH. The mechanisms underlying these changes are discussed in detail later in this chapter.

Biner et al.,[251] using immunohistochemical localization techniques on human kidney, demonstrated the presence of an amiloride-sensitive ENaC and AQP2 activity on collecting duct principal cells. Loffing et al.[272] using immunohistochemistry in rabbit kidney cortex, demonstrated that ENaC is found in the CNT cells and the CCD cells. The ENaC shifted from the apical membrane in the upstream CNT cells to a cytoplasmic location downstream in the CNT and CCD cells. In the rabbit, the AQP2 was seen only on the CCD cells. The apical membranes of the collecting duct principal cells of humans contain ENaC and AQP2, whereas the basal membranes contain Na^+-K^+-ATPase and aquaporins 3 (AQP3) and 4 (AQP4); hence, the CCD plays a critical role in the concentration of urine. The CCD also responds to the mineralocorticoid aldosterone.[273]

The collecting duct cells undergo gradual, although considerable, changes from the cortex downstream to the upper one-third of the inner medulla.[158,218] The cells of the outer medulla include principal cells similar to those seen in the cortex; however, the cells become taller with decreasing concentrations of several cellular organelles and basal infoldings. IC cells similar to type A are found in the outer medulla. From the deeper cortical levels downward into the inner medullary region, the basal labyrinth of the principal cells continues to decrease gradually, with a steeper reduction within the outer stripe.[158,215,218] The number of mitochondria also continues to decrease, whereas lysosomal elements and apical-coated vesicles seem to increase. The density of the cytoskeletal network lying under the apical cell membrane becomes more prominent and the tight junctional belt becomes deeper.[158]

From the second one-third of the inner medulla, collecting duct cell size increases steeply. These tall IMCD cells are distinct from collecting duct cells upstream according to several criteria[213,220,274] (Fig. 1.31). Their luminal membrane is covered by numerous stubby microvilli and lacks the central cilium. The lateral intercellular spaces are more extensively developed and are prominent by their dense assembly of microvilli and microfolds projecting from the lateral cell membranes. A prominent feature of principal cells of the last one-third of IMCDs is the expression of the ADH-sensitive

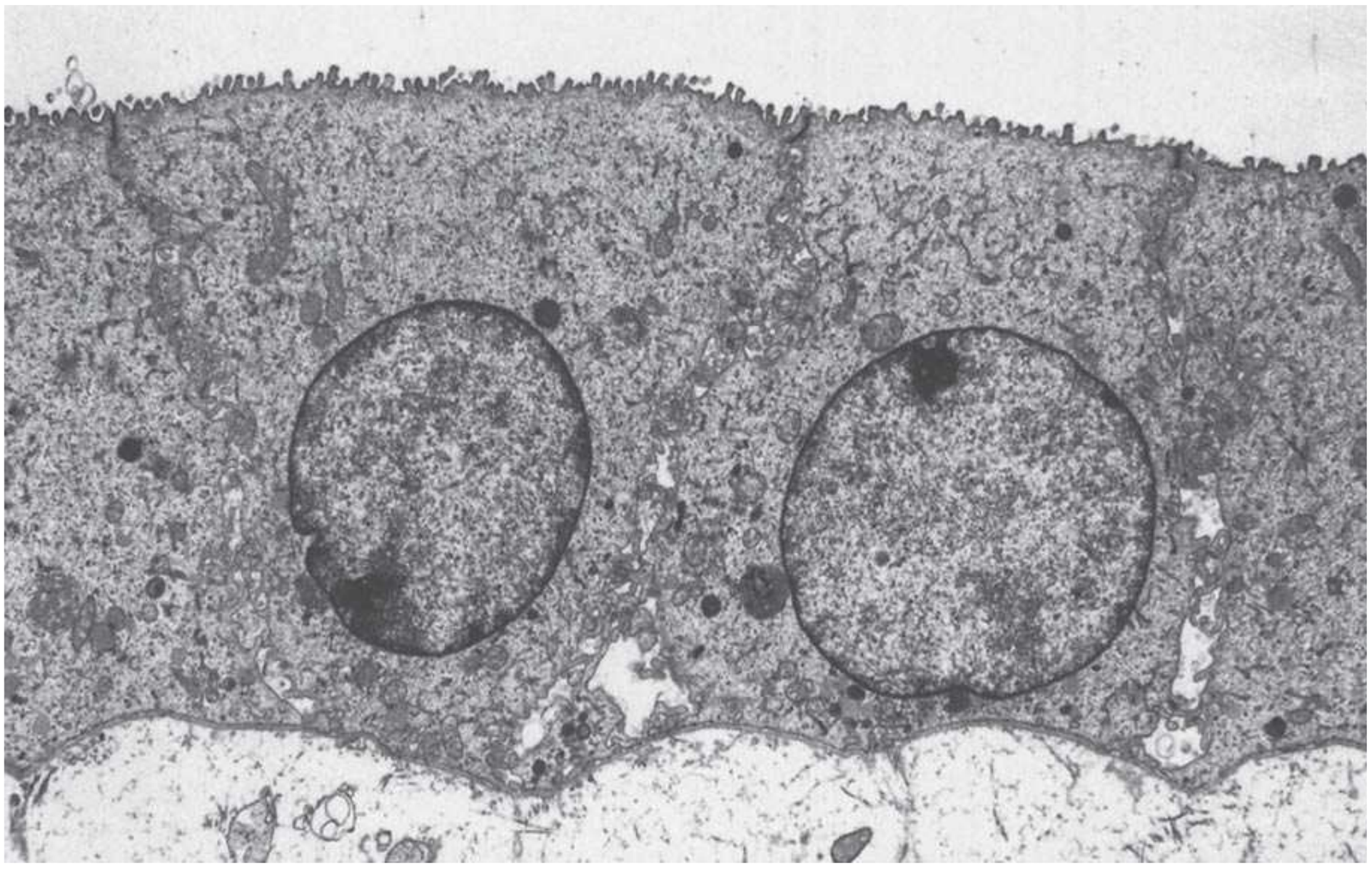


FIGURE 1.31 Transmission electron micrograph of the inner medullary collecting duct epithelium (rat) showing the high inner medullary collecting duct cells with many stubby microvilli of the luminal membrane and prominent lateral intercellular spaces filled with lateral microfolds. (Magnification ×15,500.)

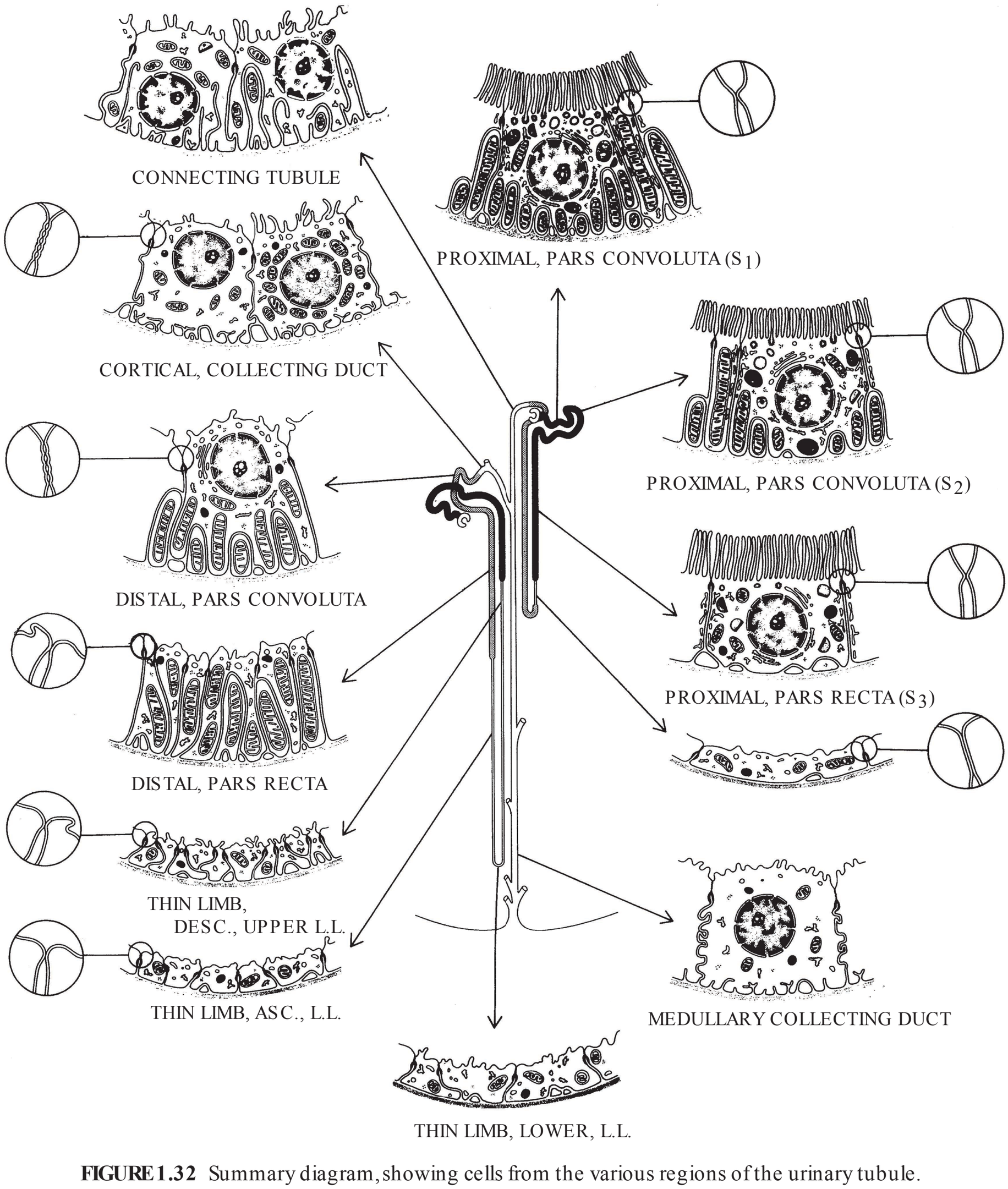


FIGURE 1.32 Summary diagram, showing cells from the various regions of the urinary tubule.

urea transporter UT-A1.[193,275] In most other respects, IMCD cells resemble the other collecting duct cells. The morphology of the various regions of the renal tubule is summarized in Figure 1.32.

Intercalated Cells

IC cells have long been identified in CCDs in a variety of species such as rats,[213,217,264,276–279] mice,[264,280] humans,[218,251] and rabbits[158,248,256,281–283] (Figs. 1.29 and 1.33). It has become obvious that IC cells are not only found in the CCD but, depending on the species, in the latter region of the DCT in humans[218,251,278] and rat,[217,241,265,278] in the connecting tubule in rabbits[158,248] and humans,[251] in the CCD (as listed previously), in the outer medullary collecting ducts in rats,[265] and in the upper part of the inner medullary cells in rats and rabbits.[158] IC cells constitute about 37% to 40% of the cells in CCD in rats and rabbits.[265,280,283] Kaissling and Kriz[158] estimate that rabbits have 33% IC cells in the CCD and 50% in the outer medullary collecting duct, whereas Hansen et al.[265] estimate that 36% to 40% of IC cells are present in the outer medullary collecting ducts in rat. IC cells show a slow decrease in the upper part of the IMCD in rats.[283]

IC cells (Fig. 1.33) are sometimes called dark cells because their cytoplasm stains more densely. They exhibit

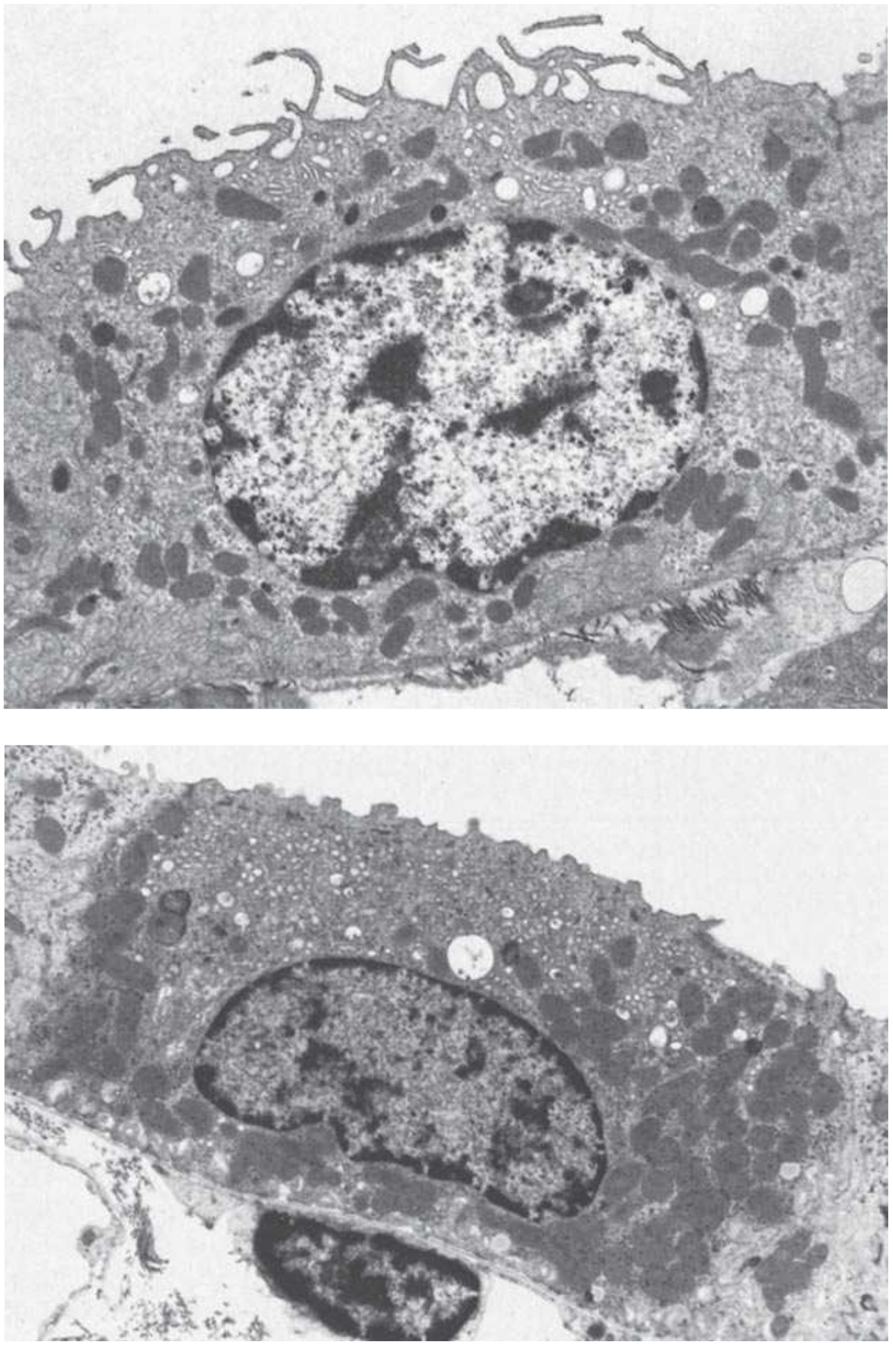


FIGURE 1.33 Transmission electron micrographs of intercalated cells (rat). **A:** Type A exhibiting apical microfolds and flat vesicles in the apical cytoplasm. (Magnification ×6,800.) **B:** Type B showing a rather smooth apical surface, many small round vesicles, and mitochondria located predominantly in the lateral and basal parts of the cell. (Magnification ×6,800.)

significant structural heterogeneity even within a single segment of the collecting duct. IC cells can also be distinguished from principal cells by differences in cell shape, cytochemical staining, and uptake of the pH-sensitive fluoroprobe, 2′,7′-bis(carboxyethyl)—5,6-carboxyfluorescein (BCECF). IC cells have a more circular, rather than hexagonal, profile that bulges into the tubule lumen when observed in isolated perfused tubules by means of interference-contrast optics. In rabbits, IC cells can be identified by positive staining with peanut lectin[284,285] and by luminal uptake of acetoxymethyl BCECF.[285] The apical surface of the IC cell is frequently adorned by luminal extensions, which include microvilli and microridges (called microplicae). Basal membrane infoldings resemble those of the collecting duct cell.

Three types of IC cells have been defined morphologically and by immunohistochemical staining, including type A, type B, and non-A–non-B.[264,280] However, there is a striking difference in the number and distribution of the various types of IC cells from animal to animal and among the nephron segments of a particular species.[264] Carbonic anhydrase II immunoreactivity was seen in all IC cells, but type A stained more intensely than type B. The immunostaining in type A cells was pronounced in the apical cytoplasm and apical microprojections. In type B cells, the staining was more diffuse throughout the cytoplasm. In the non-A–non-B cells, the staining was also diffuse.[286]

Type A IC cells tend to have a more circular apical cell profile,[278] a centrally placed nucleus, prominent apical microvilli and microplicae extending from the apical plasma membrane, and prominent apical cytoplasmic tubulovesicular

profiles (see Fig. 1.33A). Mitochondria are numerous and can be located above the nucleus, as well as between the nucleus and the basal membrane infoldings. Using freeze-fracture techniques, there are rod-shaped particles and studs present on the cytoplasmic face of the apical plasmalemma and on the tubulovesicular profiles in the IC cells of the CCD and the outer medullary collecting ducts.[280,287,288] H^+-ATPase is expressed on the apical plasma membrane and in the cytoplasmic tubulovesicular and vesicular profiles of type A IC cells. (See review of renal vacuolar H^+-ATPase.[289])

Verlander et al.[278] showed that IC cells in animals with respiratory acidosis show a striking increase in apical microprojections and tubulovesicular profiles as well as an increase in surface density of the apical plasma membrane. However, no changes were seen with the number of IC cells. When there was a stimulation of bicarbonate secretion in rats, Verlander and associates[288] documented a withdrawal of the marker for H^+-ATPase from the apical plasma membrane with its storage in apical cytoplasmic vesicles in IC cells in both the CCD and the OMCD. H^+-ATPase activity appeared to be inserted into the basal plasma membrane of type B IC cells.[288]

Luminal and/or tubulovesicular membranes exhibit two specific types of particles. Large club-shaped particles called "studs" have been observed on the cytoplasmic surface of the tubulovesicular structures on the cytoplasmic side of the apical plasma membrane; the rapid-freeze, deep-etch technique shows 10-μm spherical structures composed of multiple subunits and arranged in paracrystalline hexagonal arrays.[290] Brown et al.[291] have presented evidence that the studlike material coating the vesicles contains cytoplasmic domains of the proton-pumping H^+-ATPase. On the basis of both morphologic characteristics and immunocytochemistry, the structures appear to be (or related to) the vacuolar-type H^+-ATPase.[277,291] In addition, freeze-fracture studies have shown the presence of rod-shaped particles in vesicles and cell surface membranes,[292] which may form a component of this H^+-ATPase. Changes in membrane structure have been shown to be related to proton secretion.[248,261] When H^+ secretion is stimulated, cytoplasmic vesicles bearing rod-shaped intramembranous particles (IMPs) fuse with the apical plasma membrane, inserting IMPs into the membrane.

This supports the idea that type A IC cells demonstrate net excretion of protons into the tubular lumen that is accomplished by the vacuolar H^+-ATPase located in the apical plasma membrane and apical tubulovesicular profiles.[264,277,280,288,293,294] For this process, it has been suggested that the hydrogen ions are produced by cytosolic carbonic anhydrase in CNT cells and CCD cells of the mouse and rat[264,280,286,294,295] and human.[251,296,297] The bicarbonate that is generated is released by a band 3-like Cl^-/HCO_3^- exchanger AE1, located in the basolateral plasma membrane, into the interstitium at the base of the cell.

Medullary IC cells from rats fed a diet with a high K^+ content had a small luminal membrane area and a cell apex with numerous vesicles. The ingestion of a low K^+ diet led to an increased luminal membrane area with few apical vesicles.[268] It has, therefore, been postulated that IC cells function in potassium reabsorption.

A similar increase in the apical plasma membrane with a decrease in tubulovesicular profiles was seen in IC cells of the outer medulla in chronic metabolic acidosis[298] and acute respiratory acidosis in rats.[299] In respiratory acidosis, a marked increase in apical microprojections was seen as was an increase in the surface density of the apical membrane of type A cells. No changes were seen with type B cells in rat CCD.[278] Madsen and Tisher[213] postulated that hydrogen ion pumps located in the apical vesicles had been inserted into the apical cell membrane by vesicle fusion with the apical membrane.

Type B IC cells are present in the CCD and the CT of rats,[276,278,286,300] mice,[264,280] and rabbits.[230,256,281,301] The type B cell (Fig. 1.33B) has an angular outline[278] and a relatively smooth apical plasma membrane with short sparse microvilli (without rod-shaped studs in the apical membrane).[280] The apical membrane generally lacks studs, but the basolateral membrane exhibits studs.[278] The nucleus lies in an eccentric position and the numerous small mitochondria are densely packed and are concentrated at the basal part of the cytoplasm. The cytoplasm and the organelles stain more densely in light and electron microscopy. Cytoplasmic vesicles (mostly noncoated) are seen throughout the cell cytoplasm. In the type B cells, the H^+-ATPase is expressed in the basolateral plasma membrane and in the cytoplasmic vesicles throughout the cells.[277,280,291,300,302–305] Using freeze-fracture techniques, rod-shaped rectangular particles are found on the basal membranes in these cells and not on the apical plasma membrane.[277,300] The basal hydrogen ion secretion is thought to mediate HCO_3^- secretion into the luminal fluid by an apical Cl^-/HCO_3^- exchanger different from AE1.[256,264]

One candidate for this anion exchanger is pendrin, a Na^+-independent Cl^-/HCO_3^- exchanger. Quentin et al.[306] have shown that the Cl^-/HCO_3^- exchanger pendrin in rat kidney is specifically regulated in response to chloride balance independent of sodium and acid/base balance. Using a mouse model, Wall et al.[307] demonstrated that pendrin protein was localized in the apical cytoplasmic vesicles in both type B and non-A–non-B IC cells from a subset of cells in the DCT, the type B cells in the CNT, and the CCD. Pendrin mRNA was expressed mainly in the cortex. Pendrin immunoreactivity was highest in the apical cytoplasmic vesicles, although there was little immunogold staining along the apical plasma membrane of type B IC cells, but non-A–non-B IC cells had intense pendrin immunoreactivity along the apical plasma membrane. Kim et al.,[308] using immunoelectron microscopy, demonstrated pendrin in both the apical plasma membrane and intracellular vesicles throughout the cell. Kim et al.[286] found the carbonic anhydrase activity more diffuse in the cytoplasm of type B than type A cells.

The third type of IC cell is non-A–non-B cells that have been identified in the CT and the CCD of rats[264,276,286,309] and mice.[264,276,280] Non-A–non-B cells have vacuolar

type H^+-ATPase in both apical plasma membranes and apical vesicles, but do not have the basolateral band 3-like immunoreactivity of AE1.[264] In rabbits and mice, most non-A–non-B cells in the collecting duct have an electroneutral Na-independent Cl^-/HCO_3^- exchanger in the apical membrane.[264] Pendrin immunoreactivity is intense along the apical membrane, as well as being present in apical vesicles in some cells in the DCT, in the CNT, and CT.[307] Because both pendrin immunoreactivity and pendrin-mediated HCO_3^- secretion are present in the apical plasma membrane and apical intracellular vesicles in type B and non-A–non-B IC cells, Kim et al.[308] suggest that HCO_3^- secretion could be regulated by trafficking of pendrin between the two membraneous compartments. In addition, since non-A–non B cells seem capable of both apical HCO_3^- and H^+ secretion, the simultaneous secretion of both HCO_3^- in exchange for Cl^- and proton secretion mediated by electrogenic H^+-ATPase, would cause chloride reabsorption with no change in acid/base status.[308]

THE JUXTAGLOMERULAR APPARATUS

At the vascular pole of the renal corpuscle, the macula densa region of the CTAL comes into close proximity to the efferent and afferent arterioles and a group of cells called the extraglomerular mesangium.[310,311] The juxtaglomerular apparatus consists of four parts (Figs. 1.5 and 1.34): (1) a plaque of cells in the wall of the CTAL of the distal nephron in the region of the vascular pole of a renal corpuscle, called the "macula densa"; (2) the termination of the afferent arteriole as it enters this renal corpuscle; (3) the initiation of the efferent arteriole as it exits from the same renal corpuscle; and (4) a cone-shaped region of extraglomerular mesangial cells (also called the lacis, Polkissen, or Goomaghtigh cells) lying in the space between the macula densa and the two arterioles. This area receives a rich supply of sympathetic nerve endings.

In the human kidney, 40% of the basal lamina region of the macula densa lies adjacent to the base of the cone-shaped extraglomerular mesangium, 10% of the macula densa basal lamina is in contact with the afferent arteriole, and 5% is in contact with the efferent arteriole.[308]

The MD consists of the cells in the wall of the CTAL of the distal tubule, which lies adjacent to the glomerular vascular pole. The MD appears as a dense spot upon hematoxylin and eosin staining because the cells are narrow and the nuclei are close together. MD cells (Fig. 1.35) are not interdigitated with each other by large lateral cell processes, as in other regions of the distal tubule. In contrast, the lateral intercellular spaces between MD cells extend very straight in an apical–basal direction—their width appears to vary according to function.[312] The mitochondria are shorter and more randomly arranged. The Golgi apparatus lies on the basal side of the nucleus. The basal aspect of the MD touches

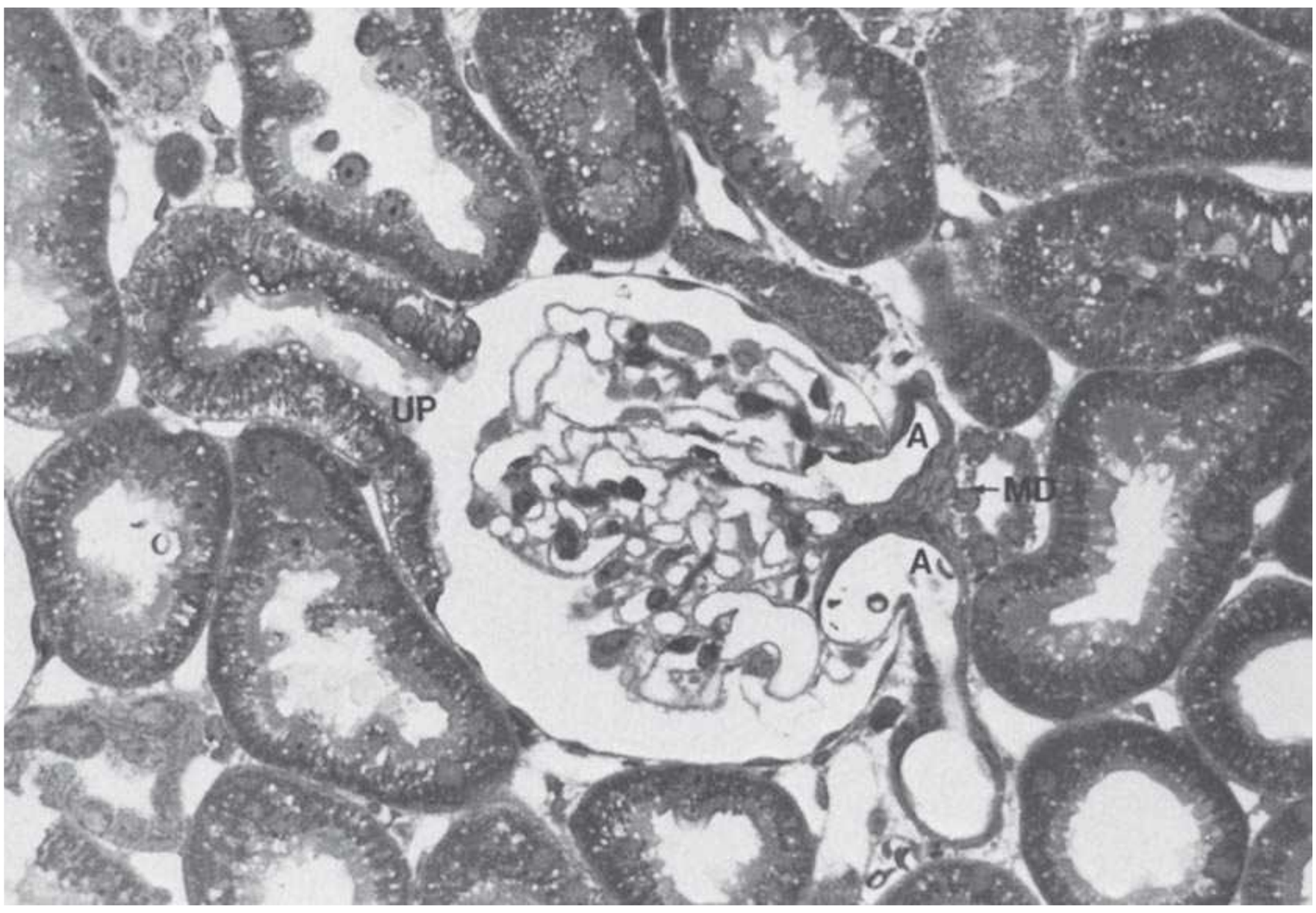


FIGURE 1.34 Light micrograph of a renal corpuscle with both urinary and vascular poles in the section. The juxtaglomerular apparatus contains the macula densa (*MD*), the two arterioles (*A*), and the extraglomerular mesangium (between the *A*s). The urinary pole (*UP*) is also apparent. (Magnification ×13,000.) (From Dobyan D, with permission.)

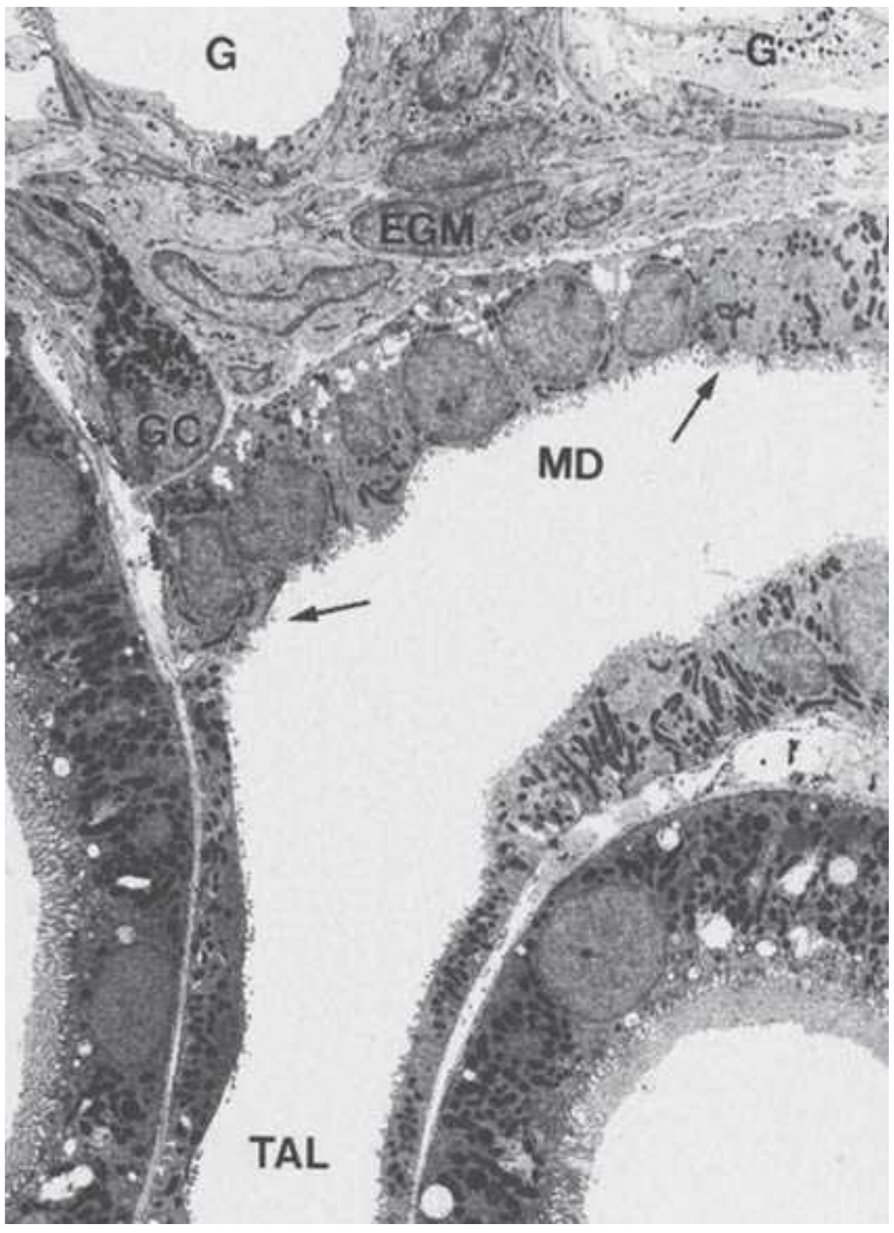


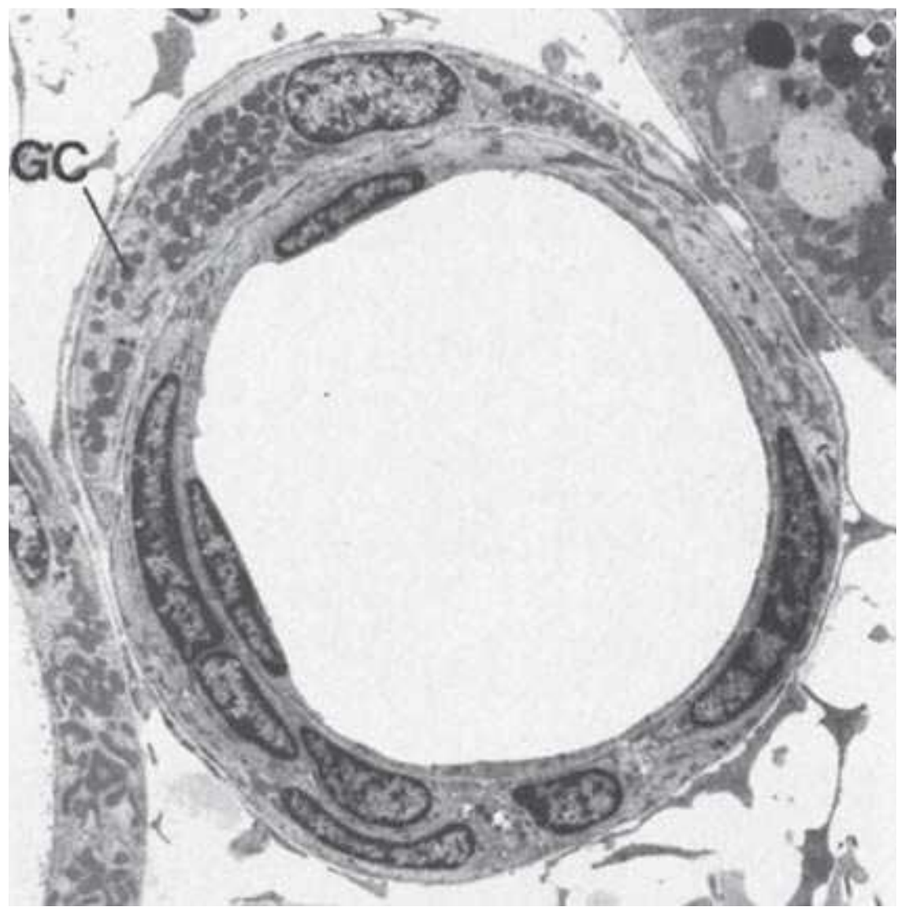
FIGURE 1.35 Transmission electron micrograph of the juxtaglomerular apparatus (rat). The transition of the thick ascending limb (*TAL*) into the macula densa (*MD*) is seen; the basal aspect of the macula densa abuts the extraglomerular mesangium (*EGM*) and also a granular cell (*GC*). *G*, glomerulus. (Magnification ×1,900.)

the extraglomerular mesangium. Additional, but variable, contacts are found with the efferent as well as the afferent arterioles.[13] The most conspicuous difference of MD cells to any other cells of the nephron is the occurrence of nitric oxide synthase I (see Fig. 1.37C).[313,314] A prominent feature of the MD is also the expression of cyclooxygenase-2.[315]

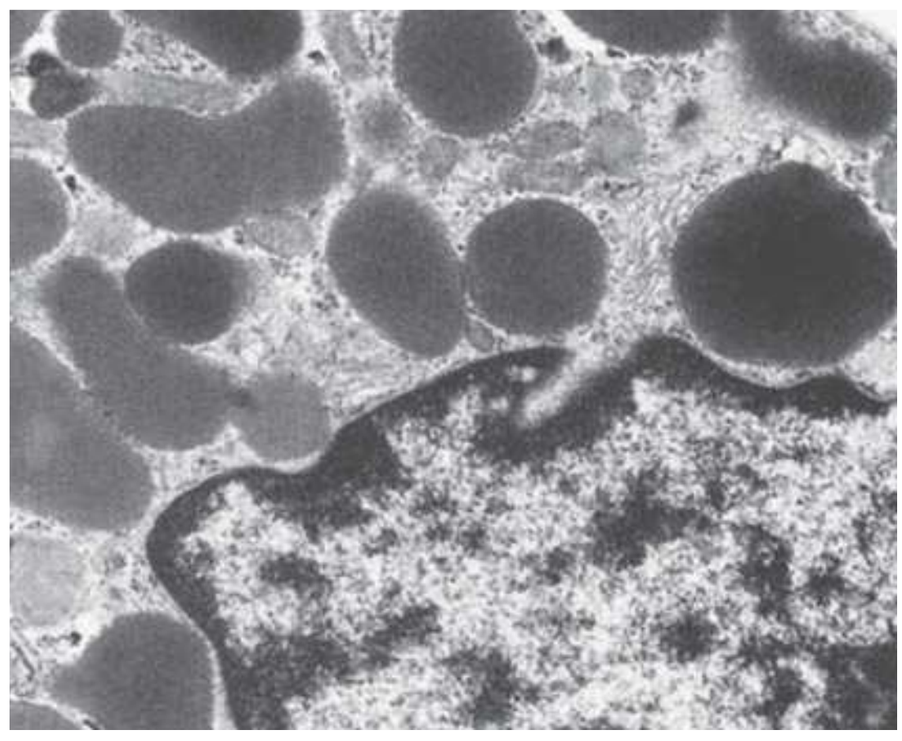
Modified smooth muscle cells in the wall of the afferent arterioles, called granular cells (formerly also called juxtaglomerular cells), contain specific membrane-bound granules (Fig. 1.36). In situ hybridization[316] and immunocytochemistry [317] have shown that these cells synthesize renin (Fig. 1.37A), which is then stored in granular form (Figs. 1.36B and 1.37B). The granules stain with the Bowie method and have a positive periodic acid-Schiff reaction. Like other smooth muscle cells, the juxtaglomerular cells also contain intracellular filaments and dense bodies, but they have more cisternae of rough-surfaced endoplasmic reticulum, a large Golgi apparatus, and mature and immature secretory granules in their cytoplasm, consistent with the ability of these cells to synthesize small peptides. The immature granules appear to have a paracrystalline structure.[80,318] The secretory product is released by exocytosis into the extracellular space within or surrounding the wall of the arteriole.[319,320] Processes of the juxtaglomerular cells contact the surrounding cells as well as the endothelial cells by means of gap junctions.[321]

The extraglomerular mesangium[310] (Goormaghtigh cells, polar cushion, Polkissen cells, and lacis cells) fills the area between the afferent and efferent arterioles and the MD (see Figs. 1.5 and 1.35). It is composed of nongranulated cells that are continuous with granular cells and smooth muscle cells of the arteriolar walls and with the intraglomerular mesangial cells.[310] The extraglomerular mesangial cells are "flatly pressed" cylinders with both ends splitting into a group of parallel processes. These extraglomerular mesangial cells are surrounded by abundant extracellular matrix. Numerous gap junctions occur between the processes of the same cell, with adjacent smooth muscle cells, with granular cells, and at the renal hilus with intraglomerular mesangial cells.[240,322,323]

The initmate and systematic juxtaposition of tubular and vascular cells within the JGA has given rise to early

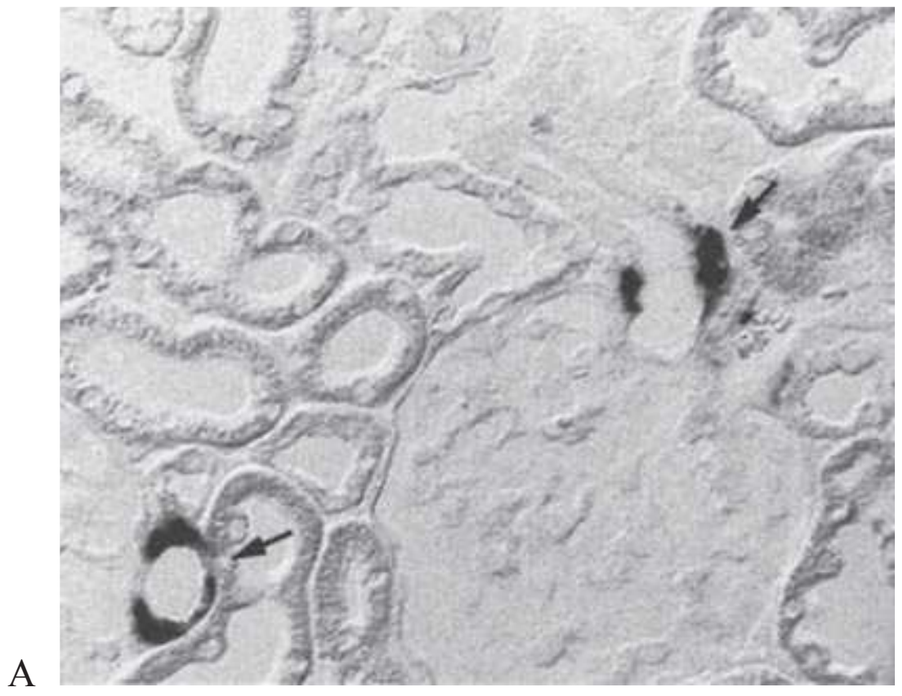


A

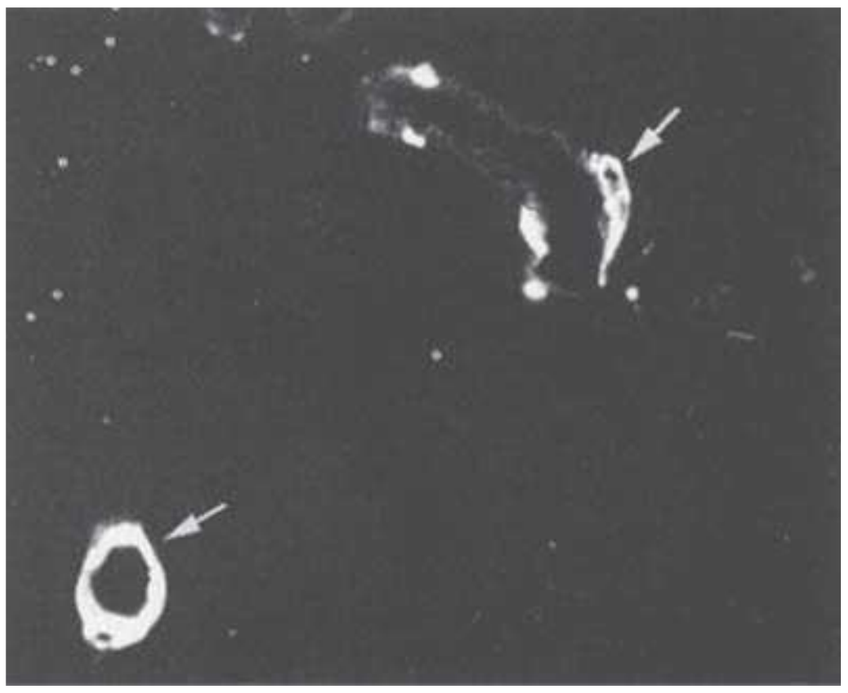


B

FIGURE 1.36 Transmission electron micrograph (rat). **A:** An afferent arteriole containing in its wall a granular cell (*GC*). (Magnification ×2,100.) **B:** Part of a granular cell containing in its cytoplasm specific membrane-bound granules. (Magnification ×14,500.)



A



B

C

FIGURE 1.37 Rat kidney containing granular cells in afferent arterioles (*arrows*) of two glomeruli. The two pictures show that renin is synthesized **(A)** and stored **(B)** in the same cells (same section). **A:** In situ hybridization using a 330-bp rat renin riboprobe (*cRNA*) labeled with digoxygenin (detection system: alkaline phosphatase). **B:** Immunocytochemistry using a rabbit polyclonal antirat renin antibody (detection system: Texas red coupled second antibody). (From Bachmann S, Heidelberg, with permission.) **C:** NADPH diaphorese reaction showing positivity of exclusively macula densa cells reflecting activity of nitric oxide synthase. (From Bosse H-M, Heidelberg, with permission.)

speculations about a functional connection in which a signal related to the composition of the tubular fluid at the MD affects glomerular vascular tone and the glomerular filtration rate.[324] It has now become clear that the JGA serves two different functions: it regulates the flow resistance of afferent arterioles in the so-called tubuloglomerular feedback mechanism and it participates in the control of renin synthesis and release from granular cells in the afferent arteriole.[325] Researchers originally assumed that the two responses might be related to each other in that renin released from the granular cells not only has systematic relevance, but locally triggers the formation of angiotensin II and thus is responsible for the afferent vasoconstriction as well; however, it now appears that the final activation of smooth muscle and granular effector cells occurs through largely independent pathways. Renin release from granular cells is the major source of systemic angiotensin II and thus plays an essential role in controlling extracellular volume and blood pressure, whereas the vasoconstriction of the afferent arteriole locally serves to modulate the filtration of this nephron.

For both mechanisms, it is well established that changes in the chloride concentration of the tubular fluid at the MD cause graded releases of mediators that reach their target by diffusion, thus acting in a paracrine fashion.[326] Note that the extraglomerular mesangium that mediates the contact between the MD and the effector cells is not vascularized, so that the buildup of any paracrine agent would not be perturbed by blood flow.

With respect to renin release, the most likely paracrine mediators of this process are prostaglandin E_2 and nitric oxide.[314,327,328] With respect to the vasoconstrictor response purinergic mediators,, either ATP or adenosine, as first suggested by Oswald and colleagues in 1980,[329] appear to play the major role.[325,330,331] For an up-to-date discussion of the function of the JGA, see the reviews by Schnermann and Levine,[325] Persson and colleagues,[332] and Komlosi et al.[333]

RENAL BLOOD VESSELS

The renal arteries arise from the lateral region of the abdominal aorta at the level of the first and second lumbar vertebrae. Each artery divides into an anterior and posterior division before traversing the renal hilus. These divisions usually form a total of five segmental branches. The anterior division gives rise to the upper, middle, and lower segmental arteries, whereas the posterior division becomes the posterior segmental artery. The apical segmental artery can arise from either division. The segmental arteries give rise to interlobar arteries within the renal sinus. The interlobar arteries enter the renal columns adjacent to the renal pyramids.

The intrarenal microvaculature has been extensively studied by several groups[10,11,158,334]; a basic pattern is established throughout the mammalian kidneys that may be described as follows. At the corticomedullary junction, the interlobar artery branches into several arcuate arteries that

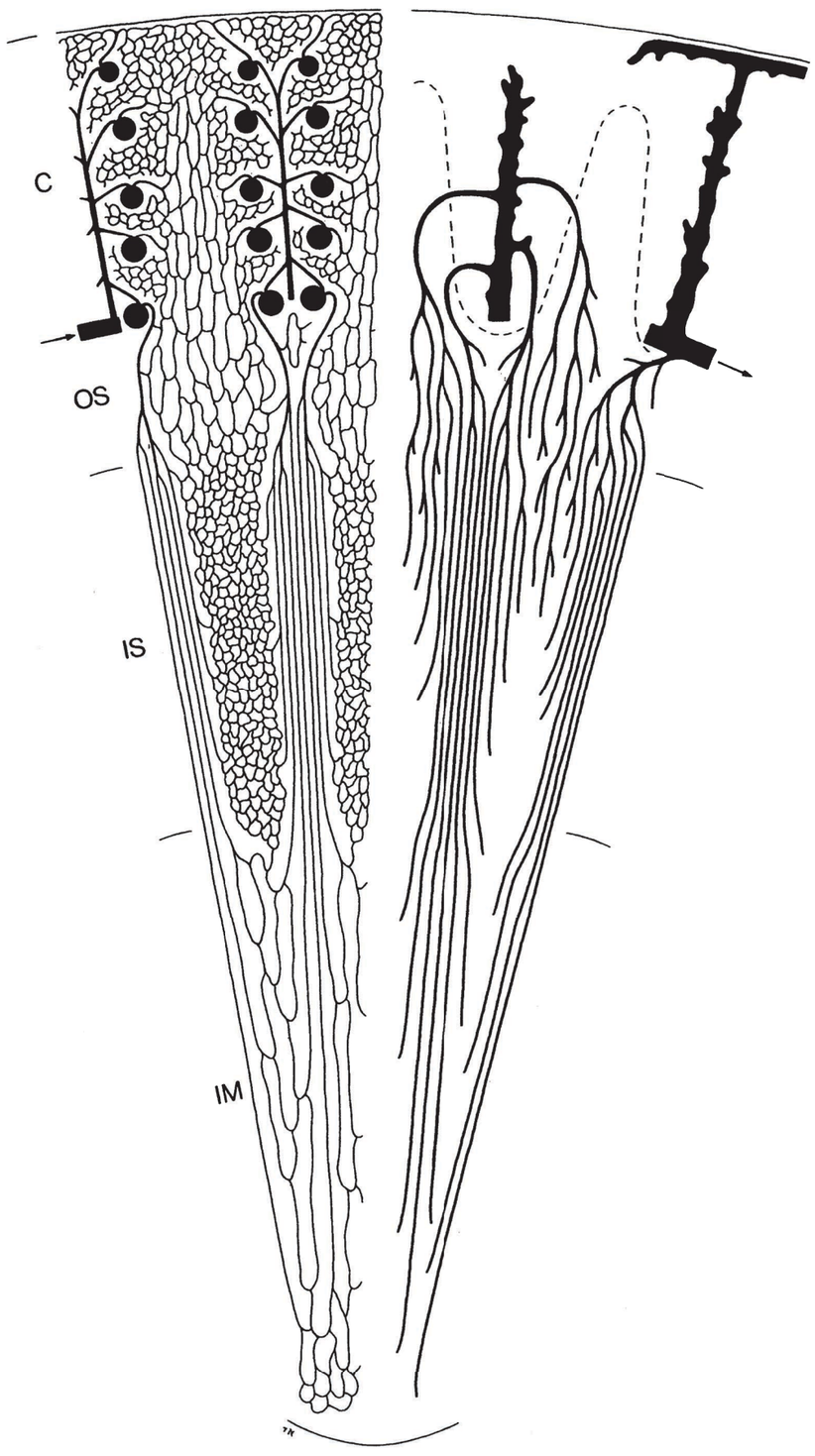


FIGURE 1.38 A basic pattern of renal microvasculature. *C*, cortex; *OS*, outer stripe; *IS*, inner stripe; *IM*, inner medulla. The *left panel* shows the arterial vessels and capillaries. An arcuate artery (*arrow*) gives rise to cortical radial arteries from which the glomerular afferent arterioles originate. Efferent arterioles of juxtamedullary glomeruli descend into the medulla and divide into the descending vasa recta, which, together with ascending vasa recta, form the vascular bundles. At intervals, descending vasa recta leave the bundles to feed the adjacent capillaries. The *right panel* shows the venous vessels. The cortical radial veins start within the superficial cortex; in the human kidney, some of them start as stellate veins on the surface of the kidney (shown on the right side). They all drain into arcuate veins. The venous drainage of the medulla is carried out by venous vasa recta; those from the inner medulla all traverse the inner stripe within the vascular bundles, whereas most of the venous vasa recta from the inner stripe ascend outside the bundles. After traversing the outer stripe as wide tortuous channels, the ascending vasa recta drain into arcuate or cortical radial veins. (From Rollhäuser H, Kriz W. Das Gefäss-system der Rattenniere. *Z Zellforsch*. 1964;64:381, with permission.)

arch across the base of the renal pyramid (Fig. 1.38). The arcuate arteries give rise to interlobular arteries (cortical radial arteries) that course peripherally, between the medullary rays and, thus, within the cortical labyrinth. The interlobular arteries also branch, and the branches give rise to afferent arterioles that supply the renal corpuscles.

Glomerular capillaries are derived from the afferent arteriole, which—strictly at the entrance level—divides into several (two to five) primary capillary branches.[335–337]

Each of these branches gives rise to an anastomosing capillary network that runs toward the urinary pole and then turns back toward the vascular pole. Thereby, the glomerular tuft is subdivided into several lobules, each of which contains an afferent and efferent capillary portion. The lobules are not strictly separated from each other—some anastomoses between lobules occur. The capillaries converge to form the more centrally located efferent arteriole, which is already established inside the glomerular tuft. Thus, the efferent arteriole has a significant intraglomerular segment that runs through the glomerular stalk (Fig. 1.5).[337] At this site a mesangial layer surrounds the vessel. After leaving the glomerulus, the efferent arteriole is reestablished as a proper arteriole.

The efferent arterioles of superficial (or subcapsular) glomeruli perfuse convoluted tubules through long pathways extending to the kidney surface or through intermediate branches near the renal corpuscle. In the midcortex, the efferents either branch near the glomerulus and perfuse convoluted tubules in that region or extend directly to the long meshed network of the medullary ray. Efferent arterioles from juxtamedullary nephrons extend downward to form the descending vasa recta (Fig. 1.38), and occasional branches to regions between the bundles. The early divisions give rise to capillaries located in the outer stripe of the outer medulla. The descending vasa recta then descend within the vascular bundles to the inner stripe and inner medulla (Fig. 1.39).[10] The medulla is drained by venous (ascending)

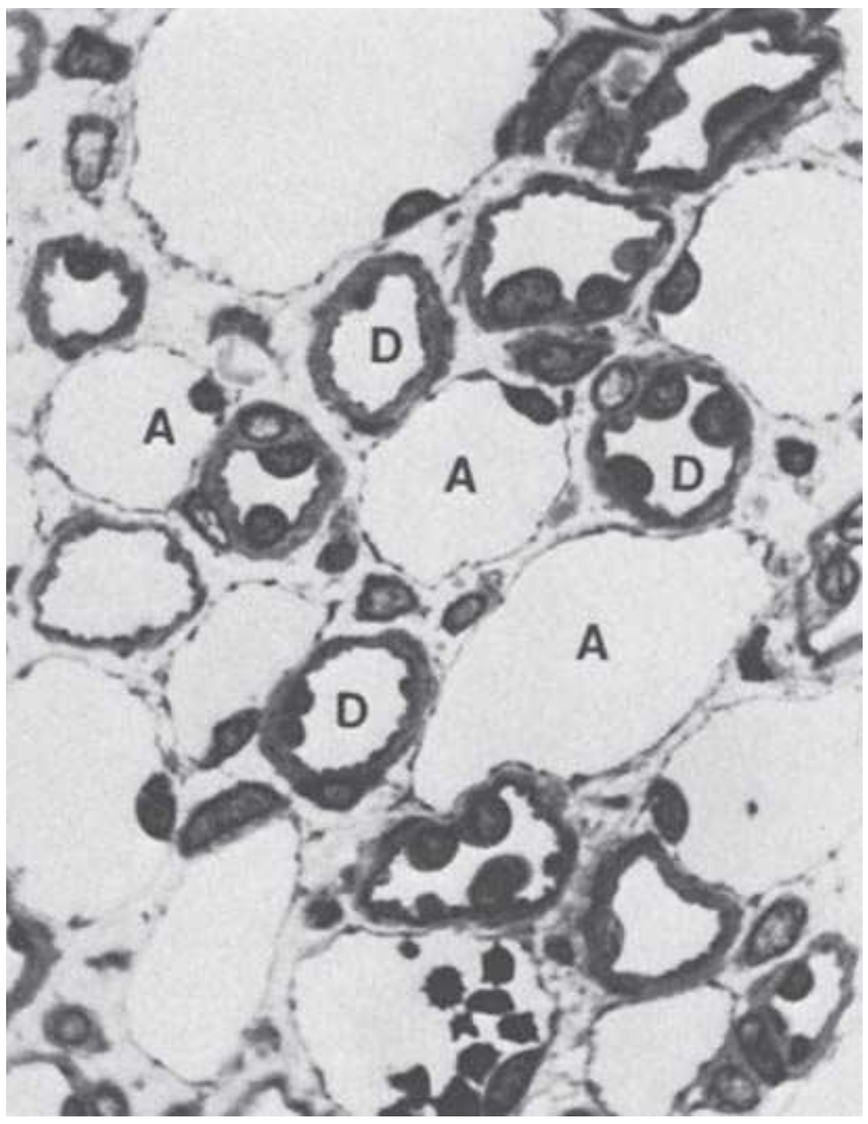


FIGURE 1.39 Light micrograph of a vasa recta bundle, showing descending (*D*) and ascending (*A*) vessels. (Magnification ×950.)

vasa recta, which lie adjacent to descending vasa recta, forming the vascular bundles that function as a countercurrent exchanger. Venous vasa recta that drain the inner medulla remain in the vascular bundles through the inner and outer stripe region. The venous vessels that drain the region of the upper and middle inner stripe ascend within the interbundle region to the outer stripe, there joining the vessels leaving the bundles to form together the major part of the blood supply to this region by a network of venous vessels. The density of capillaries that are derived directly from efferent arterioles is debated.[338] Finally, the ascending vasa recta empty into arcuate or interlobular veins (Fig. 1.38).

The cortical venous system is subject to some variation. In the human, the venous drainage starts on the renal surface beneath the capsule with small venules (stellate veins) that converge to form the interlobular veins (cortical radial veins); in most other species, the interlobular veins begin deeply beneath the cortical surface and stellate veins are lacking. Interlobular veins receive tributaries from the cortical peritubular capillary network (Fig. 1.38) and finally empty into arcuate veins that accompany the arcuate arteries. The arcuate veins receive blood from the venous vasa recta, as described in the preceding section. Interlobar veins form by confluence of arcuate veins and the latter finally form the renal vein. In contrast to the arcuate arteries, which are terminal arteries, the arcuate veins form true anastomosing arches at the corticomedullary border.

The morphology of the descending and ascending parts of the vasa recta differs markedly (Fig. 1.39).[187,339,340] The descending vasa recta are lined by a continuous nonfenestrated endothelium, with cells oriented longitudinally along the cell axis, forming 10 to 20 cell profiles in a single cross-section.[187,340] The endothelium contains the urea transporter UT-B1.[193] Pericytes are seen encircling the descending vasa recta, but they become less frequent as the vessels descend into the inner medulla, where these vessels finally convert into capillaries. As long as the descending vasa recta have pericytes nerve terminals are seen in their neighborhood.

The capillaries and the ascending vasa recta are lined by a thin fenestrated epithelium. The fenestrae are similar to those seen in peritubular capillaries, as well as in most regions of the body, being 40 to 70 μm in diameter[187] and bridged by a thin diaphragm. Uniquely, fenestrated endothelium can line quite large vessels in the kidney.

Lymphatic vessels are seen only in the cortex accompanying the arteries within the periarterial tissue sheaths (see below). The medulla has no lymphatic drainage.[341]

INTERSTITIUM

The interstitium of the kidney comprises the extravascular intertubular spaces of the renal parenchyma, with their attendant cellular elements and extracellular substances.[342,343] It is bounded on all sides by tubular and vascular basement membranes. The lymphatics are considered as part of the interstitium.

In functional studies, the interstitial volume of the kidney has been estimated to amount to 13% of the total kidney volume, whereas stereologically derived values for the cell-free interstitial space of the cortex and outer medulla of the rat range between 3% and 5%.[344–346] Thus, the functional interstitium includes more than just the peritubular spaces; the prominent periarterial connective tissue sheaths (see the following) may, in fact, account for one-half of the entire interstitial volume.[347]

The interstitium is differently developed within the various regions regions of the kidney.[342,343] In the cortex, the peritubular interstitium is distinguished from the periarterial connective tissue (Fig. 1.40). The peritubular interstitium consist of the narrow spaces between tubules and

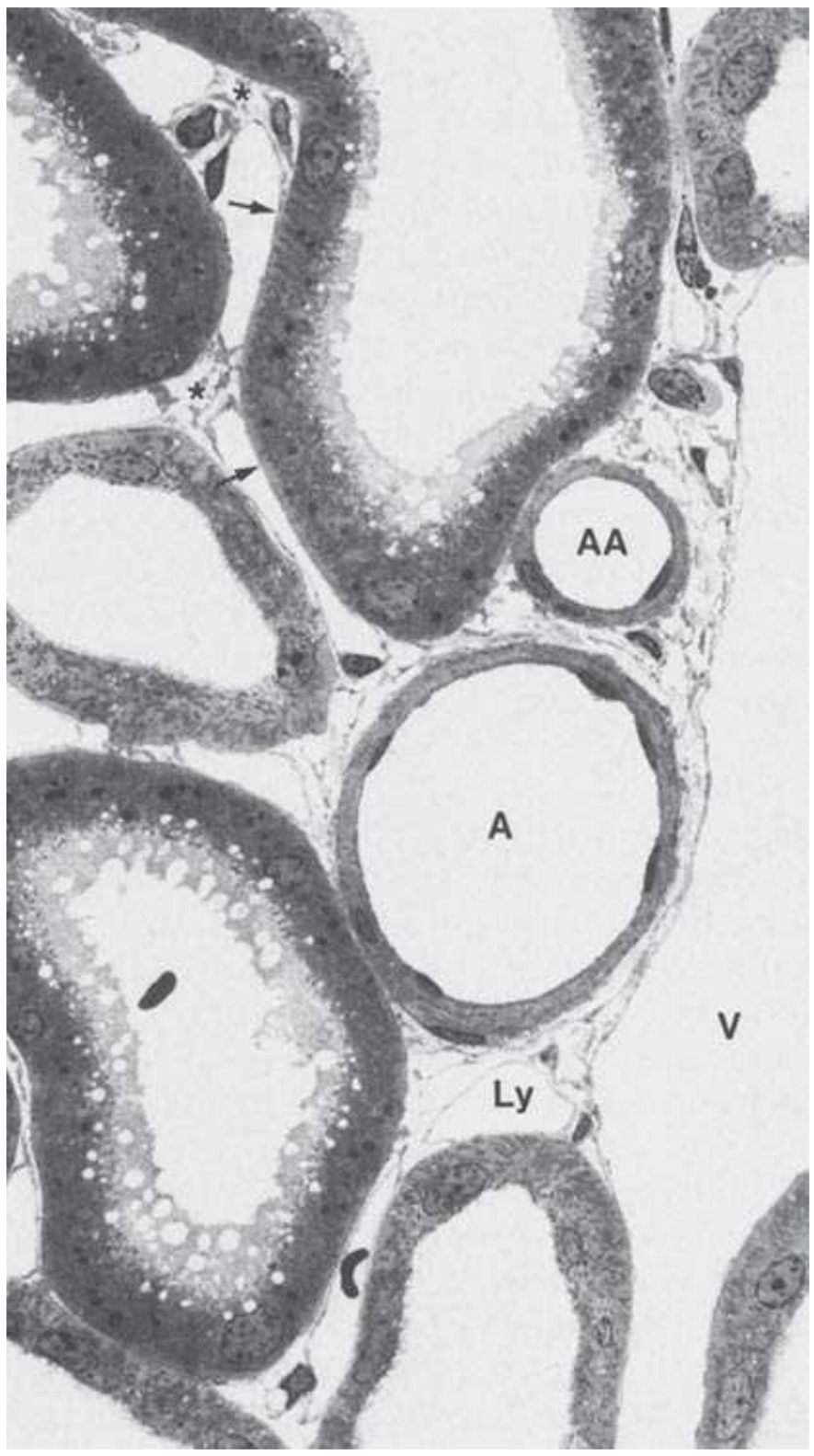


FIGURE 1.40 Low power electron micrograph of a cross-section through the renal cortex (rat). A cortical radial artery (*A*) and vein (*V*), an afferent arteriole (*AA*), and several tubular profiles are seen. The arteries are surrounded by the loose connective tissue sheath that contains the intrarenal lymphatics (*Ly*); the interstitial spaces of this sheath are continuous with the peritubular interstitium, which includes wide (*stars*) and narrow (*arrows*) portions. (Magnification ×1,000.)

around glomeruli. Sometimes, the peritubular capillaries are considered as part of the peritubular interstitium. Capillaries may abut directly to the the outer surface of tubules accounting for 54% to 67%[348] of the capillary surface, whereas only 26% of the tubular surface is directly adjacent to peritubular capillaries.[349] Thus, most of the exchanges among tubules and vessels have to pass through the interstitial compartment.

The periarterial connective tissue forms a fluid-rich loose connective tissue sheath that surrounds the intrarenal arteries and contains the lymphatic vessels of the kidney (Figs. 1.40 and 1.41).[341,347,350] The periarterial lymphatic sheath extends along the intrarenal arteries as far as the afferent arteriole, where it becomes quite attenuated. It is particularly well developed around the arcuate and cortical radial arteries. It contains the renal nerves.

The lymphatic capillaries begin within these sheaths; lymphatics do not, in general, penetrate the renal parenchyma proper and are not found in the medulla.[341,351] The lymphatic vessels converge along with the intrarenal arteries to emerge at the renal hilus. The peritubular interstitium of the cortex freely communicates with the periarterial tissue sheaths. Within the sheaths, fluid and solutes gradually may enter the lymphatic vessels as they converge toward the hilus (Fig. 1.41). In addition to lymphatic drainage, the periarterial connective tissue sheaths probably participate in the distribution of vasoactive substances alongside of the intrarenal arterioles and arteries.[350] It also serves for the intrarenal distribution of inflammatory cells.

In the medulla, the interstitium is differently developed within the three medullary regions[342]: outer and inner stripes and inner medulla. The relative interstitial volume exhibits a pronounced axial gradient from cortex to the inner medulla. The outer stripe has a very narrow, sparse interstitium, occupying 3% to 5% of outer stripe volume.[342,352] Also the vasa recta within the bundles of the inner stripe are very narrowly packed. The interstitial volume of the interbundle regions of the inner stripe is somewhat greater (10% in rats). The most elaborate and distinctive type of regional interstitium is that of the inner medulla. Here, the interstitium constitutes a much larger part of the total tissue volume (30% to 40%),[345,352] and shows a particular arrangement of its fibroblasts (see later).

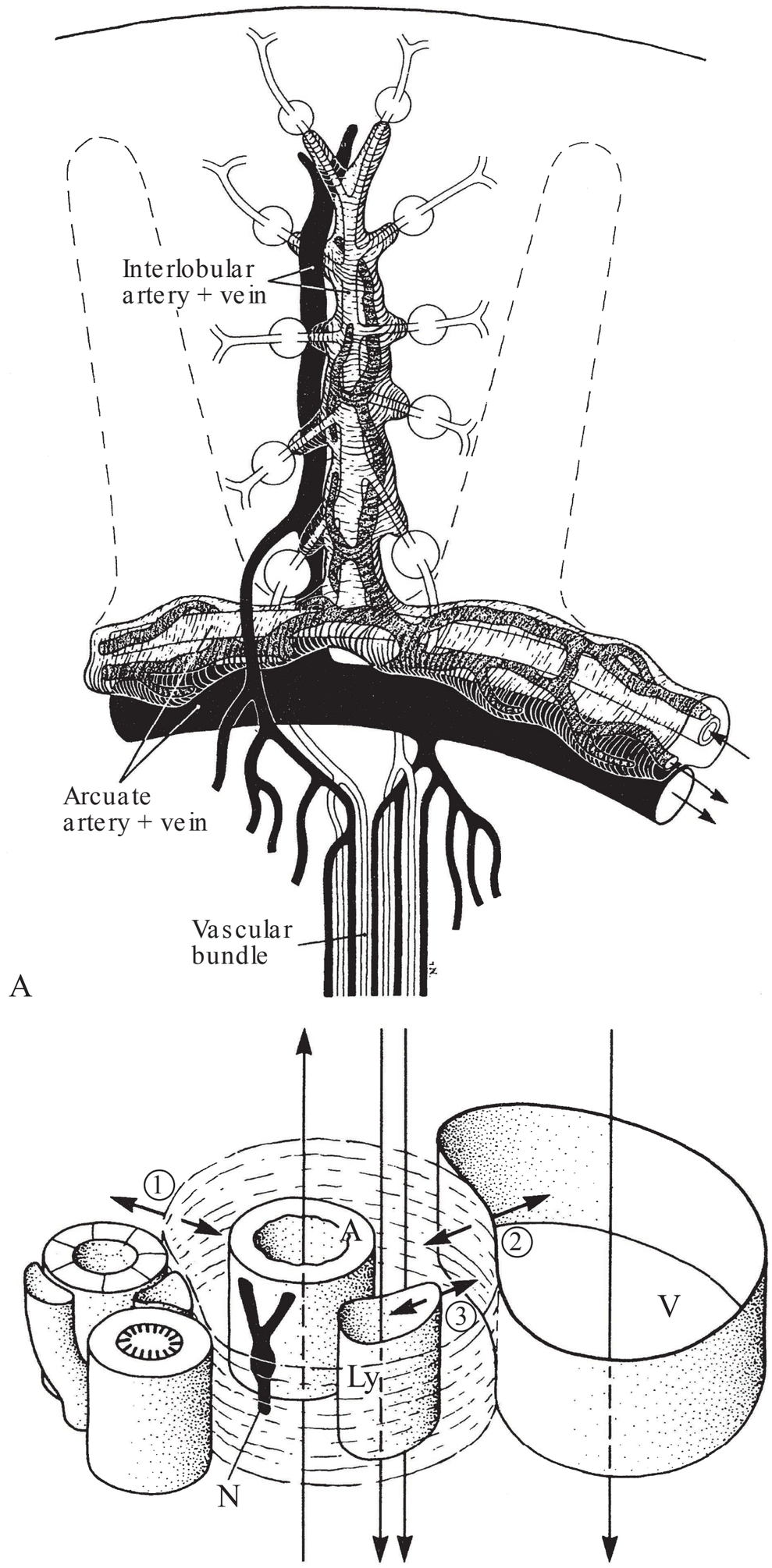


FIGURE 1.41 Schematics showing **(A)** the distribution and **(B)** the relationships of periarterial connective tissue sheath. The periarterial sheath is schematically indicated as a wide "stocking" drawn over the intrarenal arteries. In reality, there is no limiting membrane between the sheath and the surrounding interstitium. The lymphatics (*stippled area*) originate and travel within the periarterial sheath. The medullary rays are indicated by a broken line. The traverse section in **(B)** shows possible relationships between the sheath and the surrounding structures (*double-headed arrows*): (*1*) with the peritubular interstitium, (*2*) with the accompanying vein (*V*), and (*3*) with lymphatics (*Ly*). The *single-headed arrows* indicate the flow of the respective fluid. *N*, nerve. (From Kriz W. A periarterial pathway for intrarenal distribution of renin. *Kidney Int*. 987;31:551, with permission.)

The Cells of the Interstitium

The renal interstitium contains two types of cells: *fi*broblasts and dendritic cells. The fibroblasts are quite differently developed in the cortex and medulla.[342,353–356] Let us first consider the cortex. Within the peritubular interstitium roughly 50% of the cells normally present are fibroblasts and the other 50% dendritic cells, which cannot be separated from each other by simple light microscopy. The *fi*broblasts in this region are extensively branched, with long, often sheetlike processes (Fig. 1.42).[343,357,358] They contain abundant rough endoplasmic reticulum. Mitochondria,

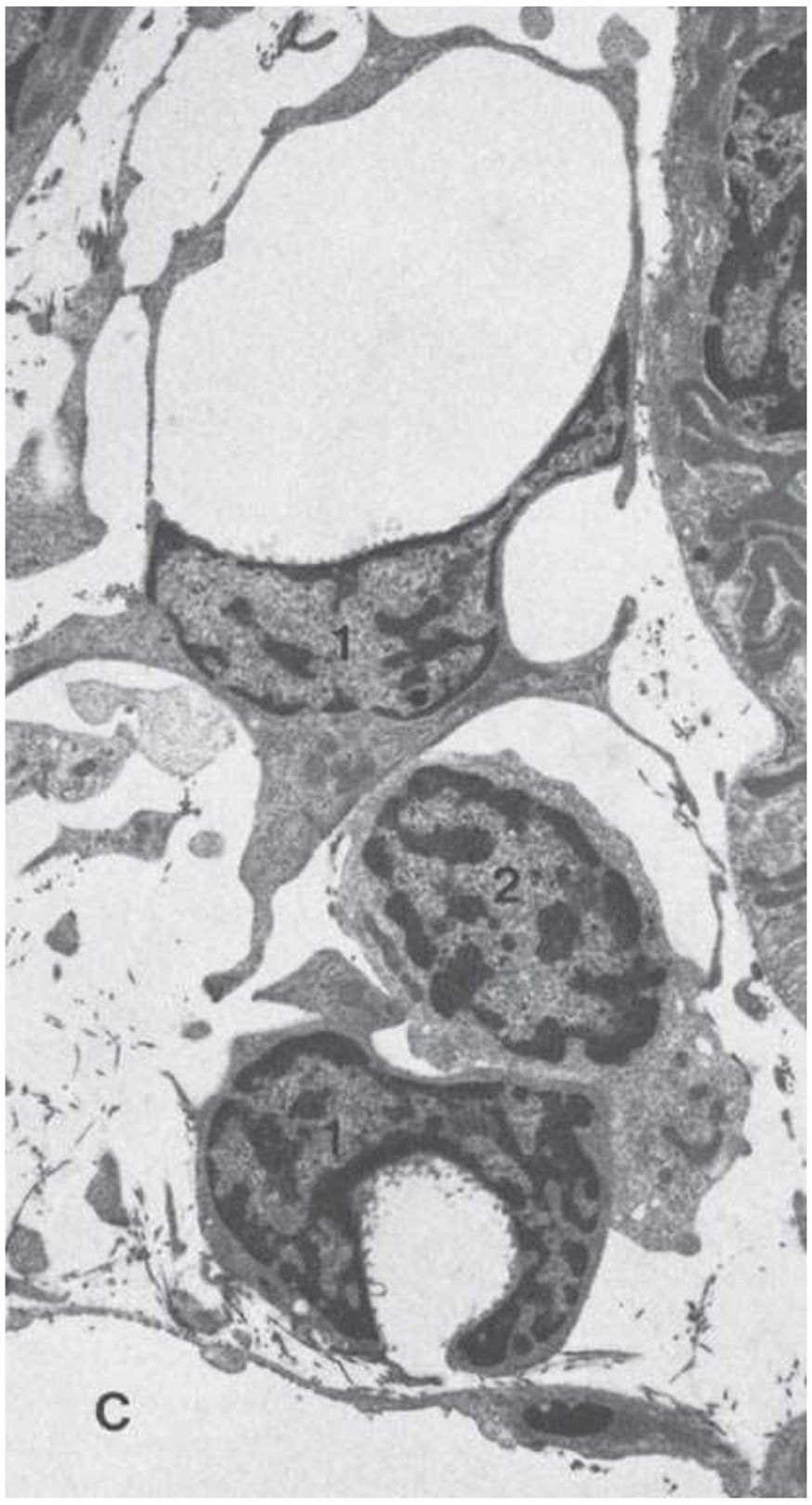


FIGURE 1.42 Transmission electron micrograph of interstitial cells in the cortical peritubular interstitium. Two types are seen: fibroblasts (*1*) with many processes and a dendritic cell (*2*). *C*, capillary. (Magnification ×5,800.)

Golgi complexes, lysosomes, and microfilament bundles are regularly encountered. Fibroblasts perform a scaffolding function interconnecting the nephrons and the peritubular capillaries. They perform this function by focal contacts of their cytoplasmic processes with neighboring fibroblasts as well as with capillaries and tubules.[343,356,357] In addition, they produce the fibrous matrix, which serves together with these cells as a common scaffold throughout the renal parenchyma. These fibroblasts can be unequivocally detected by electron microscopy and fluorescence microscopy by their strong expression of ecto-5′-nucleotidase (CD73),[343,356,357,359,360] as well as of the PDGF receptor beta (PDGFRβ).[361]

Fibroblasts of the renal cortex with their enzyme ecto-5′-nucleotidase[362,363] can generate adenosine within the cortical interstitium. In addition, a subfraction of cortical interstitial cells synthesize erythropoietin.[364–366]

In the inner medulla, the fibroblasts represent a particular variety. They contain numerous homogeneous osmiophilic lipid droplets. Hence, they are generally called lipid-laden interstitial cells (Fig. 1.43).[353,355,367] These star-shaped cells interconnect loops of Henle and vasa recta, spanning these axial structures like the rungs of a ladder.[342,358] They increase in number toward the tip of the papilla and have an abundant rough endoplasmic reticulum, with cisternae that are often dilated and filled with flocculent material. A cytoskeleton is especially well developed in their most peripheral cell processes. These cells possess receptors for angiotensin II and bradykinin.[368,369] They are responsible for

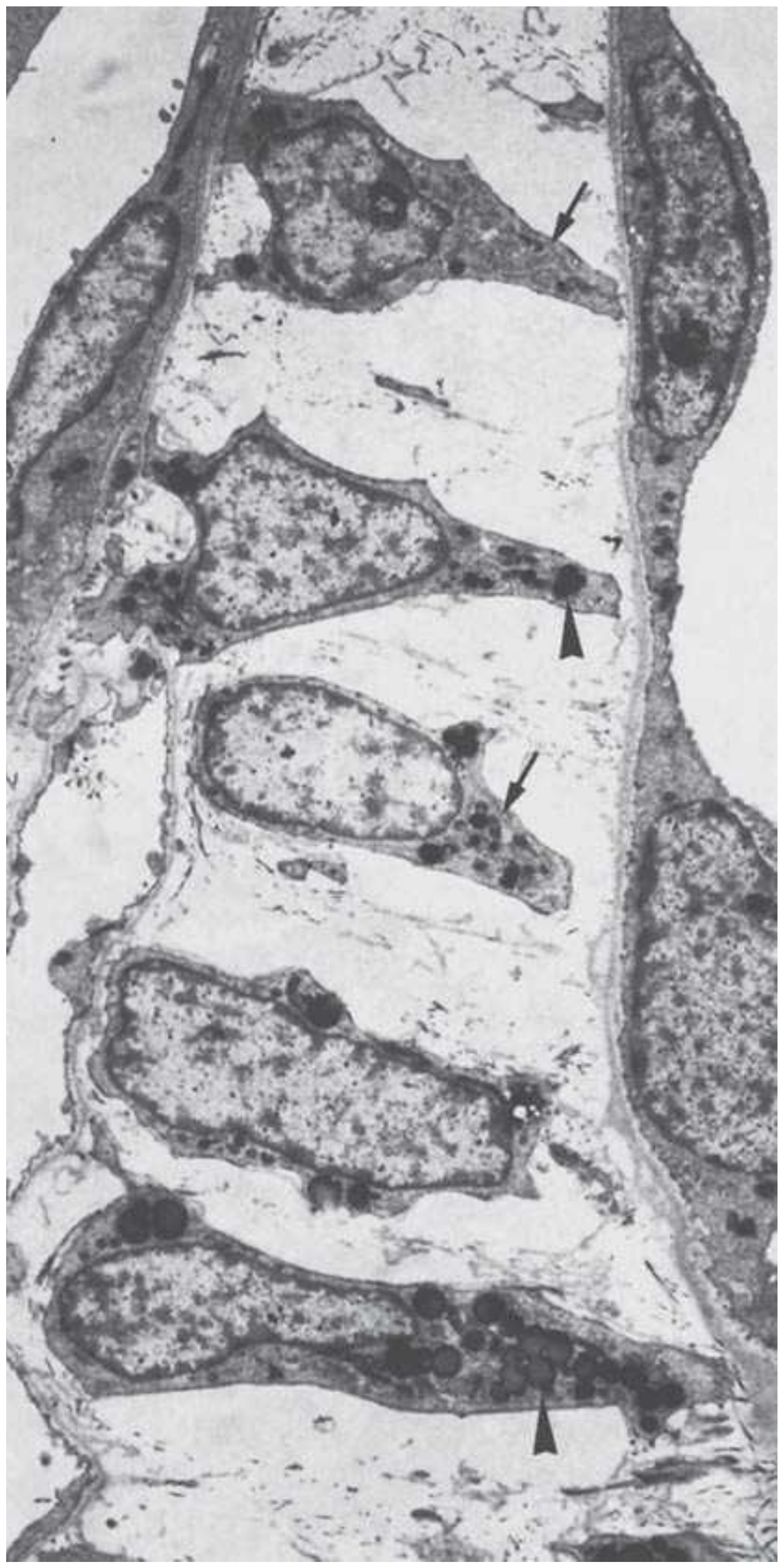


FIGURE 1.43 Transmission electron micrograph of a longitudinal section of the inner medulla (rat) showing lipid-laden interstitial cells arranged like the rungs of a ladder between parallel running tubes or vessels. Note the numerous lipid droplets (*arrowheads*) and the prominent endoplasmic reticulum (*arrows*). (Magnification ×3,300.)

the production of extracellular fibers and ground substance, including the abundant glycosaminoglycans and hyaluronic acid of the inner medulla.[370]

The lipid-laden interstitial cells participate in producing the medullary prostaglandins. The lipid droplets of these cells contain polyunsaturated fatty acids that appear to be precursors of prostaglandins and other lipid-derived hormones.[368,369] The cells produce vasodepressing lipids, in particular, prostaglandin E_2.[371,372]

The second abundant cell type in the renal interstitium is the dendritic cell (Fig. 1.42). They originate from the bone marrow and, as in other organs, are subject to a vivid turnover.[373] In the kidney, dendritic cells are found in the interstitium throughout the cortex and the outer medulla.[343,356,357] They enter the interstitium from the blood, reside for some days in interstitial spaces, and leave the interstitium with the lymph flow.

The extracellular components of the interstitium form a matrix that may be thought of as a hydrated gel of ground substance within a fibrillar reticulum.[342] Several fibers make up the interstitial reticulum. Collagen fibers (types I, III, and VI) are present in the matrix, both in isolation and in bundles. Type I collagen forms typical cross-banded fibers, generally more than 30 μm in diameter. Type III fibers (10 to 40 μm in diameter) and type VI fibers (6 to 10 μm in diameter) are often seen associated with type I fibers. In addition, unbanded microfibrils with a diameter of 15 to 30 μm and an electron-lucent core have been described.[342,354]

Myofibroblasts are absent from healthy kidneys. They differ from fibroblasts by their high production of extracellular matrix, expression of vimentin, and α-smooth muscle actin (αSMA).[343,374] The accumulation of myofibroblasts in a diseased kidney is generally suggested to occur by transformation of fibroblasts into myofibroblasts in response to stimuli from locally produced cytokines.[374,375] An alternative hypothesis postulates that myofibroblasts may develop by "epithelialmesenchymal transition" (EMT) from tubular cells[376]; however, the evidence for this origin is not conclusive.[377]

Macrophages (histiocytes) are also rarely found in the healthy kidney, except for the periarterial tissue sheaths[356] (Fig. 1.42). These round cells demonstrate primary and secondary lysosomes and characteristic surface folds. Along with interstitial affections, the number of macrophages may dramatically rise.

STRUCTURE–FUNCTION RELATIONSHIPS WITHIN THE RENAL MEDULLA

During phylogeny, the renal medulla has developed in response to the necessity to conserve water by excreting a concentrated urine.[378] Loops of Henle, collecting ducts, and a specific blood supply through vascular bundles have developed into a complex structural system that accounts for this function. Whereas the mechanisms to produce a concentrated urine up to a concentration of about 600 mOsmol/L in the outer medulla are fairly well known, the mechanisms underlying the final urine concentration in the inner medulla are still poorly understood.

Within the outer medulla the reabsorption of NaCl from the MTAL represents the driving force to produce a corticomedullary osmotic gradient that provokes the osmotic water withdrawal from the collecting duct passing through this region. ADH initiates the insertion of AQP2 channels into the apical plasma membrane of collecting duct principal cells. Together with the constitutive AQP3 and AQP4 channels in the basolateral membrane of these cells, this allows osmotic water withdrawal into the hypertonic interstitium of the outer medulla.[192] The reabsorbed water is brought back into the systemic circulation by venous vasa recta.[2]

The final step of the urine concentrating process in the inner medulla is basically identical insofar as water is reabsorbed by osmosis through ADH-stimulated water-permeable collecting ducts into a hypertonic interstitium. However, the mechanisms for creating the osmotic gradient in the inner medulla up to 1200 mOsmol/L in humans and much higher in many rodent species are much more complex than in the outer medulla and are insufficiently understood. A driving force like the sodium pump in MTALs in the outer medulla is lacking. This has led to several "passive" models,[379,380] which take into account the specific transport properties of thin limbs, collecting ducts, and vasa recta to elucidate, by mathematical modeling, how part of the osmotic energy produced by the function of MTALs in the outer medulla may be transferred into the inner medulla, leading there to an osmotic gradient toward the tip of the papilla. A review of these models is far beyond the scope of this chapter. From a structural point of view, two features appear most relevant.

1. **The shape of the inner medulla.** The inner medulla has a particular shape, tapering from a broad basis to a tiny papilla. The mass of the inner medulla is, therefore, unevenly distributed along the longitudal axis. A reconstruction study in the rat[2,381,382] has shown that the inner medulla is shaped like a mushroom, consisting of a broad head and a thin stalk. Calculations in the model have shown that the first one-half of the inner medulla comprises roughly 80% of the total inner medullary volume and, consequently, only 20% are left for the second, papillary, half. Thus, the decrease of the mass in the inner medulla along the longitudal axis is almost exponential. This shape perfectly reflects what happens with the structures within the inner medulla: loops of Henle, collecting ducts, and vasa recta all decrease rapidly in number from the base to the tip of the papilla.[381,382] It has been calculated that of an estimated 10,000 long loops entering the inner medulla at its base, only about 1,500 reach the papillary one-half

of the inner medulla.[2] Thus, the majority of thin limbs (the "short" long thin limbs) turn back shortly after entering the inner medulla; a smaller, but still substantial, number of thin limbs reach the middle part of the inner medulla; and only a small population of "long" long loops really reach the papilla.

This has led to the proposal[383] and, later, mathematical models[204,384] that this arrangement might account for a cascadelike transport of solutes toward the tip of the papilla. The large fraction of "short" long loops, by some way or another, manages the transport of solutes into the first one-third of the inner medulla, the intermediate fraction of long loops brings a proportion of these solutes further down into the middle part, and finally the small fraction of "long" long loops completes the transport of a still much smaller solute fraction down into the papilla.

2. **Structural arrangements in the renal medulla that allow the recycling of urea into the inner medulla via short loops of Henle.** The descending thin limbs of short loops in the outer medulla are arranged in a pattern that allows the shifting of urea from the venous blood coming up with the venous vasa recta from the inner medulla into tubular fluid of descending thin limbs of short loops. This possibility is perfectly developed in the complex vascular bundles in the outer medulla of rodents with high urine-concentrating abilities.[159,187,197,198] Within these vascular bundles, the SDTLs are arranged in a countercurrent fashion with ascending vasa recta coming up from the inner medulla. Thus urea, by countercurrent exchange, may directly enter the descending thin limbs through the urea transporter UT-A2[193,275] (the fenestrated endothelium of venous vasa recta may readily be expected to be highly permeable for urea). In a renal medulla with "simple" vascular bundles (as they are found in most species), countercurrent exchange of urea first occurs from ascending to descending vasa recta (which contain the urea transporter UT-B1), which, afterward, will deliver their blood to the capillary plexus of the interbundle region surrounding the thin limbs of short loops on their descending direction. Thus, even if probably less effectively, urea from the inner medulla has access to the SDTLs.[193,197,385,386]

Starting with the MTAL, thus abruptly at the end of the ATL, the uriniferous tubule is impermeable to urea until the terminal collecting duct in the inner medulla, which, starting abruptly, expresses the ADH-dependent urea transporter UT-A1.[193,275] Because of water reabsorption upstream from this segment, urea becomes concentrated within the tubular urine to levels always higher than the adjacent interstitium. In the terminal CD segment, a major part of this urea diffuses into the papillary interstitium and where it mixes with other solutes (NaCl), contributing to the osmotic driving force that reclaims water from the collecting duct. The locus of urea delivery in the papilla (i.e., at the "ultimate bend" of the complex countercurrent exchange system of the inner medulla) is optimal because (1) by countercurrent exchange in vasa recta and thin limbs, urea is largely trapped and distributed in a longitudinal gradient within the inner medulla and (2) the fraction of urea that escapes the inner medulla is subject to recycling via short loops back into the inner medulla. Thus, this latter fraction of urea is available another time within the papillary interstitium ready to withdraw and/or to balance water reabsorption from the collecting duct. Recycling is not simply trapping because this fraction of urea on its nephron way back into the inner medulla is concentrated a second time, essentially by the work of the sodium pump in the MTALs. In essence, urea recycling by short loops (not by long loops) represents an effective transport of osmotic energy from the outer into the inner medulla.

Whether these mechanisms are sufficient to build an osmotic gradient up to several osmoles in desert rodents, or whether there are additional sources complementing the solute gradients (e.g., continuous production of osmotic active substances), is presently unknown. Even if this question does not belong to the most urgent problems in medicine, it represents a highly challenging biologic enigma.

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CHAPTER

2 Clinical Importance of Nephron Mass

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INTRODUCTION

The relationship between renal salt handling, intravascular volume homeostasis, and hypertension is well established, which points to the kidney as the central organ in the development of hypertension.[1] Based on the concept of developmental programming, where an environmental stimulus experienced during a critical period of early development can induce long-term structural and functional adaptive changes in the developing organism, Brenner et al. proposed that a low nephron number, acquired during fetal life, may predispose an individual to hypertension and renal disease.[2,3] This hypothesis was attractive because low birth weight, a marker of an adverse intrauterine environment, if associated with a congenital reduction in nephron number, could potentially explain the variability in hypertension and renal disease prevalence observed among populations of different ethnicity where those with lower birth weights tend to have a higher burden of renal disease.[4–7] The initial hypothesis suggested that a kidney with fewer nephrons would have a reduced filtration surface area with a limited capacity to excrete sodium, thereby contributing to the development of hypertension. Although this "nephron number" hypothesis was initially quite controversial, and with time has proved to be not entirely this straightforward, the association between nephron number and predisposition to hypertension and renal disease has been borne out in many animal experiments and human studies.[8–12] In this chapter we put forward the existing evidence comprising animal and human studies, which link nephron mass, birth weight, and other clinical variables with clinical outcomes. Extrapolation from animals to humans has many limitations and, therefore, where possible we have included human studies to corroborate or refute animal findings. Although this field has grown significantly within the last decade and many questions remain unanswered, a clearer and consistent picture is emerging that shows nephron mass does have clinical importance.

An individual's nephron mass is determined by a complex interplay between genetics and environment, evolving throughout their lifetime, bearing the imprint of their past, being reflected in their present, and affecting their future risk of hypertension and renal disease. Although traditionally it has been thought that all kidneys have about 1 million nephrons, recent studies have found that total glomerular number varies up to 13-fold in human kidneys, much more, for example, than height or weight (Table 2.1).[13] The terms "nephron endowment," implying number of nephrons present upon completion of nephrogenesis; "nephron number," implying the number of intact nephrons at the time of measurement; and "glomerular number," including the number of tubular and atubular glomeruli, have all been used interchangeably.[13] In this chapter we use the term "nephron number" more generally to describe the total number of nephrons in a kidney at the time of discussion. The term nephron mass is used more broadly as a clinical term to incorporate nephron number, kidney weight, kidney size, and kidney volume. A discussion of the importance of acquired reduction in nephron mass in later life is beyond the scope of this chapter.

DEVELOPMENTAL DETERMINANTS OF NEPHRON NUMBER

Low Nephron Number

Kidney development in humans proceeds from the 9th to the 36th week of gestation.[9,14] Accurate determination of nephron number is difficult because nephron number cannot be determined in humans in vivo. The unbiased fractionator-sampling/dissector method is thought to be the most objective nephron counting method, and is currently utilized in most human studies.[15,16] This method, however, requires postmortem kidney samples and is very labor intensive. An in vivo glomerular counting method comparing the fractionator technique with a combined renal biopsy/magnetic resonance imaging (MRI) method in explanted canine kidneys has been attempted.[17] This study found a good correlation of glomerular number on average between the two methods, but, within kidneys, there was a 36% variance, calling individual applicability into question. Large-scale human

2.1 Nephron Numbers in Humans

Reference	Population	Sample Size	Mean	Range	Fold
Nyengaard and Bendtsen	Danish	37	617,000	331,000–1,424,000	4.3
Merlet-Benichou et al.[a]	French	28	1,107,000	655,000–1,554,000	2.4
Keller et al.	German	20	1,074,414	531,140–1,959,914	3.7
	Hypertensive	10	702,379	531,104–954,893	1.8
	Normotensive	10	1,429,200	884,458–1,959,914	2.2
Douglas-Denton et al.	African Americans	105	884,938	210,332–2,026,541	9.6
	White Americans	84	843,106	227,327–1,660,232	7.3
Hoy et al.	Australian non-Aborigines	21	861,541	380,517–1,493,665	3.9
	Australian Aborigines	19	713,209	346,161–1,129,223	3.1
McNamara et al.[b]	Senegalese	47	992,353	536,171–1,764,241	3.3
Hoy et al.	African and white Americans, Australian Aborigines, and non-Aborigines and Senegalese	420	901,902	210,332–2,702,079	12.8

[a]Used acid maceration technique. All other studies used unbiased stereology.
[b]Values for 47 participants were combined from two publications.
Reprinted with permission from Puelles VG, Hoy WE, Hughson MD, et al. Glomerular number and size variability and risk for kidney disease. Curr Opin Nephrol Hypertens. 2011;20:7–15. See original manuscript for detailed references.

studies of nephron number and association with phenotype are therefore not easily feasible.

Average nephron number has been reported to range from 617,000 (range 331,000–1,424,000) to 1,429,200 (range 884,485–1,959,914) per kidney among normal adult Caucasian Europeans.[10,18] Other studies including subjects of multiple ethnic origins from the United States, Africa, and Australia showed somewhat similar results, with a mean number around the mid 800,000 glomeruli per kidney, with a very wide range, from 210,332 to 2,702,079 as shown in Table 2.1.[13] The range appears widest in kidneys from subjects of African origin.[13,19] In general, nephron numbers are lower in older subjects, attributed to age-related glomerulosclerosis and obsolescence.[18,20] Whether the high variability in nephron number across populations reflects true differences or is confounded by small sample sizes or limitations of counting methods will become clearer with time as more studies accumulate or as better techniques evolve.

Various animal models have been used to study the impact of developmental programming on nephrogenesis. The details and pathophysiology of these models, and mechanisms whereby nephron numbers are reduced, are beyond the scope of this chapter and are outlined in detail elsewhere.[8,21,22] Extrapolating from the animal studies, from a clinical point of view, the factors associated with development of low nephron number can be divided into two groups: modifiable and nonmodifiable, as outlined in Table 2.2.

Modifiable Factors

Modifiable factors associated with low nephron number include prenatal events—factors occurring during gestation and postnatal events occurring in the neonate.

Prenatal Factors. Maternal diets deficient in protein, total calories, or iron have all been shown to reduce nephron numbers in offspring of experimental animals, most often in association with low birth weight.[12,23–26] Figure 2.1 shows a reduction in nephron numbers in low birth weight rats that were subjected to maternal low protein diets during gestation. Maternal dietary deficiencies are common in pregnant mothers in developing countries and therefore likely clinically relevant in a large proportion of the world.[27] Maternal vitamin A deficient diets are associated with a dose-dependent reduction in nephron number in animals.[28]

2.2 Factors Associated with Charges in Nephron Number and Kidney Size

REDUCED NEPHRON NUMBERS OR KIDNEY SIZE

Timing	Condition	Source of Data	Effect on Nephron Number (NNx)/Kidney Size	References
Prenatal, modifiable	Maternal low protein diet or total calorie restriction	Animal	↓ NNx, 16%–40%	12, 246
	Maternal vitamin A restriction	Animal	↓ NNx, in proportion to reduction in vitamin A	28, 29
		Human	Small infant kidney size	
	Maternal iron restriction	Animal	↓ NNx, 22%	25
	Gestational glucocorticoid exposure	Animal	↓ NNx, 20%–38%	37, 180
	Uterine artery ligation/ embolization	Animal	↓ NNx, 20%–30%	81
	Maternal diabetes/ hyperglycemia	Animal	↓ NNx, 10%–35%	50, 51, 247
	Gestational drug exposure	Animal		
	■ Gentamicin		↓ NNx, 10%–20%	40–45, 248
	■ β lactams		↓ NNx, 5%–10%	
	■ Cyclosporine		↓ NNx, 25%–33%	
	■ Ethanol		↓ NNx, 10%–20%	
	■ COX2 inhibitors		↓ NNx	
	■ Indomethacin		↓ NNx	
Prenatal, nonmodifiable	Genetics			
	■ RET(1476A) polymorphism	Human	10% ↓ newborn kidney volume	63, 64
	■ PAX2 AAA haplotype	Human	10% ↓ newborn kidney volume	
	Prematurity	Human	NNx ↓ with gestational age, limited post natal nephrogenesis	54, 69, 132
			Reduced kidney size in growth restricted children	
Postnatal	Nutrition	Animal	NNx ↓ with postnatal nutrient restriction alone	32
	Renal failure	Human	? cause or consequence of NNx ↓	54

NORMALIZATION OR INCREASE IN NEPHRON NUMBER

Timing	Condition	Source of Data	Effect on Nephron Number (NNx)/Kidney Size	References
Prenatal	Maternal vitamin A supplementation	Animal	Normalization of NNx in LPD model	82
	Maternal amino acid supplementation	Animal	Normalization of NNx in LPD model	24

(continued)

2.2 Factors Associated with Charges in Nephron Number and Kidney Size (continued)

Timing	Condition	Source of Data	Effect on Nephron Number (NNx)/Kidney Size	References
	Ouabain administration	Animal	Normalization of NNx in LPD model	84
	Maternal uninephrectomy	Animal	NNx ↑	88, 89
	Genetics			
	■ ALDH1A2rs7169289(G) allele	Human	22% ↑ newborn kidney size	83
Postnatal	Reinstitution of good nutrition	Animal	Catch-up of NNx in LPD model	81
	Overfeeding	Animal	NNx ↑ in normal birth weight rats	152

↑, increase; ↓, decrease; ?, unknown.
Adapted from Luyckx VA, Brenner BM. The clinical importance of nephron mass. J Am Soc Nephrol. 2010;21:898–910.

Vitamin A deficiency was examined in a cohort of Indian compared to Canadian mothers and found to be associated with significantly smaller newborn renal volume, which the authors suggest likely reflects lower nephron number.[29] Retinoic acid, the active metabolite of vitamin A, functions as a transcription factor regulating expression of Ret, a tyrosine kinase receptor critical for kidney development.[30] Interestingly, vitamin A levels are reduced by smoking and alcohol intake, both known to reduce birth weight.[31] Uteroplacental insufficiency, induced by uterine artery ligation late in gestation, also results in low offspring birth weight and low nephron number.[32,33] This model may share some similarities

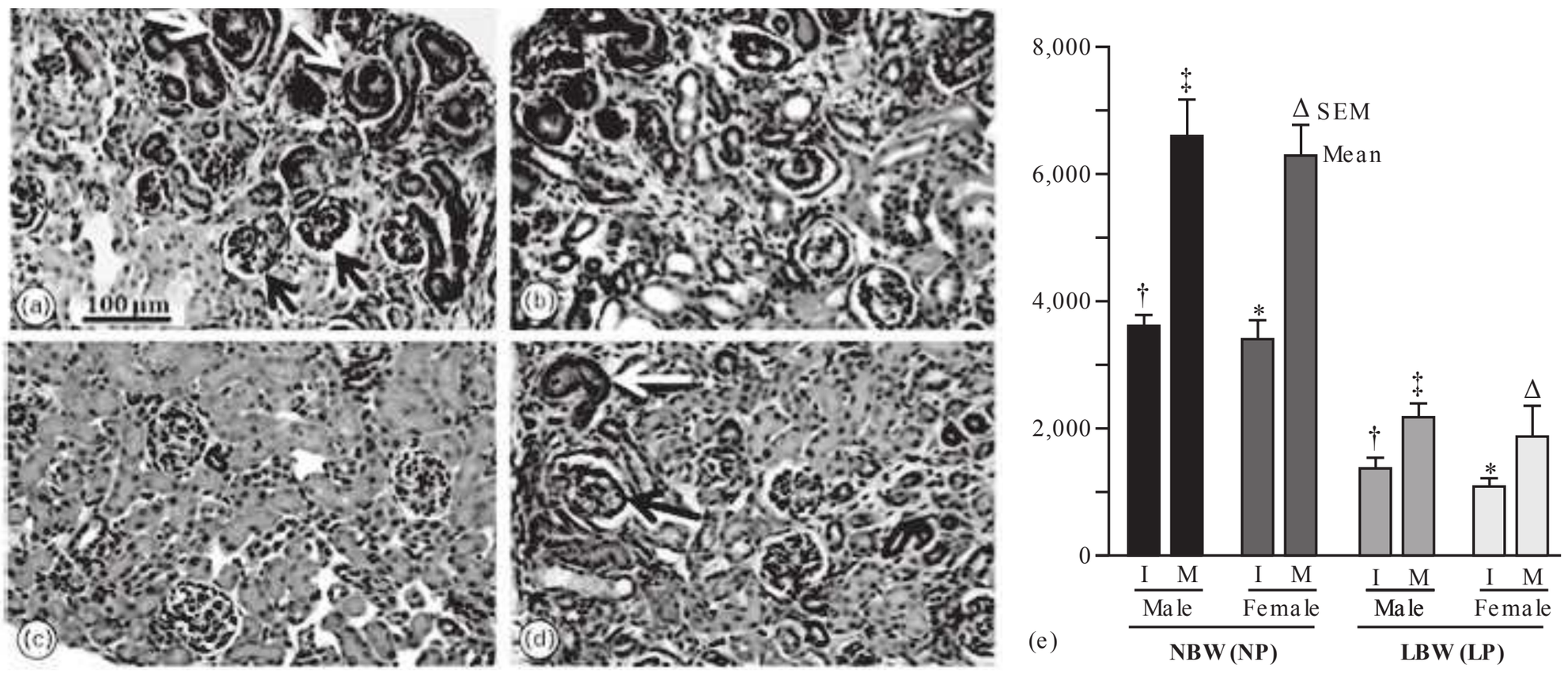

FIGURE 2.1 Relationship between glomerulogenesis, nephron number, and birth weight in rats subjected to maternal normal (NP) or low (LP) protein diets. Renal cortex at days 0 (d0) and 10 (d10) in rat offspring of NP-fed dams (normal birth weight, NBW) and LP-fed dams (low birth weight, LBW). *(a)* Normal glomerulogenesis in NBW offspring at d0, with comma-shape structure (immature renal corpuscle, *white arrow*) and inner vascularized structure (mature renal corpuscle, *black arrow*); *(b)* LBW d0 with fewer corpuscular structures and moderately dilated tubules; *(c)* NBW d10 showing only mature renal corpuscles; *(d)* LBW d10 showing immature and mature renal corpuscles; and *(e)* number of glomerulus-like structures (immature [I] and mature [M]) measured in NBW and LBW offspring ($n = 5$ per group). Symbols indicate group comparisons, $P < 0.05$. (Reprinted with permission from Villar-Martini VC, Carvalho JJ, Neves MF, et al. Hypertension and kidney alterations in rat offspring from low protein pregnancies. *J Hypertens Suppl.* 2009;27:S47–51.)

with preeclampsia in humans in terms of the reduction of uterine blood flow and the restriction of fetal nutrient supply.

Increased fetal glucocorticoid exposure is a likely mechanism whereby maternal low protein diet reduces nephron number, via reduced activity of placental 11β-hydroxysteroid dehydrogenase activity, shown in both animals and humans.[34,35] Similarly, administration of glucocorticoids during gestation in rats and sheep leads to reduced nephrogenesis, although this effect was not seen in the Marmoset monkey.[36–38] Glucocorticoids are thought to reduce nephron number by impacting ureteric bud invasion of the metanephric mesenchyme, thereby limiting branching morphogenesis.[8] The impact of maternal glucocorticoid utilization during pregnancy on human nephrogenesis is not known. Ingestion of other medications during pregnancy may also impact nephrogenesis in many ways.[39] Gestational administration of aminoglycosides, beta lactams, cyclosporine, cyclooxygenase inhibitors, and nonsteroidal anti-inflammatory drugs have all been associated with reduced nephron number in experimental models.[39–43] Similarly, chronic and acute gestational exposure to alcohol impairs embryonic ureteric bud branching, resulting in fewer nephrons in offspring.[44,45] In humans one abstract suggested an impact of maternal alcohol consumption on kidney development in Australian Aboriginal children.[9]

Conceivably, therefore, all of these prenatal experimental conditions may impact human nephrogenesis and minimization of these exposures prior to and during pregnancy would optimize fetal nephrogenesis. The timing of an insult during gestation is also relevant to its impact on nephrogenesis, with the greatest effect in animals generally seen with interventions in the latter half of gestation.[8]

Maternal factors also impact fetal development during gestation. Low birth weight is associated with multiple maternal factors although nephron number has not specifically been examined in most cases.[46,47] Manalich et al. found a strong correlation between low birth weight and low nephron number in a cohort of Cuban newborns.[48] Maternal hypertension and maternal smoking were correlated with low birth weight, although direct correlation with nephron number was not reported. In experimental animals, maternal diabetes or hyperglycemia has been shown to result in approximately 30% lower offspring nephron number in some, but not all, studies, although differences in methods of nephron number counting may account for some of the variability.[49–51] In other studies, maternal diabetes was associated with smaller kidneys, higher blood pressures, microalbuminuria, and reduced glomerular filtration rates in rat offspring.[51,52] In young adults, renal functional reserve was found to be reduced in those who had been exposed to maternal diabetes during gestation, compared to those with paternal diabetes (i.e., excluding a genetic component), or those with nondiabetic parents.[53] The reduced renal functional reserve was interpreted by the authors as a possible surrogate for a reduced nephron number acquired in utero in the offspring of diabetic mothers.

Postnatal Factors. Although nephrogenesis is thought to be complete at birth in humans, this may not be the case for babies born prematurely, and therefore a window in which nephrogenesis may still be vulnerable likely exists soon after birth in these infants.[54] Consistent with this possibility, early postnatal growth restriction alone in normal birth weight rats was associated with a reduction in nephron number, demonstrating the importance of early postnatal nutrition on nephrogenesis.[32] The relevance of these findings to the human, however, is questionable because nephrogenesis normally proceeds for 10 days after birth in rodents and therefore this period is analogous to late gestation in humans. These data may, however, have relevance to humans born prematurely. Indeed, in a cohort of children born either very low birth weight (<1,000 g) or premature (<30 weeks gestation), extrauterine growth restriction was associated with significantly lower glomerular filtration rates at a mean of 7.6 years of age, suggesting an impact of postnatal nutrition on renal development.[55] Another study of postmortem kidneys from premature infants who died after 40 days of life found glomerular number to be significantly lower in those who developed renal failure compared to those who did not. These findings may suggest that renal failure itself inhibits glomerulogenesis; however, it is also possible that fewer glomeruli made these extremely ill infants more susceptible to renal failure. In another cohort of critically ill premature infants, renal failure was a significant complication and associated with a high mortality, although not associated with birth weight.[56] In contrast, another study did find neonatal acute kidney injury to be an independent predictor of mortality in very low birth weight infants.[57] Prematurity itself is a recognized risk for renal failure in infants, and has been shown to be associated with increased risk of subsequent hypertension and chronic kidney disease (CKD).[58] Taking these human studies together, postnatal events do impact renal development in premature infants and may have potentially adverse short- and long-term consequences.

Nonmodifiable Factors

Nonmodifiable factors also impact nephrogenesis, and may occur in isolation or together with other potentially modifiable factors described previously (Table 2.2).

Genetics. Rare congenital and genetic abnormalities associated with abnormal kidney development manifest with renal dysfunction, often presenting very early in life.[11,59] Approximately 40% to 60% of childhood end-stage renal disease (ESRD) results from some form of congenital renal hypoplasia.[60] More subtle renal developmental abnormalities—which may not manifest as overt syndromes but, rather, with later life renal dysfunction—may well be the result of gene polymorphisms impacting nephron number. Renal hypoplasia and reduced nephron number have been described with full or partial deletion of over 25 genes in mice, which are reviewed in detail elsewhere.[13,21,61] The important steps in kidney development

include specification of the metanephric blastema from the intermediate mesoderm, formation of the ureteric bud and its outgrowth from the wolffian duct, and ureteric bud branching. Genes participating in specification of the metanephric blastema from the intermediate mesoderm include Odd-1, Eya 1 Pax 2, Wt-1, Six 1, Gdnf, and Sall 1, of which Odd-1 and Eya -1 are critical.[21,60] Genes regulating formation of the ureteric bud and its outgrowth from the wolffian duct include Pax2, Liml, Bmp4, and Gdnf. [21,60] Gdnf (glial cell-derived neurotrophic factor) signals through the Gfrα1 receptor and the c-Ret receptor tyrosine kinase and, during branching, morphogenesis is only expressed on the tips of ureteric branches, selectively inducing branching at this location.[21] Among the most important pathways impacting nephrogenesis, therefore, are Gdnf/Ret and Pax2. In mice, deletion of Gdnf and c-Ret leads to renal agenesis or severe hypoplasia.[21,60] Deletion of Pax2, the "master organizer" of renal development, is incompatible with life.[60] The impact of genetic polymorphisms in these pivotal genes has been studied in humans. Haploinsufficiency of the PAX2 gene causes the autosomal dominant renal coloboma syndrome, associated with significant reduction in nephron number and "oligomeganephronia."[60,62,63] Taking this finding further, looking for a more subtle impact in the wider population, Quinlan et al. found that the common AAA haplotype of PAX2, present in 18.5% of newborns in a Canadian cohort, was associated with reduced allele-specific mRNA expression in vitro, and a 10% reduction in newborn kidney volume, compared with the GGG haplotype.[63] Similarly, a polymorphic variant of RET, RET(1476A), was also associated with reduced mRNA synthesis, an almost 10% reduction in kidney volume, and higher levels of the renal function marker cystatin C at birth compared with the RET(1476G) variant in Caucasian newborns.[64] These authors found that newborn kidney volume is proportional to nephron number, therefore PAX2 and RET polymorphisms are likely associated with reduced nephron number in humans.[64] Among 15% of Caucasians inheriting both alleles, newborn kidney sizes were 23% smaller.[65] Surprisingly, however, none of 19 common GDNF gene variants or three single nucleotide polymorphisms related to a putative GDNF-PAX binding site were associated with small kidney size among 163 Caucasians newborns.[65] One rare coding GDNF variant (R93W) was not found in any subject and therefore, the clinical impact of this potential mutation is not known.[65] These early and small studies suggest that genetic polymorphisms in genes that are critical in nephrogenesis may contribute to the wide spectrum of nephron number found in the general population.

Prematurity. Unlike in rodents, postnatal nephrogenesis does not occur in humans, except in extremely premature infants; therefore, nephron number is predominantly determined in utero. Rodriguez et al. examined kidneys from 56 extremely premature infants compared with 10 full-term infants at autopsy.[54] Radial glomerular counts were lower in premature compared with full-term infant kidneys and glomerular number correlated with gestational age, as has been reported previously.[54,66] In addition, they found evidence of active glomerulogenesis (indicated by the presence of basophilic S-shaped bodies under the renal capsule in kidneys) in premature infants up to, but not beyond, 40 days of life.[54] This was the first study to demonstrate ongoing nephrogenesis in humans postnatally. Similarly, in preterm baboons, nephrogenesis was found to continue after birth and nephron number was within the normal range; however, there was a greater proportion of abnormal glomeruli in the superficial cortex compared to full-term controls, suggesting compromised nephrogenesis after premature birth.[67] In contrast, Hinchliffe et al. did not find an increase in nephron number in growth restricted infants who died as stillbirths at varying gestations, or at 1 year of age, suggesting a lack of nephrogenesis after birth.[66,68] Gestational age was found to correlate with nephron number, which reached a maximum around 36 weeks.[69]

Gender. Gender likely plays a complicated role in developmental programming. In the largest series of kidneys analyzed to date, glomerular number in adult females was found to be reduced by up to 12% compared to males.[13,70] In a cohort of Cuban newborns, however, nephron number was not affected by gender.[48] In experimental models, reviewed in detail elsewhere, males generally tend to be more severely affected than females in terms of reduction in nephron number, as well as subsequent manifestation of hypertension and renal dysfunction.[71,72] These differences may in part result from differences in postnatal growth rates between males and females, gender-specific differences in adaptation to adverse events, and gender-specific regulation of genes and pathways impacting renal development, function, and hypertension.[33,72] Similarly, a large study in humans found an association of CKD with low birth weight in adult males, but not in females, suggesting a possible impact of gender on subsequent disease expression, although mechanisms are not yet clear.[73]

Ethnicity. Hoy and colleagues have shown a reduction in nephron number among Aboriginal compared with non-Aboriginal Australians (Table 2.1).[70] Among African Americans and Caucasian Americans, nephron number was not significantly different in both groups and correlated with birth weight, although the distribution appeared to be more bimodal in the African American cohort.[74] No low birth weight subjects were included in this study, but low birth weight is more prevalent among African Americans; therefore, in the general U.S. population, a greater proportion of African Americans may have lower nephron number. This remains to be studied. Nephron number among Senegalese Africans and African Americans was similar.[75] Among Cuban neonates, nephron number was again not different between black compared with white subjects.[48] To our knowledge kidneys of subjects from other ethnic groups have not been studied. Ethnicity, therefore, may have an impact on nephron number, although it is difficult to dissect out an impact independent of its association with birth

weight, socioeconomic factors, genetic polymorphisms, and many other potential confounders.

Intergenerational Factors. Among both white and African American women, mothers who had been of low birth weight had a significantly increased risk of having low birth weight offspring, independent of economic environment, suggesting a cross-generational effect of maternal low birth weight.[76] Similarly maternal, but not paternal birth weights, were associated with offspring birth weight, arguing for an intergenerational programming effect of the maternal environment.[77] Interestingly, in a large population-based study, mothers experiencing preeclampsia, especially when associated with premature birth and low birth weight in the offspring, are at increased risk of subsequent need for renal biopsy and/or ESRD.[47,78] A reduced maternal glomerular filtration rate (GFR) <90 mL per minute and hypertension are significant risk factors for preeclampsia, small for gestational age infants, and premature delivery.[79] The question arises why the mother herself may have been predisposed to these adverse pregnancy-related and renal outcomes. It is conceivable that a vicious cycle may occur where a low birth weight mother would be predisposed to programmed adverse pregnancy outcomes, in turn impacting fetal nephrogenesis and thereby future pregnancy outcomes and renal health of the subsequent generations. To our knowledge this specific association has not been studied in humans. In rats, the first generation offspring of mothers fed low protein diets during gestation had low birth weights, low nephron number, and developed spontaneous hypertension at 8 weeks of age. Offspring of these first generation females, although maintained on normal diets throughout gestation, also exhibited low nephron number and hypertension, demonstrating intergenerational programming.[80] Interestingly the effect was lost by the third generation, suggesting that the intergenerational cycle can be interrupted by optimization of risk factors such as maternal nutrition.

Strategies for Augmentation of Nephron Number

Although total filtration surface area in individuals with fewer nephrons may not be reduced, as a result of compensatory hypertrophy of the existing nephrons (see later), low nephron number is still associated with an increased risk of hypertension and renal dysfunction in later life. Strategies to optimize nephron number may therefore have an important impact on clinical disease (Table 2.2). Interventions would likely need to be applied during gestation to have an optimal effect. Ideally, optimization of all modifiable risk factors prior to pregnancy would appear the simplest and most widely applicable intervention. Clinically feasible interventions are being studied to potentially "rescue" nephron number and reduce subsequent hypertension.

Postnatal Nutrition

Provision of adequate postnatal nutrition in low birth weight rat pups, achieved by cross-fostering onto normal lactating females at birth, led to restoration of nephron number and prevented the development of subsequent hypertension compared to pups with continued growth restriction.[81]

Vitamin A Supplementation

Because vitamin A deficiency is associated with a nephron deficit, administration of a single dose of retinoic acid during early nephrogenesis restored nephron number to control levels in rat pups exposed to low protein diet in utero.[82] Postnatal administration of retinoic acid to preterm baboons, however, was not able to stimulate nephrogenesis compared to preterm controls, suggesting a more proximal window for the effect of vitamin A on nephrogenesis, although these results may have been confounded by routine antibiotics given to all animals, which may have negatively impacted nephrogenesis, confounding a potentially small vitamin A effect.[30]

Genetics

In a cohort of Caucasian newborns, a common variant of the ALHD1A2 gene involved in retinoic acid metabolism, ALHD1A2rs7169289(G), was associated with a 22% increase in newborn kidney size, and higher cord blood retinoic acid levels, compared to the wild-type ALDH1A2 rs7169289(A) allele.[83] These authors suggest this gene polymorphism could be protective for nephrogenesis in the setting of vitamin A deficiency.

Prevention of Low Nephron Number

The ubiquitous plasma membrane protein Na^+/K^+-ATPase functions as an ion pump as well as a signal transducer. Ouabain is a highly specific Na^+/K^+-ATPase ligand that triggers the release of calcium waves, which are important regulators of early development.[84] Interestingly, erythrocyte membrane Na^+/K^+-ATPase activity was found to be reduced in a cohort of low birth weight males at age 20, making this a potentially relevant pathway.[85] The impact of ouabain administration was studied experimentally as a modulator of nephrogenesis under protein-deficient conditions in vitro and in vivo.[86] Ouabain was found to abrogate the effect of serum starvation on ureteric bud branching in cultured metanephroi, and to prevent reduction in nephron number in offspring of low protein diet-fed dams.[84] The ouabain was administered throughout pregnancy in this study and, therefore, the potential of ouabain to rescue or restore nephron number once an adverse event is already established has not been studied. Similarly, supplementation of maternal diet during gestation with glycine, urea, or alanine prevented the reduction in nephron number induced by maternal low protein diet in all offspring, but blood pressure was only normalized in those receiving glycine.[24] Interestingly, nephron number in the offspring of mothers subjected to water restriction during gestation was increased, but also did not abrogate development of subsequent hypertension—again suggesting possible divergent programming mechanisms for nephron number and blood pressure in some models.[87]

Maternal Nephrectomy

Uninephrectomy in rat mothers prior to pregnancy has been associated with an increase in offspring nephron number at birth; however, at 6 weeks, nephron numbers were not different from offspring of nonnephrectomized dams.[88,89] These authors suggest a possible circulating renotrophic factor in response to maternal uninephrectomy, possibly inducing hypertrophy of the contralateral kidney, which may accelerate nephrogenesis in the fetus but may not affect ultimate nephron number. These observations may be relevant in human cases such as maternal renal transplantation or maternal kidney donation, although timing of pregnancy in relation to nephrectomy may be an important variable. This area deserves more investigation.

CLINICAL SURROGATES FOR NEPHRON NUMBER

In vivo, nephron number can only be grossly estimated by MRI or kidney biopsy.[18,70,90] Associations of nephron number with readily available clinical variables have been described and are outlined in Table 2.3.

Anthropomorphic Features

Birth Weight

Low birth weight is defined by the World Health Organization as a birth weight under 2,500 g. Very low birth weight is usually defined a below 1,500 g. Low birth weight could result from prematurity itself (i.e., birth before the 37th week of gestation with an appropriate weight for gestational age), or from intrauterine growth restriction (IUGR) at any gestation.[46] A small for gestational age infant is defined as having a birth weight below the 10th percentile of normal for that gestational age.[46] Full-term IUGR is the most strongly associated with adult disease.[91] Risk factors for low birth weight are diverse and, in poorer countries, maternal malnutrition, poor prenatal care, and infections are common, whereas in the developed world, factors such as high risk pregnancies, assisted reproduction, multiple gestations, and advanced maternal age are becoming more frequent.[46,92] High birth weight is defined variably as a birth weight >4,000 g or >4,500 g, and is associated with maternal obesity, maternal diabetes, prolonged gestation, and reduced maternal smoking.[93] High birth weight has also been associated with adverse renal outcomes in the offspring, especially as a consequence of maternal diabetes.[94,95]

2.3 Clinical Surrogates for Low Nephron Number

Clinical Feature	Association with Nephron Number	Population	Reference
Low birth weight	↑ of 257,426 glomeruli per kg increase in birth weight	U.S. white and black, children and adults	19
Prematurity	↓ glomerular number in premature compared to term infants	U.S. premature and full term neonates	54, 68
Gender	Nephron number is 12% lower in females	U.S. white and black Aboriginal Australian	70
Age	↓ 3,676 glomeruli per kidney per year of age >18 years	U.S. white and black Aboriginal Australian	70
Adult height	↑ 28,000 glomeruli per centimeter increase in height	Australian Aboriginal German, white	10, 70
Kidney mass	↑ 23,459 glomeruli per gram of kidney tissue	Infants <3 months of age	64
Glomerular volume	Inverse correlation between glomerular volume and nephron number	U.S. white and black Aboriginal Australian German adults, Cuban infants	10, 13, 48
Ethnicity	↓ Aboriginal Australians compared to U.S. white and black	U.S. white and black Aboriginal Australian	70

↑, increase; ↓, decrease.

Low birth weight is the strongest current clinical surrogate for nephron number. Nephron number has consistently been shown to correlate strongly with birth weight in humans, with an extrapolated increase of 257,426 glomeruli per kilogram increase in birth weight.[19,48,54,70] The relationship of birth weight to nephron number is preserved among Australian Aboriginals, African Americans, and Whites and therefore may be generalizable to other populations.[19,70] The specific relationship between nephron number and low birth weight has only been examined in infants. Low birth weight was associated with lower nephron number than normal birth weight, and was similar among black and white subjects.[48,68] In experimental animals, however, not all low birth weight animals have been found to have reduced nephron number and, conversely, low nephron number has been reported in the absence of low birth weight.[96,97] Birth weight alone, therefore, is not a universal surrogate for nephron number. To our knowledge, nephron number has not been specifically studied in high birth weight humans or animals. Other anthropomorphic correlates that have been associated with nephron number are highlighted in Table 2.3.[18,20,70,98]

Kidney Size

Renal Mass

From autopsy studies, nephron number has been found to correlate directly with kidney weight in both adult and infant cohorts.[18,64] Zhang et al. calculated a predicted increase of 23,459 glomeruli per gram of kidney mass in infants under 3 months of age.[64] In living subjects, kidney weight is not routinely obtainable, but donor kidney mass measured prior to transplantation, as a measure of nephron "dose," has been shown to have clinical relevance (see later).

Kidney Volume

Kidney volume can be measured in vivo, making it an attractive potential surrogate for nephron number. Renal volume is dependent on nephron number, but is also strongly

2.4 Differences in Nephron Number, Glomerular Volume, and Total Glomerular Surface Area in the Right Kidney, U.S./Australian Adults (18+ years), Means (SD)

		All	No hypertension	Hypertension	No Hypertension versus Hypertension
US whites	Nglom	855 183 (295 247)	894 339 (275 956)	747 727 (271 155)	P = 0.026
	Nglom adj[a]	866 722 (289 896)	912 876 (283 744)	779 808 (288 510)	P = 0.046
US blacks	Nglom	921 708 (318 089)	931 463 (290 529)	912 480 (350 329)	P = 0.776
	Nglom adj[a]	946 379 (322 516)	949 934 (288 768)	952 441 (353 197)	P = 0.97
Aborigines	Nglom	733 484 (217 763)	843 423 (199 384)	631 321 (105 298)	P = 0.04
	Nglom adj[a]	776 422 (253 631)	912 539 (218 432)	653 241 (178 609)	P = 0.110
US whites	Mean Vglom, gmean	7.1 (6.6–7.6)	6.87 (6.3–7.5)	7.82 (6.9–8.9)	P = 0.096
US blacks	Mean Vglom, gmean	7.7 (7.2–8.3)	6.92 (6.3–7.6)	8.64 (7.8–9.5)	P = 0.0012
Aborigines	Mean Vglom, gmean	7.7 (6.5–9.0)	6.88 (5.4–8.7)	8.0 (5.3–11.9)	P = 0.426
US whites	Vglomtot, cm^3	5.68 (5.3–6.1)	5.79 (5.2–6.5)	5.34 (4.7–6.3)	P = 0.484
US blacks	Vglomtot, cm^3	6.74 (6.3–7.3)	6.16 (5.6–6.8)	7.34 (6.6–8.2)	P = 0.020
Aborigines	Vglomtot, cm^3	5.40 (4.6–6.3)	5.89 (4.4–7.4)	4.96 (3.9–6.3)	P = 0.365

Nglom, Nglom adj, and Vglomtot: arithmetic means (SD). Vglomtot is the combined volume of all glomeruli in the kidney. Vglom in $\mu m^3 \times 10^6$, geometric mean (95% confidence interval). Nglom for US whites versus US blacks, P = 0.133. Nglom adj for US whites versus US blacks, P = 0.079. Nglom for Aboriginal versus other, P = 0.047.

[a]Nglom adj: adjusted for proportions of sclerosed glomeruli seen on light microscopy.

Reprinted with permission from Hoy WE, Bertram JF, Denton RD, et al. Nephron number, glomerular volume, renal disease and hypertension. Curr Opin Nephrol Hypertens. 2008;17:258–265.

correlated with current body size.[18] In fetuses and at birth, kidney volume is presumed to be directly proportional to nephron number; however, once normal kidney growth and adaptation occurs after a few months of life, impacted by body surface area, age, gender, glomerular hypertrophy, or nephron loss through injury, the relationship likely becomes less linear.[63] Despite this caveat, several authors have investigated utility of renal volume in relation to birth weight. Evaluation of fetal renal function by ultrasound in growth restricted fetuses in utero found reduced hourly urine output, greater oligohydramnios, reduced renal perfusion, and smaller kidney volume compared to normally growing fetuses.[99–101] Although these findings could simply reflect globally reduced renal perfusion, abnormal renal development cannot be excluded. Another study utilizing serial ultrasounds in a cohort of small for gestational age compared to appropriate for gestational age fetuses found that kidneys were smaller in the small for gestational age cohort, although kidney length was relatively preserved compared to width and circumference after 26 weeks of gestation.[102] Follow-up of kidney size and growth postnatally in 178 premature or small for gestational age children, compared with 717 term appropriate for gestational age controls, at 0, 3, and 18 months found that weight for gestational age correlated with kidney volume at all three time points.[103] Slight catch-up in kidney growth was observed in the growth-restricted, but not the premature infants. Among a cohort of low birth weight Australian Aboriginal children aged 5 to 18, renal volumes were found to be lower when adjusted for body size compared to normal birth weight children.[104] These authors also found that the reduction in kidney volume was driven more by a shorter depth than length of the kidney. Kidney length and volume were also both found to be smaller in a cohort of low birth weight children aged 10 to 12 years, and correlated weakly with lower GFR (Fig. 2.2).[105] In contrast, others found no difference in kidney volume, adjusted for body surface area and gender, between individuals who had been appropriate weight for gestational age at term, small for gestational age at term, or preterm at age 9 to 12 years.[106] Among young adults born premature (either appropriate or small for gestational age) compared with term age-matched controls, prematurity was associated with smaller kidneys at age 20 years, whereas IUGR had only a small, nonsignificant effect.[107] Kidney volume may therefore be less reliable as a surrogate for nephron endowment as subjects age. In addition, as a note of caution, renal volumes were not comparable between ultrasound and MRI in a neonatal population, suggesting that the same imaging modality should be used if measurements are to be compared.[108]

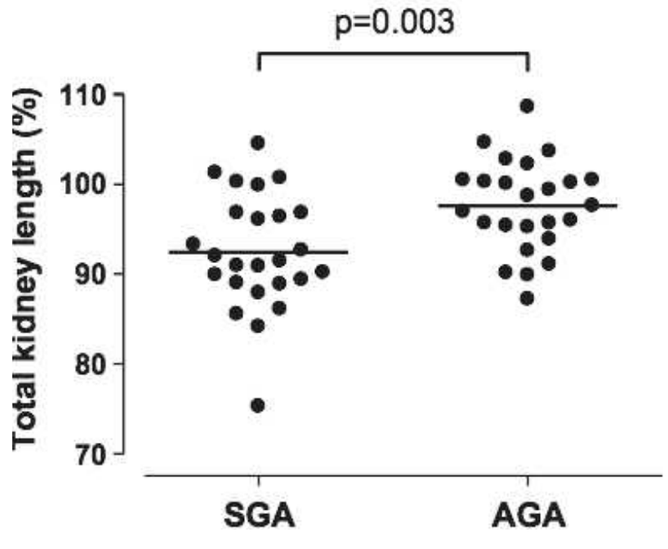

FIGURE 2.2 Correlation between growth restriction and kidney length. Kidney length (expressed as percent expected from the literature) is significantly shorter in Caucasian children aged 11.3 ± 2.1 years who were born small weight for gestational age (SGA) compared with appropriate weight for gestational age (AGA) at birth. (Reprinted with permission from Simonetti GD, Raio L, Surbek D, et al. Salt sensitivity of children with low birth weight. *Hypertension*. 2008;52:625–630.)

Histologic Features

Glomerular Volume

Glomerular number has been found to vary up to 13-fold among all the cohorts studied, and glomerular volume has been found to vary up to 6.7-fold (Table 2.1).[13] Because nephron number is fixed at birth, glomerular size is likely the major variable determining adaptation of filtration capacity to match the body's demands.[19,20,48,98,109,110] Indeed, calculated total filtration surface area in kidneys with varying nephron number tends to be very similar, suggesting compensatory hypertrophy in those with fewer nephrons as shown in Table 2.4.[9] Consistent with this, mean glomerular volumes vary directly with body size, and inversely with nephron number and birth weight in all populations studied (Fig. 2.3).[10,48,70,111] Most studies, however, report mean glomerular volumes for a whole kidney. Further investigation into glomerular heterogeneity has found significant inter- and intraindividual variability in glomerular size.[13,112] Individual glomerular volume was found to vary up to eightfold within a single subject.[13] Glomerular size tended to be larger and more variable in the outer cortex compared to those deeper within the kidney.[13] In multiple analyses, hypertension, obesity, age, and low nephron number emerged as major predictors of larger glomeruli and greater heterogeneity.[13,70,75,111–113] Interestingly, consistently, African American and African subjects have higher mean glomerular volumes and greater heterogeneity of glomerular volume compared to white subjects, independent of nephron number (Fig. 2.4).[13,75] Furthermore, between Senegalese and African American subjects, glomerular volumes were higher in the U.S. cohort after controlling for body size.[75] Increased glomerular volume and heterogeneity therefore may result from different mechanisms among African origin populations compared to Caucasians. Whether this may have posed an evolutionary survival advantage which may be maladaptive in the current environmental circumstances given the greater burden of renal disease among African Americans, or whether this may reflect differences in glomerular perfusion, circulating glucose concentrations, or chronic inflammation, remains to be elucidated.[75,114] It is possible that the

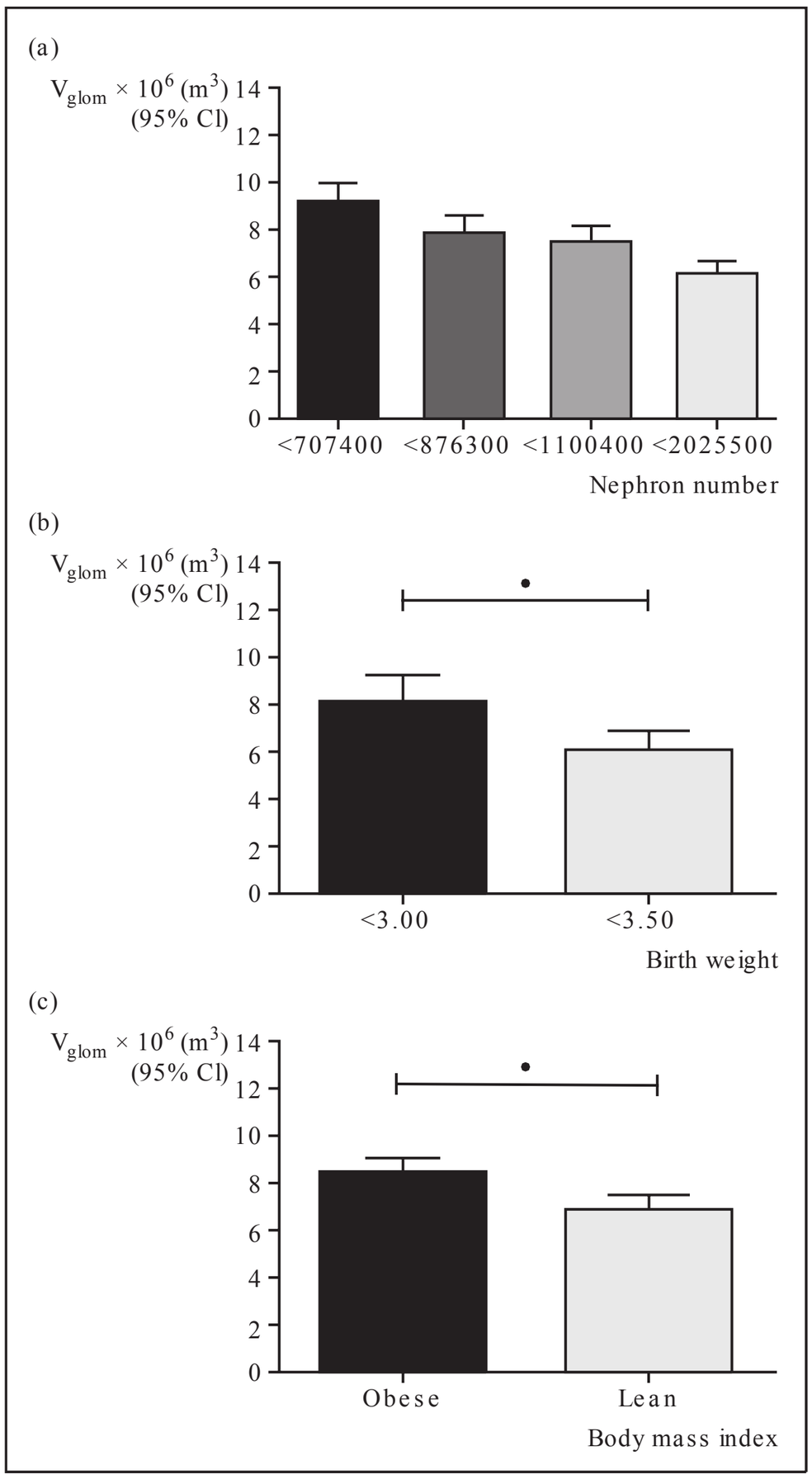

FIGURE 2.3 Relationship of glomerular volume (V_{glom}) to nephron number, birth weight, and body size. **A:** Inverse association of mean glomerular volume (V_{glom}) with nephron number, $n = 252$; **(B)** inverse association of mean glomerular volume (V_{glom}) with birth weight, $n = 58$; and **(C)** direct association of mean glomerular volume (V_{glom}) with body mass index (BMI; obese $\geq$30 kg per m^2, $n = 95$; lean $<$25 kg per m^2, $n = 78$). Subjects were U.S. whites and African Americans. * $P < 0.05$. (Reprinted with permission from Puelles VG, Hoy WE, Hughson MD, et al. Glomerular number and size variability and risk for kidney disease. *Curr Opin Nephrol Hypertens.* 2011;20:7–15.)

greater average adult height among African American subjects may augment final nephron number, diminishing the relationship with birth weight, but may not compensate for, or may exacerbate, other potentially programmed changes such as glomerular size. Compensatory hyperfiltration may be adequate in the short term to augment glomerular filtration, but sustained hyperfiltration, especially in the setting of additional renal stresses (e.g., rapid growth, hypertension, diabetes) will eventually become maladaptive and contribute to ongoing nephron loss. A kidney starting with fewer nephrons would therefore reach a critical deficit within a shorter time. Consistent with this, evaluation of glomerular size in donor kidney biopsies again found higher maximal planar area of glomeruli to be a predictor of poorer transplant function and, again, glomerular size was higher in African American subjects.[115] From a clinical point of view, therefore, an otherwise unexplained increased in glomerular volume should raise the suspicion of a coexisting reduction in nephron number, although this relationship is less clear in African origin populations.

Pathologic Changes

Most renal biopsy results in subjects with lower nephron number have commented on glomerular size. In general the degree of glomerulosclerosis present in kidneys with low nephron number has been found to increase with age and hypertension, but has not been a prominent feature in most studies.[116] The pattern of glomerulosclerosis has been described as a global ischemic collapse rather than classical focal and segmental glomerulosclerosis that might have been expected with hyperfiltration causing cumulative glomerular injury.[74] A recent case series of six very low birth weight subjects, aged 15 to 52 years, who had been born prematurely, however, did find evidence of secondary focal and segmental glomerulosclerosis, associated with glomerulomegaly in all biopsies.[117] Although all biopsies were performed because of a clinical indication, and therefore may not be a generalizable sample, the authors suggest that low birth weight was a common denominator predisposing to hyperfiltration and glomerulosclerosis. Analyses of histologic changes in human biopsies may be confounded by multiple factors. In kidneys of rats exposed to gestational low protein diet, fewer and more immature glomeruli were present on day 10 at the end of nephrogenesis, exhibiting a markedly thickened glomerular basement membrane and abnormal podocyte structure (Fig. 2.1).[118] Similarly, in prehypertensive Gdnf heterozygous mice, which have a 30% reduction in nephron number, glomerular enlargement was observed, associated with an increase in cellular proliferation, thickened glomerular basement membrane, reduced podocyte density, and a mild expansion of the tubulointerstitium.[119] In rats rendered diabetic at 12 weeks, and followed until 40 weeks, glomerular changes associated with diabetes were similar in those that had been of low birth weight compared to normal birth weight. However, again, in addition to lower nephron number, podocyte density was reduced and the area covered by each podocyte was greater in the low birth weight diabetic animals.[120] Interestingly, proteinuria tended to be higher in the low birth weight group, likely suggesting a greater degree of hyperfiltration. Taken together, these authors postulate that early subtle structural abnormalities in kidneys with reduced nephron number may enhance susceptibility to subsequent renal injury.

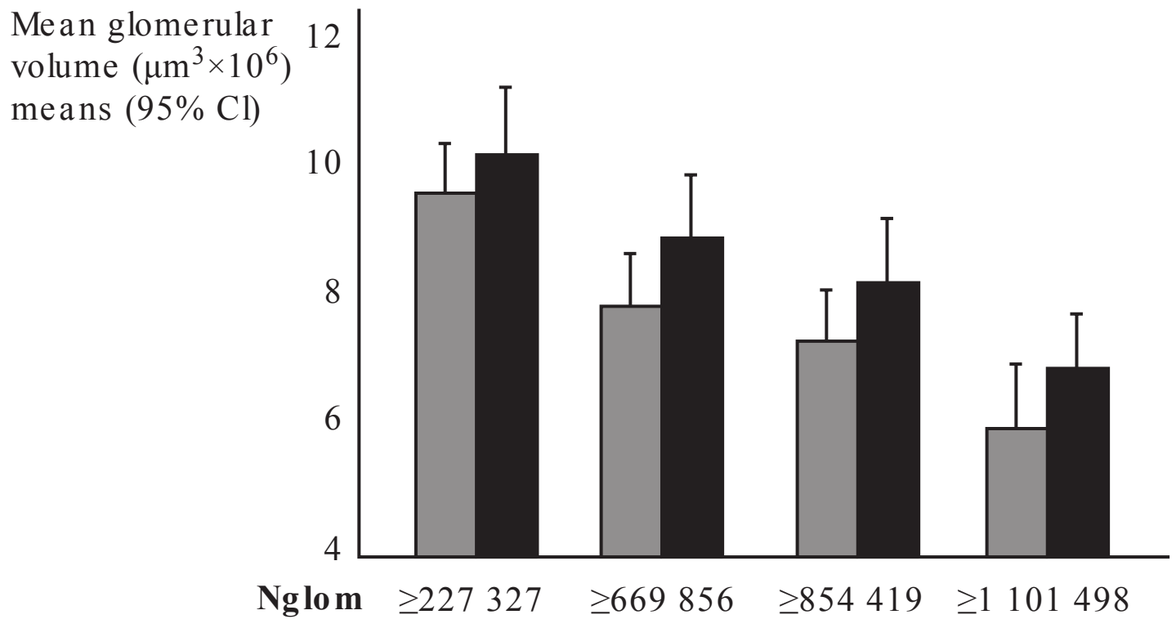

FIGURE 2.4 Relationship between mean glomerular volume and glomerular number (Nglom) in U.S. whites and African Americans. Mean glomerular volume by quartiles of glomerular number. *Light bars* are U.S. whites and *dark bars* are African American adults. U.S. whites, P for trend <0.0001; African Americans *P* for trend $= 0.0008$. (Reprinted with permission from Hoy WE, Bertram JF, Denton RD, et al. Nephron number, glomerular volume, renal disease and hypertension. *Curr Opin Nephrol Hypertens*. 2008;17:258–265.)

CLINICAL IMPACT OF NEPRHON MASS

Blood Pressure

Birth Weight and Blood Pressure

Many studies in humans and animal models have supported the observation that low birth weight is associated with higher blood pressures in later life (Fig. 2.5).[12,121–127] Higher blood pressures have been reported in newborns of lower birth weight, therefore this programming effect is evident early and tracks through to adulthood (Fig. 2.6).[127] Importantly, low birth weight children tend to have higher blood pressures, although not in the hypertensive range, compared to normal birth weight children. With increasing age, however, blood pressure differences between low birth weight and normal birth weight subjects become amplified and do reach hypertensive levels with time.[128,129] Prematurity and gestational age have also been associated with higher blood pressures in young adults; however, low birth weight for gestational age was a more significant predictor of blood pressure at birth and 18 years of age than low birth weight of prematurity.[107,130–133] This observation suggests that ongoing intrauterine stress may have a greater impact than premature birth. Consistent with this possibility, abnormal placental morphology, a marker of adverse intrauterine conditions, has been associated with higher blood pressures in children at 7 years of age.[134] The importance of the intrauterine environment has also been highlighted in both monozygotic and dizygotic twin studies, where the lower birth weight twin has been found to have a greater increase in blood pressure in infancy and higher blood pressure in adulthood.[135,136] These data have been interpreted to suggest that genetic factors play a smaller role than fetal growth in developmental programming. Although this may be the case, a significant modulation of the relationship between birth weight and blood pressure has been found by genotype of beta adrenergic receptors, suggesting possible developmental interaction with gene expression as well.[137] The relationship between birth weight and blood pressure has not been universally found, however, and in particular appears to be weaker, but not always absent, in African American children.[128,138–142] However, interestingly, the association between low birth weight and higher blood pressures does appear preserved in African and Caribbean black children, suggesting that early childhood growth rates, genetic, and/or other environmental factors are also important.[143–145] Current body mass index (BMI) is a frequent confounder in these studies, and may play a greater role among black compared to white subjects.[137]

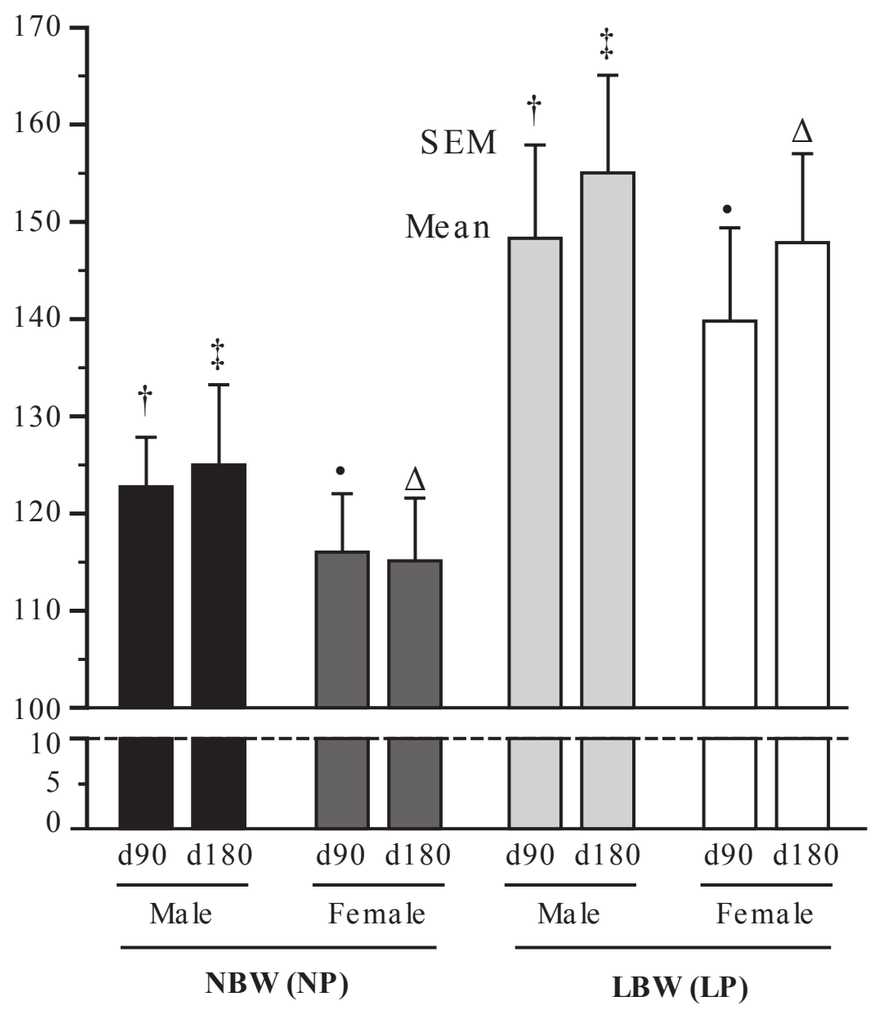

FIGURE 2.5 Relationship between birth weight, nephron number, and hypertension in rats subjected to maternal normal (NP) or low (LP) protein diet. Systolic blood pressure in 90-day-old (d90) and 180-day-old (d180) male and female rats with normal (NBW) or low (LBW) birth weights. Symbols indicate group comparisons, $P<0.05$. (Reprinted with permission from Villar-Martini VC, Carvalho JJ, Neves MF, et al. Hypertension and kidney alterations in rat offspring from low protein pregnancies. *J Hypertens Suppl*. 2009;27:S47–51.)

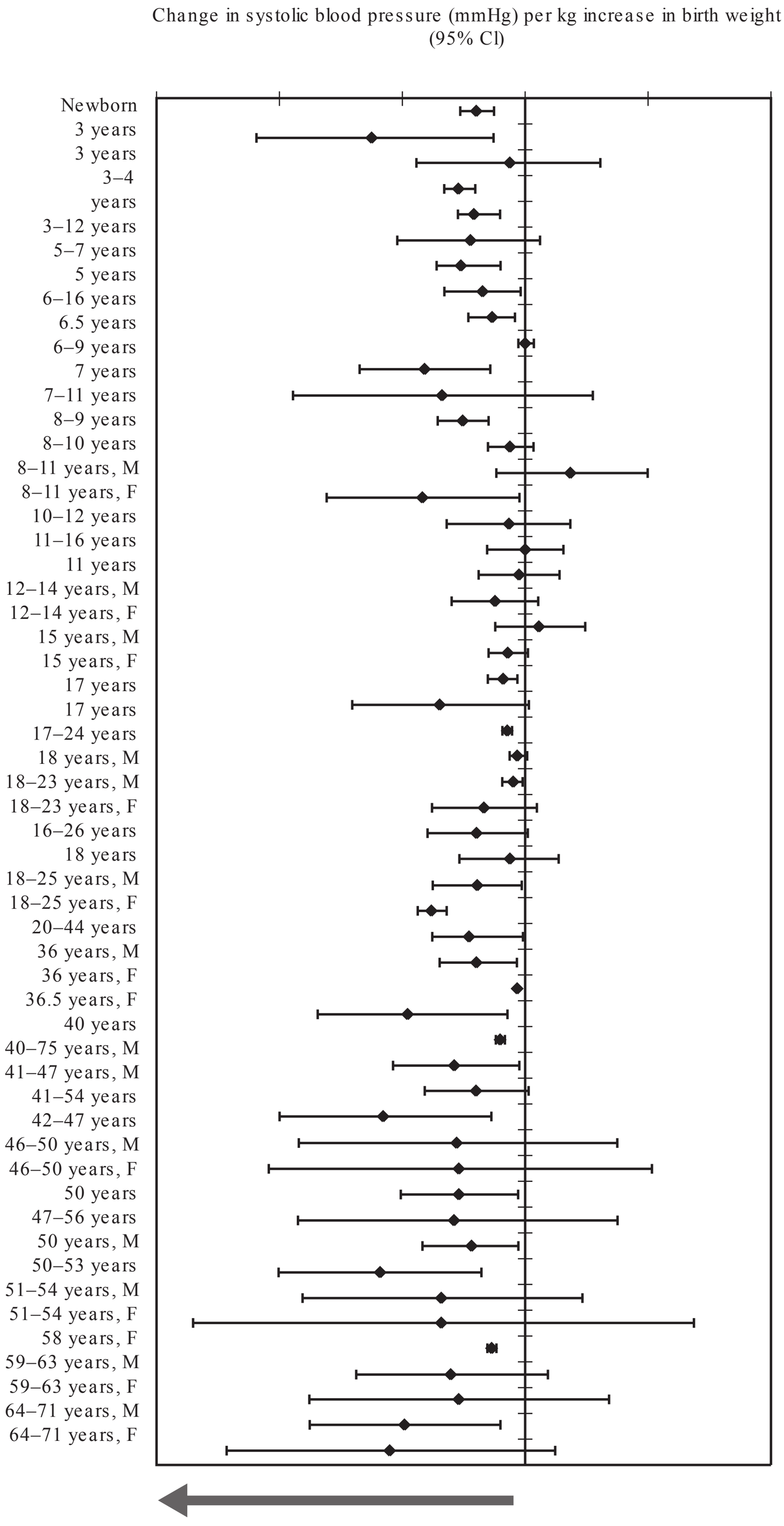

FIGURE 2.6 Studies reporting multiple regression analyses in children, adolescents, and adults. Higher blood pressures are generally associated with lower birth weights across all ages and in both genders. *CI*, confidence interval. See original source for detailed references. (Reprinted with permission from Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens*. 2000;18:815–831.)

Nephron Number and Blood Pressure

The association between low birth weight and later life hypertension has been well documented in various animal models utilizing gestational interventions such as maternal dietary protein restriction, dexamethasone administration, or uterine artery ligation, as described previously.[12,28,37,146–150] The link between low birth weight and subsequent spontaneous hypertension in these models appears to be, at least in part, attributable to an inborn nephron deficit.[12,37,148] Most interventions result in a 20% to 30% reduction in glomerular number in offspring, and hypertension emerges by early adulthood.[12,151] Conversely, optimization of postnatal nutrition after growth restriction has been found to normalize nephron number and abrogate the development of hypertension in rats, suggesting that nephron number per se is an important contributor to the pathogenesis of hypertension.[24,81,152] In contrast, however, augmentation of nephron number does not always protect against high blood pressure. Restoration of nephron number by supplementation of maternal low protein diet with glycine, urea, or alanine only normalized blood pressure in the offspring supplemented with glycine.[24] Similarly, a 20% increase in nephron number, induced by postnatal overfeeding in normal birth weight rats, did not prevent hypertension and glomerulosclerosis with age, although concomitant obesity may have been a confounder in this study.[152] Based on these studies, therefore, developmental programming of hypertension is dependent on more than a reduction in nephron number, although under certain circumstances nephron number does appear to be the predominant predisposing factor.

Evidence in humans suggests a similar association between nephron number and risk of hypertension. In a cohort 35- to 59-year-old European Caucasians who died in accidents, mean nephron number was significantly lower, and glomerular volume significantly higher in the 10 subjects with a history of essential hypertension, compared to 10 normotensive matched controls.[10] There was no evidence of disproportionate glomerulosclerosis or renal injury, leading the authors to suggest that an intrinsic deficit in nephron number was the most likely factor associated with development of essential hypertension. Birth weights were not available in this study, therefore potential associations with nephron number could not be speculated. A possible limitation of this study is the high mean glomerular number in the nonhypertensive group compared to that reported in other Caucasian populations; however, mean nephron number in the hypertensive group was similar to that in a hypertensive U.S. Caucasian cohort. Similarly, lower nephron numbers have been associated with higher blood pressures among Caucasians and Australian Aboriginal subjects, although the relationship is not as consistent among subjects of African origin (Fig. 2.7).[10,70,98] Conversely, the prevalence of hypertension was found to be lower among Caucasians and Australian Aboriginals with higher nephron numbers, suggesting a protective effect of higher nephron numbers in these populations.[70,113] An important caveat is that these studies are all performed on postmortem kidney samples mostly from adults of varying ages, and therefore may not reflect nephron endowment at birth. Much larger sample sizes would be required to control for all possible confounding variables between subjects, which are not easily feasible. In addition, associations have been well described between birth weight and nephron number, nephron number and hypertension, and birth weight and hypertension but, to date, to our knowledge, no study has analyzed nephron number, birth weight, and hypertension in individual subjects.

Timing of nephron loss appears to impact subsequent risk of hypertension and renal disease. In humans, congenital conditions associated with significant reductions in nephron mass (e.g. unilateral renal agenesis or bilateral renal hypoplasia) result in worsening proteinuria, hypertension, and renal dysfunction with time.[11] In contrast, nephrectomy later in life, resulting in a comparable loss of nephron mass, does not necessarily result in progressive renal functional decline.[153] Similarly, nephrectomy in adult animals in varying experimental settings does not invariably lead to hypertension and renal dysfunction.[154] Removal of a kidney on postnatal day 1 in rats, or fetal uninephrectomy in sheep (i.e., loss of nephrons during active nephrogenesis), however, does lead to adult hypertension in the absence of evidence of renal injury.[155–157] Taken together these animal and human observations suggest that loss of nephrons during renal development, as opposed to after nephrogenesis is completed, may have a more critical impact on the long-term risk of hypertension.

In support of this hypothesis, glomerular numbers in adult rats were similar after uninephrectomy at day 3 or day 120 of age; however, a greater proportion of immature glomeruli were present in kidneys of rats having undergone removal of the contralateral kidney at day 3.[158] In addition, mean glomerular volume compared to controls was 59% higher in rats undergoing neonatal nephrectomy compared with 20% higher in rats nephrectomized as adults, suggesting more vigorous compensatory hypertrophy and hyperfunction in response to neonatal nephrectomy, which may become maladaptive over the long term. Developmentally acquired low nephron mass may, therefore, be considered along the broader continuum of renal hypoplasia and associated with long-term consequences.[13]

Glomerular Volume and Blood Pressure

Glomerular volume varies inversely with nephron number and, in U.S. White, Black, and Australian Aboriginal subjects, is associated with increased risk for high blood pressure (Fig. 2.8).[70] In addition, among the U.S. Black and Australian Aboriginal populations, large glomeruli on renal biopsy are associated with poorer renal outcomes in native and transplanted kidneys.[98,115,159,160] Among Black subjects, glomerular size appears to be an independent predictor of higher blood pressure, whereas in White and Australian Aboriginal subjects, the relationship appears also dependent on low nephron number.[113]

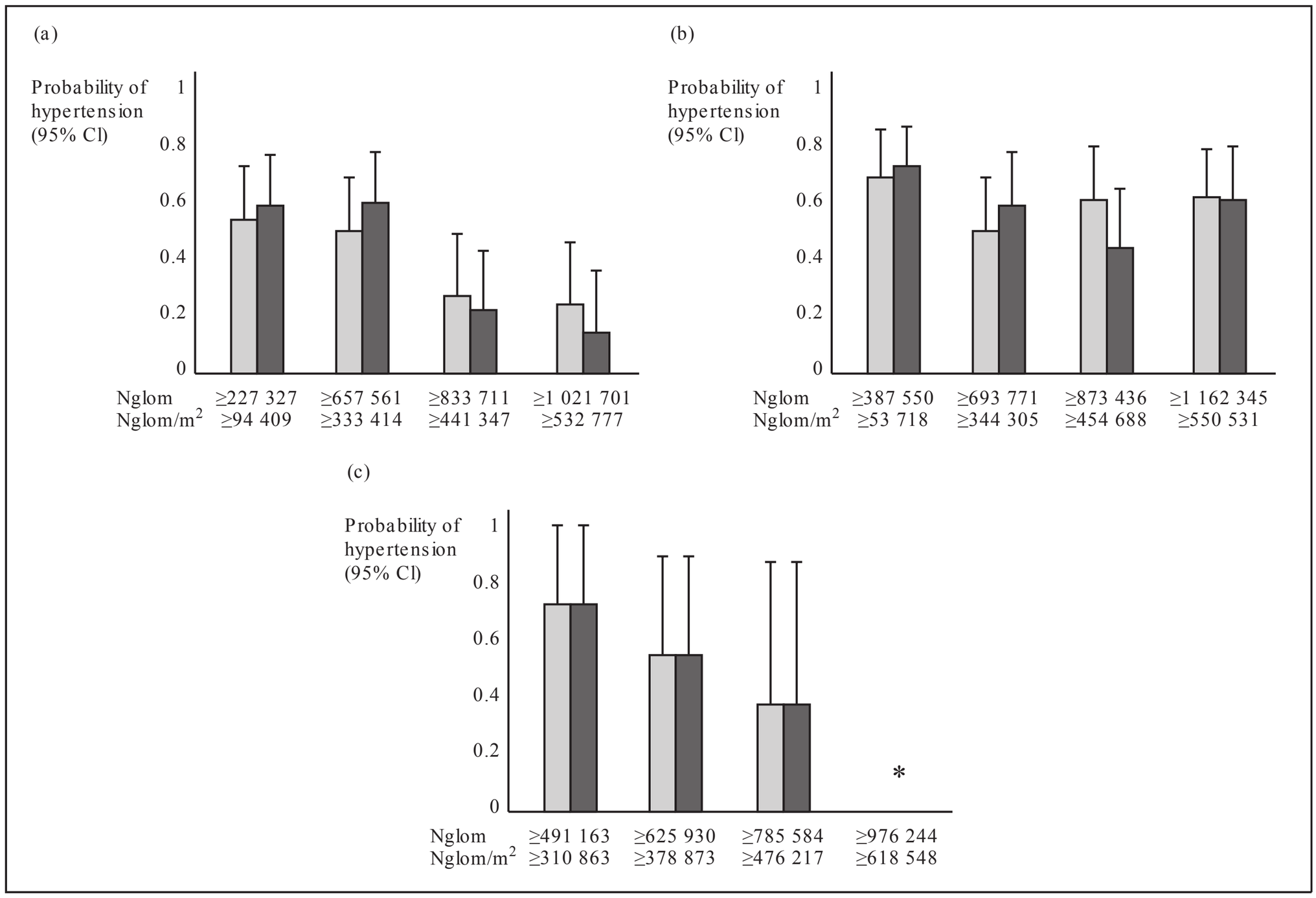

FIGURE 2.7 Probability of hypertension by glomerular number (Nglom) and ethnicity. Probability of hypertension in **(A)** U.S. white adults, by Nglom (*light bars*), $P = .097$; adjusted for sex, $P = .042$. By Nglom per m^2 body surface area (BAS), Nglom per m^2 (*dark bars*), $P = .0012$; adjusted for sex, $P = .0006$. **B:** African American adults, by Nglom, $P = .625$; adjusted for sex, $P = .71$. By Nglom per m^2, $P = .246$; adjusted for sex, $P = .245$. **C:** Australian Aboriginal adults, by Nglom, $P = .167$; adjusted for sex, $P = .173$. By Nglom per m^2, $P = .167$; adjusted for sex, $P = .109$. *Among subjects with Nglom in this category none had hypertension. *CI*, confidence interval. (Reprinted with permission from Hoy WE, Bertram JF, Denton RD, et al. Nephron number, glomerular volume, renal disease and hypertension. *Curr Opin Nephrol Hypertens*. 2008;17:258–265.)

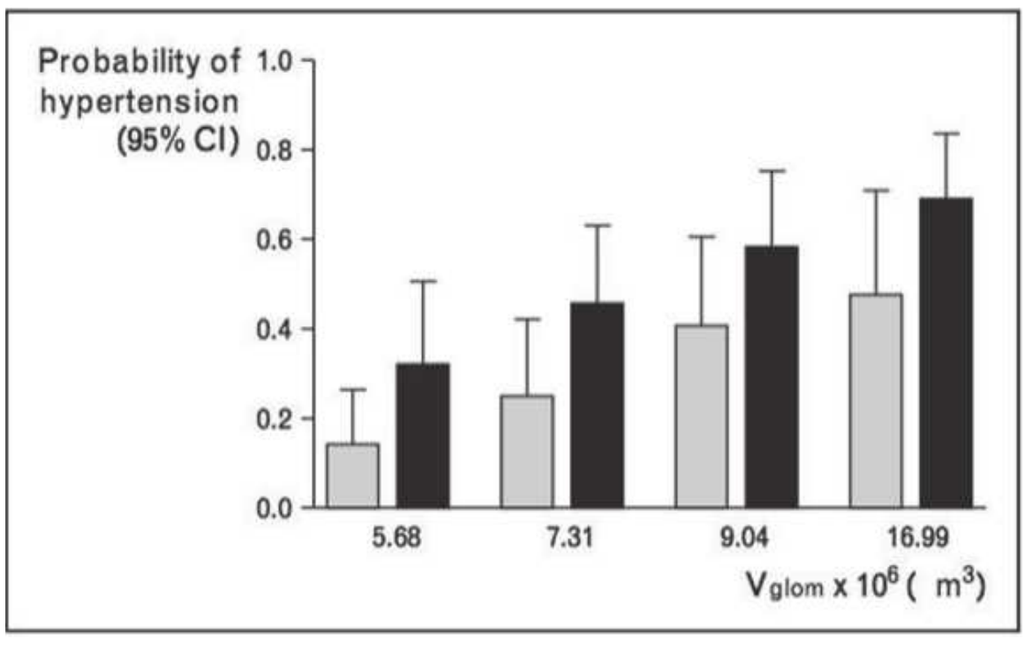

FIGURE 2.8 Probability of hypertension by glomerular volume (V_{glom}) in U.S. white and African American adults. Increasing probability of hypertension with increasing (V_{glom}) in U.S. white (*light bars*) and African American (*dark bars*) subjects aged 18 years or older ($n = 252$). (Reprinted with permission from Puelles VG, Hoy WE, Hughson MD, et al. Glomerular number and size variability and risk for kidney disease. *Curr Opin Nephrol Hypertens*. 2011;20:7–15.)

The observation that in some animal models normalization of nephron number did not prevent development of subsequent hypertension, argues against nephron number being the sole programmed link between early developmental stress and higher blood pressures.[161,162] Given that the filtration surface area in kidneys with low nephron numbers has not been found to be significantly lower than in kidneys with a higher number of nephrons, and may even be increased in subjects with hypertension (Table 2.4), other nonglomerular contributors to sodium avidity in low birth weight kidneys have been investigated.[8,10,70] Blood pressure is dependent on renal, neuroendocrine, and vascular factors, all of which may be subject to simultaneous developmental programming.[8] Programmable vascular factors which have been studied include altered structure and function of large vessels, impaired vascular reactivity, and endothelial dysfunction, which are reviewed in detail elsewhere.[163–165] Neuroendocrine factors include altered stress responsiveness, cortisol levels, insulin resistance, and sympathetic nervous system activity.[71,166,167] Within the kidney, in addition to programming of nephron

number, alterations in sodium transport and modulation of the renin-angiotensin system have also been well described.

Renal Programming of Blood Pressure

Salt Sensitivity and Birth Weight

The original programming hypothesis postulated a reduction in filtration surface area and limitation in sodium excretory capacity in a kidney with fewer nephrons.[2] Consistent with this, prenatal dexamethasone administration in rats was associated with lower GFR, higher urine albumin excretion, reduced urinary sodium excretion, and higher tissue sodium content compared to controls.[37] Interestingly, in a study of Caucasian men aged 20 years, those who had been of low birth weight had an increase in systolic blood pressure and no change in GFR, but an increase in fractional excretion of sodium compared to normal birth weight controls.[85]

Salt-sensitive hypertension has subsequently been shown in low birth weight rats (induced by uterine artery ligation), and in adult rats that had been exposed to maternal gestational diabetes.[52,168,169] In contrast, however, blood pressures did not increase more in low birth weight rats (induced by maternal protein restriction) compared to normal birth weight rats on a high salt diet.[170] Timing of the dietary sodium challenge may have an impact, however.[171] Blood pressure rises more consistently in response to a high salt diet in older rats, suggesting loss of potential adaptive mechanism with age, or greater susceptibility to salt sensitivity as nephron numbers decline with age.[172] Plasma volume and blood pressures were found to be higher in low birth weight compared to normal birth weight juvenile rats on normal diets, however, suggesting positive sodium balance at baseline.[173] Interestingly, although a further increase in sodium intake did not increase blood pressures or plasma volume expansion more than in normal birth weight rats, GFR was significantly increased in the low birth weight rats on a high sodium diet, suggesting a shift in the pressure natriuresis curve.[173] These rats were subjected to global malnutrition in utero, therefore a low sodium diet during gestation, which also may have modulated their renal sodium handling. Interestingly, manipulation of sodium intake postnatally was found to impact long-term blood pressure in young low birth weight rats.[174] Low birth weight rats were placed on either low, normal, or high salt diets from weaning to 6 weeks of age, followed by normal diets thereafter. Later life hypertension was abrogated by early low salt diet and worsened by early high salt diet. In addition, salt sensitivity after 40 weeks was lost in the rats subjected to early low salt diets.[174] Early low salt diet therefore appears to be able to "re-program" the kidney and prevent hypertension in low birth weight rats.

Filtration surface area, blood pressure, and response to sodium loading was assessed in GNDF heterozygous mice in which nephron numbers are reduced, and 20% of animals have unilateral renal agenesis.[175] Total nephron number was reduced by 25% in mice with two kidneys (HET2K) and 65% in mice with single kidneys (HET1K) compared to the wild type. The degree of glomerular hypertrophy was similar in HET1K and HET2K, resulting in normalization of glomerular surface area in HET2K but a persistent reduction in the HET1K. In this model, reduced nephron number alone was not associated with increased blood pressures, but both groups of HET mice became significantly hypertensive on a high sodium diet, with blood pressures being highest in the HET1K mice.[175] Interestingly, at baseline urine sodium excretion was progressively higher in HET2K and HET1K mice, suggesting augmented natriuresis that maintained normal blood pressures. In the same model, tubule sodium transporters were not found to be increased, in contrast to other programming models. This suggested a different adaptation in baseline sodium handling in this genetic model, which may be overwhelmed in the face of a high sodium diet.[119,175]

In healthy human adults, salt sensitivity was found to correlate inversely with birth weight, independent of GFR (Fig. 2.9).[176] Similarly, in low birth weight children, the prevalence of salt sensitivity was found to be high and to correlate

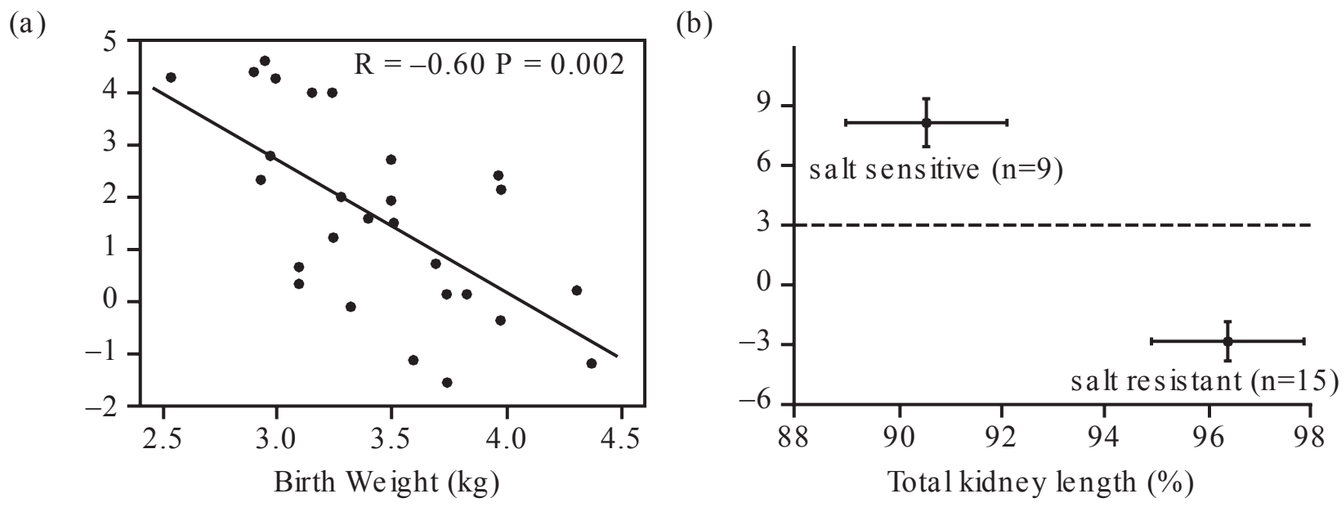

FIGURE 2.9 Salt-sensitivity in humans relative to birth weight and kidney length. **A:** Correlation between birth weight and salt sensitivity in 27 normotensive subjects aged 37.2 ± 14.5 years. **B:** Correlation between salt sensitivity and percent expected kidney length in children aged 11.3 ± 2.1 years. Kidney lengths were smaller in children who had been small weight for gestational age (SGA) compared to appropriate weight for gestational age (AGA) children at birth, suggesting a correlation of salt sensitivity with birth weight. (**A**, reprinted with permission from de Boer MP, Ijzerman RG, de Jongh RT, et al. Birth weight relates to salt sensitivity of blood pressure in healthy adults. Hypertension 2008;51:928–932. **B**, reprinted with permission from Simonetti GD, Raio L, Surbek D, et al. Salt sensitivity of children with low birth weight. *Hypertension*. 2008;52:625–630.)

inversely with kidney size, again independent of GFR, suggesting that renal function itself was not a confounder (Fig. 2.9).[105] Developmental programming of blood pressure in most animal models and humans does, therefore, appear to be associated with altered sodium handling by the kidney.

Renal Sodium Transport

The expression of renal sodium transporters has been investigated in several animal models of developmental programming as a contributor to salt sensitivity. In offspring of mothers fed a low protein diet or given dexamethasone during gestation, chloride transport in the thick ascending limb (mTAL) and the lumen positive transepithelial potential difference were significantly higher compared to control rats, demonstrating increased bumetanide sensitive Na^+-K^+-2 Cl^- (NKCC2) transporter activity in the mTAL.[177] Rats were already hypertensive at the time of study and, consistent with these findings, administration of furosemide reduced blood pressure in the prenatal low protein diet group compared to controls, demonstrating that the increased NKCC2 activity was contributing to the higher blood pressure.[177] Interestingly, in this study, at 6 weeks of age, expression of the NKCC2 was increased in the medulla of the low protein diet group but not the prenatal dexamethasone group, despite changes in sodium chloride transport being evident in both.[177] Similarly, NKCC2 and thiazide sensitive Na^+-Cl^- cotransporter (NCC) protein levels were significantly elevated in low birth weight, induced by maternal low protein diet, rat kidneys at 4 weeks of age—that is, before the manifestation of hypertension. This occurred even though expression of the proximal tubule sodium hydrogen exchanger (NHE3) and the epithelial sodium channel (ENaC) expression were not changed.[178]

In another study, prenatal dexamethasone was associated with increased NKCC2 protein expression at 8 weeks, along with NCC and NHE3, but not ENaC, and their expression was reduced by renal denervation, suggesting modulation of sodium transporter expression by the renal nerves.[179] Other investigators found mRNA expression of the glucocorticoid receptor, and the glucocorticoid responsive α1- and β1-subunits of Na^+/K^+-ATPase to be increased in offspring of rats fed low protein diets during gestation.[180] Similarly, in animals subjected to maternal diabetes, renal Na^+/K^+-ATPase expression was increased, as well as the β and γ subunits of ENaC.[168] When these animals were subjected to a high salt diet, expression of NHE3 and NCC increased, but expression of NKCC2 decreased.[168] In yet another model of low nephron endowment, there was no difference in NCC or ENaC expression by immunohistochemistry in kidneys of Gdnf heterozygous compared to wild type mice.[119] Various programming models of low nephron number and hypertension, therefore, are associated with some disparities in renal sodium transporter expression, but in general it appears sodium transporters are upregulated and likely contribute to increased blood pressures. Whether the alteration in sodium transport is a direct result of reduced nephron numbers, single nephron hyperfiltration and glomerulotubular balance, or is an independent simultaneously programmed change in the renal tubules is not yet clear.

The intrarenal renin-angiotensin system is important for nephrogenesis as well as blood pressure regulation.[8,72] Administration of inhibitors of the renin-angiotensin system during renal development results in abnormal kidneys and reduced nephron numbers.[8] Maternal low protein diet, prenatal dexamethasone, and uterine artery ligation all induce intrauterine growth restriction and have been associated with altered expression of components of the renin-angiotensin system.[8,181–185] The observed alterations in renal renin, angiotensinogen, angiotensin converting enzyme activity, angiotensin II, and angiotensin receptor subtype 1 and 2 levels all appear to be modulated at different times of development with some changes being present at birth and others manifesting in later life, as reviewed elsewhere.[8] In addition, some of the observed variation likely results from differences in timing and nature of the programming insult. The observation that programmed hypertension could be modulated by postnatal administration of inhibitors of the renin-angiotensin system supports a role for this system in generation of increased blood pressures, potentially involving altered sodium transport, reduced nitric oxide activity, and reactive oxygen species generation.[183,185] Furthermore, evidence of lack of suppression of this system in the setting of increased renal sodium transport and plasma volume expansion also suggests dysregulation by prenatal programming.[8] To our knowledge, levels of renin-angiotensin system activity have not been investigated in low birth weight humans.

Catch-up Growth and Blood Pressure

Developmental programming does not only encompass the intrauterine period but, as discussed previously, early postnatal events may also be critical in renal development. Similarly, early childhood growth, especially after intrauterine growth restriction, is emerging as a significant risk factor for subsequent hypertension and cardiovascular disease.[186]

In low birth weight male rats with reduced nephron numbers, induced by maternal gestational protein restriction, postnatal overfeeding resulted in accelerated development of hypertension and a significant reduction in GFR in adulthood.[187] The rapid postnatal weight gain in the low birth weight overfed rats therefore exacerbated the programmed risk of hypertension, acting as a "second hit" superimposed on the low nephron number. Interestingly, appetite, obesity, and energy expenditure are also developmentally programmed, compounding the risk of cardiovascular disease in growth restricted individuals.[188] In a cohort of adolescents, rapid weight gain in the first 2 weeks of life was associated with reduced flow-mediated dilation of the brachial artery measured at ages 13 to 16, underscoring the long-term impact of early nutrition on vascular function.[189] Similarly, weight gain within the first 5 months of life was associated with increased systolic and diastolic blood pressures at 25 years of age, although only systolic blood pressure was inversely associated with birth weight.[190] The combination of low birth

2.5 Difference in Systolic Blood Pressure (mm Hg) at 22 Years per SDs Increase in Birth Weight, Infant Weight Gain (First Year), and Early Childhood Weight Gain (1–5 Years)[a]

Weight Growth Variable	Regression Coefficient (95% CI)	
	Without Adjustment for Adult BMI	With Adjustment for Adult BMI
Birth weight	−1.3 (−2.3 to −0.3)	−1.2 (−2.2 to −0.3)
Conditional infant weight gain	0.5 (−0.6 to 1.5)	−0.1 (−1.2 to 0.9)
Conditional early childhood weight gain	1.6 (0.6 to 2.7)	0.6 (−0.5 to 1.7)
Adult BMI[b]	. . .	2.6 (1.5 to 3.7)

[a]With and without adjustment for adult body mass index.
[b]Geometric SDs.
CI, confidence interval; BMI, body mass index; SDs, standard deviations.
Reprinted with permission from Law CM, Shiell AW, Newsome CA, et al. Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. Circulation. 2002;105:1088–1092.

weight followed by accelerated childhood growth has also been consistently found to be associated with higher blood pressures in childhood and adulthood (Table 2.5).[191,192]

Several potential mechanisms have been investigated to explain this amplification of cardiovascular risk by rapid weight gain after growth restriction. In obese sheep that had been exposed to maternal nutrient restriction during gestation, adipose tissue showed an increase in number of necrotic cells, increased secretion of pro-inflammatory factors, and increased evidence of endoplasmic reticulum stress compared to obese controls.[188] In addition, the nutrient restricted obese sheep exhibited abnormal myocardial structure and function.[188] One possible connection between growth restriction and accelerated catch-up growth is the development of premature senescence.[193] Chronic diseases such a hypertension, chronic kidney disease, and cardiovascular disease have all been associated with increased expression of senescence markers. In animal models, low birth weight followed by accelerated postnatal growth was associated with more rapid telomere shortening and accelerated senescence in kidneys and aortas, as well as premature death.[194–196] These markers were not correlated directly with blood pressures and nephron numbers in the same animals. However, investigators utilized a standard programming model of maternal dietary protein restriction, therefore low birth weight animals were expected to have low nephron numbers and high blood pressures.

In another study, there was no difference in expression of the senescence marker p16 between low birth weight and normal birth weight rats, suggesting that senescence is not likely a contributor to the reduced nephron numbers.[193] p16 was significantly increased by weaning in kidneys and hearts of low birth weight rats, and continued to rise progressively with age, suggesting ongoing tissue stress and accelerated senescence precipitated by catch-up growth. Increased senescence in the low birth weight kidneys may result from ongoing hyperfiltration injury in kidneys with fewer nephrons, exacerbated by a rapid increase in body size. Interestingly in humans, leukocyte telomere length was not different between small for gestational age and appropriate for gestational age newborns, but by 5 years low birth weight children had significantly shorter telomeres than normal birth weight children, consistent with accelerated senescence.[197,198] However, growth trajectories of these children were not reported. Markers of endoplasmic reticulum and mitochondrial stress were increased in low birth weight rat kidneys after rapid catch-up growth, which points to increased reactive species generation as a possible mediator of increased senescence.[193] Consistent with these animal findings, in humans there is evidence of increased oxidative stress in children born small for gestational age compared to controls, which was relatively higher in those who experienced catch-up growth.[199,200] The link between nephron mass, catch-up growth, premature senescence, and the development of hypertension and renal disease in humans after developmental programming has not been studied.

Renal Function and Nephron Mass

Glomerular Filtration Rate

A reduction in nephron number, and therefore filtration surface area, in the absence of compensatory hyperfiltration, would be expected to be associated with a reduced whole kidney GFR. Consistent with this possibility, amikacin clearance, as a surrogate for GFR, was lower in premature or growth restricted neonates on day 1 of life (i.e., before

any adaptation would have occurred).[201] Similarly, nephron number and GFR were measured in newborn growth restricted piglets.[202] GFR was reduced in proportion with nephron number, suggesting a lack of glomerular adaptation early after birth. With increasing age, however, as the single nephron GFR increased with hyperfiltration, differences in total GFR have not been consistently seen between normal and low birth weight subjects. Among 6- to 12-year-olds, estimated creatinine clearance was lower and serum creatinine higher in very low birth weight compared to age-matched normal birth weight subjects.[203] In contrast, estimated GFRs were not different between three groups of 9- to 12-year-olds born with low gestational age or either small for gestational age or appropriate for gestational age at term.[106] Interestingly, estimated GFR calculated using cystatin C showed a significant linear trend with birth weight compared to creatinine-based GFR in a cohort of children divided into quartiles by birth weight. This suggested the potential pitfalls with creatinine measurements and/or the need to adapt GFR calculation formulae in young low birth weight subjects.[204]

Utilizing iothalamate clearance, GFR was measured in a cohort of children at a mean age of 7.6 years who were born with a birth weight under 1,000 g or before 30 weeks of gestation, and stratified according to whether they had experienced either intrauterine or extrauterine growth restriction or normal perinatal nutrition.[55] GFRs were significantly lower in both pre- and postnatally growth restricted children, although still within the normal range for their age. This observation further highlights the potential negative impact of poor extrauterine nutrition on early renal development in premature infants. In young adults born very premature, the creatinine-based Cockroft-Gault GFR was found to correlate positively with birth weight.[131] GFRs measured by 24-hour urine creatinine clearance within adult twin pairs were lower in the lower birth weight twin, again suggesting an independent effect of the intrauterine environment on programming of renal function.[205] Overall, from several studies, GFRs were estimated to increase by 3.8 to 7.2 mL per minute in males and 2.6 to 5.7 mL per minute in females per 1-kg increase in birth weight.[206,207]

An interesting small study evaluated total GFR, effective renal plasma flow, and filtration fraction before and after low dose dopamine or an oral amino acid load to test renal functional reserve in 20-year-olds born (1) premature with appropriate weight for gestational age, (2) premature and small for gestational age, or (3) term and appropriate for gestational age. Intriguingly, although the changes did not reach statistical significance, the stimulated increase in GFR was less in small for gestational age compared with appropriate for gestational age and control subjects. Effective renal plasma flow was lower in both small for gestational age and appropriate for gestational age preterm subjects, suggesting at least a small decrease in renal functional reserve capacity.[208]

GFR and effective renal plasma flow were also studied before and after an intravenous amino acid infusion in young adults who had diabetic mothers (i.e., had been exposed to maternal diabetes during gestation) compared to those with diabetic fathers. Subjects were matched for age, gender, BMI, and birth weight. The offspring of diabetic mothers had a significantly reduced renal reserve capacity, suggesting a renal programming effect due to maternal diabetes.[53] Evaluation of renal functional reserve may therefore be a sensitive method to detect subtle changes in renal function due to a reduced nephron number that may not be evident with baseline GFR measurements.

Proteinuria is a marker of glomerular hyperfiltration and renal injury and, as such, has been investigated in several low birth weight populations. Among children aged 8 to 11 years of age, low birth weight was associated with significantly higher blood pressures and 24-hour urine albumin excretion compared to normal birth weight controls.[209] Multiple other studies have also shown a largely consistent relationship between low birth weight and proteinuria, although in some studies albuminuria was associated with thinness at birth, an indicator of intrauterine stress, rather than birth weight.[94,131,207,210,211] Among Australian Aborigines, albuminuria was strongly associated with low birth weight and the relationship was amplified with increasing age.[212] Macroalbuminuria in this cohort was also associated with a high risk of renal failure and death.[213] In a Finnish population with type 1 diabetes, however, after a mean duration of diabetes of 19 years, no association was found between proteinuria and birth weight stratified as low, <10th percentile; high, >90th percentile; and intermediate, between 10th and 90th percentiles.[214]

In a similar study, among Danish women with type 1 diabetes, with a median duration of 27 years, 75% of those with birth weight under the 10th percentile had nephropathy, defined as persistent urine albumin excretion >300 g per day, compared with 35% of those with birth weights above the 90th percentile.[215] The effect was not present in men, although in another study, short stature in men was associated with a higher risk of macroalbuminuria.[216] A U-shaped association between birth weight and proteinuria was found among Pima Indians with type 2 diabetes in the United States, suggesting that high birth weight and low birth weight are both risk factors for renal disease in this population.[94] Interestingly 64% of subjects with high birth weight were offspring of diabetic mothers versus none of those with low birth weight. The association of high birth weight with proteinuria was lost after adjustment for maternal diabetes, suggesting potentially different programming mechanisms in low birth weight and high birth weight with respect to proteinuria.[94]

Differences between these studies in diabetic subjects may reflect altered genetic susceptibility to renal disease in the Pima population, differences between type 1 and type 2 diabetes, as well as different durations of diabetes. As mentioned previously, podocyte abnormalities are present in kidneys with developmentally programmed low nephron numbers, which may contribute to the development of proteinuria in low birth weight subjects.[118]

Chronic Kidney Disease

Although GFRs have been found to be statistically lower in low birth weight populations, this has not always been outside of the normal range, calling into the question true clinical relevance.[55,131] A recent meta-analysis examined 31 studies that reported risk of CKD—including various end points, including proteinuria, diabetic nephropathy, and reduced GFR—relative to birth weight.[207] Overall they found a 70% increased risk of CKD in low birth weight individuals, regardless of end point studied.[207] Again, the effect was stronger in males. Among 12,364 participants in the Kidney Early Evaluation Program, among men a U-shaped curve was found for risk of CKD, defined as an estimated GFR <60 mL per minute or albumin excretion ≥30 g per g, and birth weight, with increased risk with birth weights <2,500 g and ≥4,500 g.[73] No association was found among female participants, however.

The clinical relevance of the risk of CKD with low or high birth weights is borne out in studies examining risk of ESRD. Retrospective analysis in over 2 million Norwegians found the relative risk of ESRD to be 1.7 in males and females born below the 10th percentile in weight.[217] Interestingly, when looking at absolute birth weights and risk, the relative risk for ESRD was 2.0 with birth weights <2.5 kg, but was only increased in females with birth weights ≥4.5 kg.[217] In a predominantly black, southern U.S. population, a U-shaped curve was again described for risk of ESRD with low and high birth weights, this time in both men and women.[218]

Growing numbers of epidemiologic studies therefore support the relationship between high birth weight or low birth weight and risk of subsequent renal disease (Table 2.6). A direct link between nephron number and renal disease in individual human subjects has not been made, however. Nephron number is unlikely to be the sole cause of renal dysfunction in most patients, and other susceptibilities likely compound the risk. A low nephron number, however, may lower the threshold to reach a critical loss of renal function in response to superimposed renal injury or stress. In support of this possibility, low birth weight has been associated with poorer renal outcomes in patients with nephrotic syndrome, membranous nephropathy, IgA nephropathy, minimal change disease, and diabetic nephropathy.[95,219–222]

Interestingly in a model of diabetes superimposed on low nephron number, the increase in glomerular volume in response to hyperglycemia was more exaggerated and maladaptive in the low compared to normal birth weight rats.[223] Similarly, mesangioproliferative glomerulonephritis was associated with significantly increased glomerulosclerosis in low birth weight animals with reduced nephron numbers.[224] Potential molecular mechanisms whereby kidneys with fewer nephrons adapt differently than normal kidneys include an imbalance between apoptosis and cell proliferation, accelerated senescence, reactive oxygen species generation, and mitochondrial dysfunction.[162,193]

RELEVANCE OF NEPHRON MASS IN TRANSPLANTATION

Impact of Kidney Donation

In experimental models of low birth weight, as described previously, nephron numbers are often reduced by 25% to 30%, and animals develop spontaneous hypertension and

2.6 Clinical Findings Associated with Birth Weight, Nephron Number, and Kidney Size in Humans

Low Birth Weight/ Prematurity	Low Nephron Number	Reduced Renal Size	High Birth Weight/ Maternal Diabetes
↑ Blood pressure[127] Salt sensitivity[105,176] Proteinuria[131,210] ↓ GFR[55,131,201] ↓ Renal functional reserve[208] Accelerated progression of primary renal disease[219–222] Chronic kidney disease[73,207] End-stage kidney disease[217,218] Death[210]	↑ Blood pressure[70] ↑ Glomerular volume[13] ? Predisposition to renal failure in neonates[54]	↑ Blood pressure[105] Salt sensitivity[105] ↓ GFR[105] ↓ Renal allograft survival if small kidney into large recipient[237]	Proteinuria[95] ↓ Renal functional reserve[53] End-stage kidney disease[217,218]

↑, increase; ↓, decrease; ?, unknown.

renal dysfunction.[12,37] In humans, donation of a kidney implies loss of 50% of nephron mass, and therefore may carry some long-term risk. However, many studies have shown that kidney donation is safe and former kidney donors have similar or even better life expectancy and lower risk for ESRD than the general population.[153] This paradox might be due to the very thorough screening of potential donors and the selection of only the very healthiest subjects. In addition, the studies on long-term outcomes have predominantly been done in Caucasians, are limited by significant loss to follow-up, and do not use similarly selected, healthy control groups for their comparative analysis.

An early retrospective study of 52 kidney donors followed after at least 10 years did find a higher risk of hypertension and mild proteinuria compared to age-matched controls and other potential donors, although creatinine clearance did not deteriorate as a function of time.[225] Interestingly the risks of hypertension and proteinuria were greater in men. In contrast, subsequent studies in predominantly Caucasian donors did not find a significantly increased risk of hypertension and proteinuria, suggesting that uninephrectomy is safe.[153] Concerns have been raised, however, about possible harm of living kidney donation in other ethnic groups. After a median of 16 years postdonation, the incidence of new onset hypertension, CKD, and ESRD was significantly higher among Australian Aboriginal kidney donors compared to Caucasians.[226] Similarly, among Canadian donors, hypertension was present in 42% of Aboriginals compared with 19% of Caucasians by a mean of 14 years of follow-up, and in 100% of Aboriginal donors by 20 years postdonation.[227] Estimated GFR was not different between Caucasians and Aboriginals, but proteinuria was more common among Aboriginal donors. In U.S. cohorts, black kidney donors were found to have significantly more hypertension and CKD compared to white donors.[159,228]

In all of these cohorts donors are presumed to have been screened and found healthy prior to donation, therefore, uninephrectomy in populations with high susceptibility to hypertension and kidney disease may carry more risk than has thus far been appreciated. Aboriginal Australian and U.S. black populations have lower birth weights than their Caucasian counterparts, and Canadian Aboriginals have higher birth weights (World Health Organization). This suggests that programming of nephron number may be a factor contributing to increased hypertension and renal risk postnephrectomy. Birth weight and early development history should therefore be incorporated into a potential donor's evaluation, and consideration given to measurement of renal functional reserve, although at present it is likely premature to suggest that decisions on donor eligibility be based on this information.

Impact on Allograft Function

The importance of nephron mass as a nonimmunologic determinant of long-term transplant outcomes has been debated since the nephron number hypothesis was first put forward.[229] Indeed, in rat models it has been elegantly shown that transplanted nephron mass (i.e., kidneys with varying nephron numbers) has a significant impact on allograft outcome, independent of immunologic barriers.[230] As nephron numbers cannot be determined in vivo, various surrogates have been examined in humans to assess the impact of transplanted nephron mass, measurable to some degree ex vivo, relative to recipient demand, on allograft outcomes (Table 2.7). Such surrogates include ratios of recipient to donor body surface area (BSA) or body weight, of kidney volume to recipient BSA, and of kidney weight to recipient body weight.[231–235] As mentioned previously, kidney mass and kidney volume do reflect nephron number to some degree, but these data should be interpreted with caution, realizing that BSA is not always proportional to kidney weight, and that two kidneys of the same size may differ in nephron number.

Despite these caveats and the variability of methods employed, the evidence shows fairly consistently that small kidneys, or kidneys from small donors, transplanted into larger recipients, tend to have poorer outcomes.[231–235] Duration of follow-up, however, is also a crucial variable, as seen in the donor literature, where differences in hypertension and proteinuria emerge only after many years. Interestingly, an early report in a cohort of renal allograft recipients, with a mean of 32 months of follow-up, failed to find any impact of graft weight on short-term graft survival.[236] With longer follow-up, however, in the same cohort, subjects with a low donor kidney weight to recipient body weight ratio (DKW/RBW) showed a greater adaptive early increase in GFR, which remained stable for 7 years, followed by a more rapid loss of GFR as compared to the high DKW/RBW group, thus demonstrating the importance of long term follow-up.[237] These data suggest that smaller kidneys transplanted into larger recipients underwent early hyperfiltration that could not be sustained indefinitely, resulting in ongoing nephron loss. Over time, the low DKW/RBW group required more antihypertensives, had more proteinuria, and on kidney biopsy showed a greater degree of glomerulosclerosis. Overall, the risk of transplant failure was 55% higher in low DKW/RBW compared to the high DKW/RBW group at 2 years.[237] The authors conclude that mismatch between allograft and recipient weight is an independent predictor of long-term graft survival.

These results are consistent with another analysis, which was restricted to recipients of living donor kidneys who had not experienced any complications within the first year of transplantation. Progressively higher levels of urine protein excretion and lower GFRs occurred as DKW/RBW fell.[238] Similarly, a large retrospective analysis of 32,083 recipients of a first cadaveric kidney transplant, utilizing the ratio of donor to recipient BSA, found that large recipients of small kidneys had a 43% increased risk of late allograft failure compared to the reference group which was medium sized recipients receiving kidneys from medium sized donors.[234]

Another study examined the outcomes of kidneys from donors over age 60, presumed to have fewer nephrons by

2.7 Impact of Donor and Recipient Mismatch on Renal Allograft Outcomes

Measurement	Allograft Outcome	Donor	Reference
Donor kidney weight : recipient body weight	↑ Risk of late allograft loss, proteinuria, hypertension, and glomerulosclerosis at 6.2 years with lower ratios	Cadaveric	237
Donor kidney weight : recipient body weight	↓ Creatinine clearance and ↑ proteinuria at 3 years with lower ratios	Living	238
Donor–recipient body weight ratio	↓ Graft survival at 5 years in low ratio group	Living	232
Donor : recipient BSA	↑ Late allograft loss in large recipients who received kidneys from small donors	Cadaveric	234[a]
Transplant cross-sectional area (ultrasound): recipient body weight (Tx/W)	↓ Creatinine, trend toward improved outcome with larger ratios at 12 months	Cadaveric	241
Kidney weight (g)	↑ Creatinine clearance with higher kidney weight at 12 months	Living	231

[a]More studies reviewed in this reference.
↑, increase; ↓, decrease; BSA, body surface area.

virtue of increased age, relative to recipient BMI, and found that 5-year allograft survival was significantly lower in recipients with higher BMI or BSA, again suggesting an impact for mismatch between fewer transplanted functioning nephrons and higher donor demand on long-term outcomes.[239] Not all studies have found consistent results, however, with some failing to find an impact of differing donor to recipient BSA ratios on outcomes of paired cadaver kidneys.[233] The high and low ratios did overlap in this study, however.

A more recent study of paired kidneys did find a donor–recipient size mismatch to be a risk factor for delayed graft function.[240] Other investigators have examined the ratio of transplanted kidney cross-sectional area, as measured by ultrasound, relative to recipient weight as a predictor of outcomes. They found lower serum creatinines and a trend toward improved graft survival at 5 years in those with higher ratios.[241] Overall, therefore, transplanted nephron mass does appear to have a long-term impact on allograft function. Clinically, however, nephron mass at transplantation is likely impacted by many other factors in addition to nephron endowment (e.g., loss through peritransplant injury, immune-mediated injury, donor age, and other donor factors). Kidneys transplanted with fewer nephrons are likely to have less functional reserve and therefore are at risk of declining function over time. Awareness of this association may impact a clinician's decision about medication choices, peritransplant interventions, and ultimately potentially organ allocation.

THE IMPACT OF PROGRAMMING ON RELATED ORGAN SYSTEMS

The interaction of gestational diabetes exposure, birth weight, and proteinuria, for example, demonstrates that developmental programming may impact multiple organ systems simultaneously.[53,95,215] In a Swedish cohort of 18,230 twins, low birth weight was found to increase the risk of adult type 2 diabetes with an adjusted odds ratio of 1.44 per 500 g decrease in birth weight.[242] Similarly, in a Chinese cohort, low birth weight was found to be inversely associated with risk of type 2 diabetes, and both high and low birth weights were associated with increased risk of hypertension and abdominal obesity.[243] Low birth weight combined with abdominal obesity was the strongest predictor of diabetes. Obesity and diabetes are both risk factors for CKD and, therefore, their interaction with low birth weight likely augments this risk. Interestingly, low birth weight rats, induced by maternal low protein diet, suffered more severe cardiac dysfunction after myocardial ischemia and reperfusion compared to normal birth weight rats.[244] In another study, graded surgical reduction in nephron number in normal rats was associated with progressively

higher systolic blood pressures, left ventricular hypertrophy, and left ventricular systolic and diastolic dysfunction compared to sham operated controls.[245] In sheep, fetal uninephrectomy resulted in hypertension and more severe renal dysfunction and albuminuria with age.[156] In addition, cardiac functional reserve, measured in response to dobutamine infusion, was significantly reduced by 6 months of age in the neonatally nephrectomized sheep and left ventricular mass was significantly increased. In the clinical arena, patients with CKD often have coexisting diabetes and/or cardiac dysfunction, and CKD itself is a known cardiac risk factor. Whether some of this association is related to the consequences of renal dysfunction per se—that is, hypertension, volume expansion, proteinuria—or may reflect parallel programming of multiple organ systems in the same individual has yet to be elucidated.

CONCLUSION

The concept that nephron mass, at least in part, is determined during the perinatal period and has a long-term impact on an individual's subsequent risk of hypertension and renal disease is now accepted (Fig. 2.10). Nephron number alone is not often enough to result in overt disease, but does appear to be a strong modifier of risk in various ethnic groups, as well as under certain clinical conditions (e.g., diabetes mellitus). As such, surrogate markers for low nephron number and adverse perinatal conditions should be screened for in the clinical setting and appreciated as risk factors that may not be modifiable in the adult. However, they may highlight the need to minimize further insults and optimize other risk factors for hypertension and renal disease. Low birth weight is currently the most useful clinical surrogate for low nephron mass and the developmentally determined risk of hypertension and renal disease.

Much more work is needed to determine the impact of high birth weight. The exciting experimental findings that low nephron numbers can be rescued under some circumstances points to the importance of improving maternal health before and during gestation, optimizing of neonatal nutrition, and avoiding nephrotoxins in the early perinatal period, as well as raising hope for potential translation of therapeutic interventions to the human in the future. From a public health point of view, close attention should be paid to improving perinatal care and early childhood nutrition as potential tools to stem the growing tides of renal and cardiovascular disease in future generations.

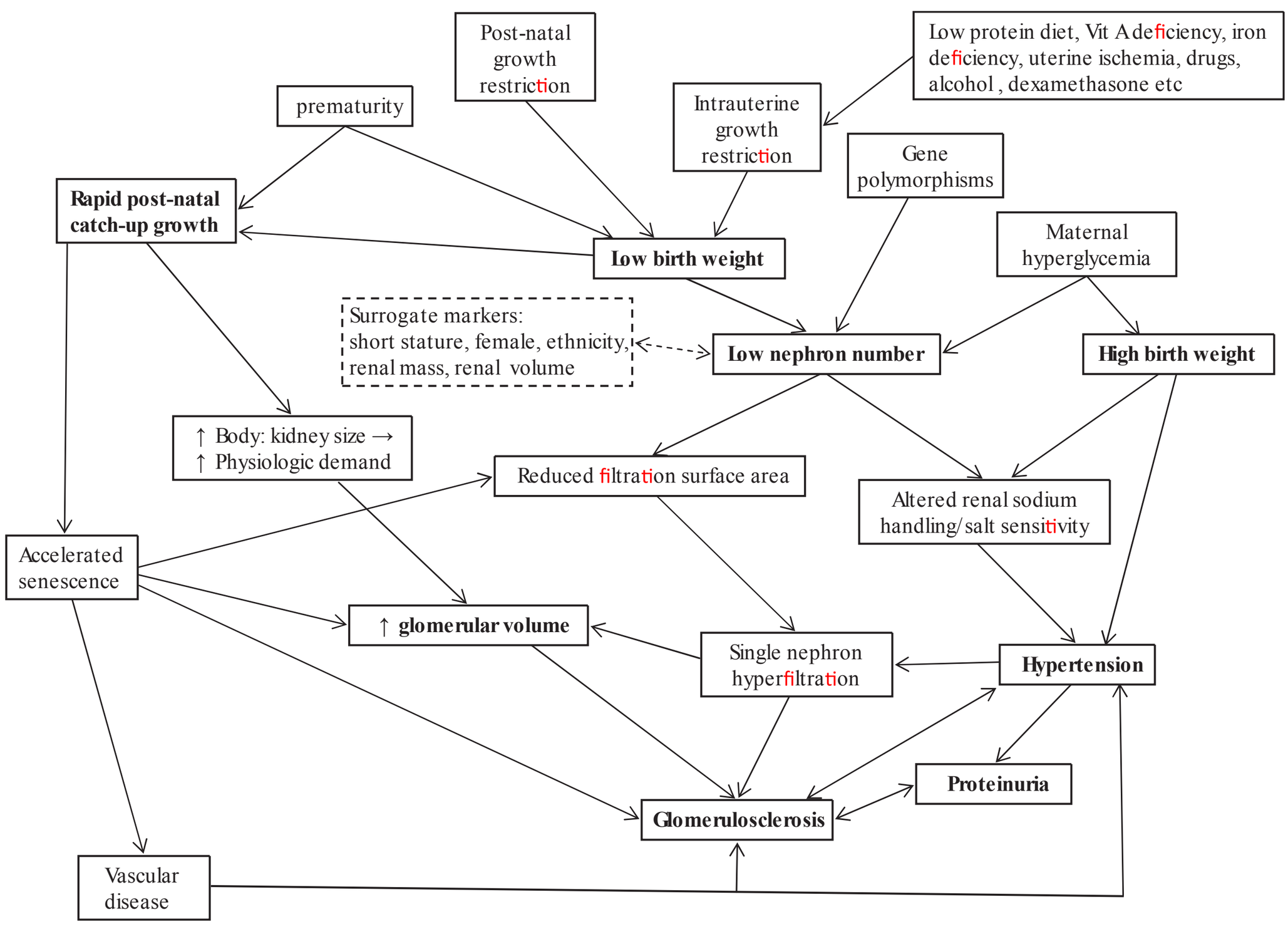

FIGURE 2.10 Diagram of proposed mechanisms impacting developmental programming of renal disease and hypertension. (Adapted from Schreuder M, Delemarre-van de Waal H, van Wijk A. Consequences of intrauterine growth restriction for the kidney. *Kidney Blood Press Res*. 2006;29:108–125.)

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CHAPTER

3 Renal Circulation and Glomerular Hemodynamics

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The capability of the kidneys to achieve their sophisticated homeostatic function is optimized by an intricate microvascular system that adjusts vascular resistance to maintain an appropriate control of the intracapillary and interstitial forces that govern renal blood flow in the cortex and medulla as well as the glomerular filtration rate. A combination of intrinsic and extrinsic regulatory mechanisms are responsible for controlling the one-fifth of the cardiac output that circulates through the kidneys. Essentially all the renal blood flow (RBF) traverses through the glomerular capillaries, where about 20% of the plasma is filtered. The complex glomerular filtration apparatus is truly unique in having both a very high hydraulic conductivity and a remarkably low permeability to plasma proteins. One major function of the renal vasculature is to regulate the intraglomerular forces so that an adequate, yet not excessive, volume is filtered into the urinary tubules. In this chapter, we discuss the characteristics of the filtering and the reabsorbing microcirculatory structures in the normal kidney. Particular emphasis is placed on the dynamic interactions among the intrarenal paracrine and extrarenal homeostatic mechanisms that participate in regulating these processes. To allow a better appreciation of basic mechanisms, some structural relationships and fundamental concepts related to vascular smooth muscle, endothelial cells, and other components of the renal microvascular network are discussed. A detailed discussion of the anatomic features of the kidney is provided in Chapter 1.

THE MAGNITUDE OF RENAL BLOOD FLOW AND GLOMERULAR FILTRATION RATE

The multiple intrarenal parallel arteriolar pathways provide the kidneys with a very low vascular resistance. They normally receive about 20% of the cardiac output. This amounts to a blood flow of 1,000 to 1,200 mL per minute in a 70- to 75-kg person. RBF is even more impressive when considered per unit of kidney weight, because the kidneys account for only 0.5% of the total body weight, or about 300 g. Thus, as shown in Table 3.1, blood flow per gram of kidney weight is about 4 mL per minute, which is 5 to 50 times greater than the flow through other organs and circulatory beds. Based on a total of 1 million glomeruli in each kidney or a glomerular density of 7,000 glomeruli per gram, the average blood flow and glomerular filtration rate (GFR) per glomerulus is 570 nL per minute and 62 nL per minute, respectively. This large flow, coupled with the maintenance of a high hydrostatic pressure in glomerular capillaries, allows the filtration of about 20% of the plasma, which amounts to an average GFR of 120 mL per minute, or 170 L per day.[1,2]

The extraordinarily high RBF is in marked excess of that simply required to provide the renal parenchyma with adequate supplies of oxygen and nutrients. For this reason, it is generally recognized that RBF is regulated primarily to maintain the glomerular and peritubular intrarenal hemodynamic environments at levels compatible with the optimum delivery of filtrate to the nephrons and appropriate reabsorption of fluid back into the systemic vasculature.

The Relationship of Renal Blood Flow to Oxygen Consumption

Although oxygen (O_2) use is not a major determinant of RBF, O_2 consumption by the kidneys is still quite high because of the very high metabolic activity of the tubules. Over 99% of the filtered fluid, electrolytes, and essential organic nutrients are normally reabsorbed by the tubules and returned to the circulation via the peritubular capillaries. The tubular reabsorptive processes depend on the integrity of the epithelial transport systems, in particular the energy requiring Na-K-ATPase. Such tubular enzyme systems account for the majority of the O_2 consumption by the kidneys.

RBF is about 400 mL/min/100 g of tissue, and the arteriovenous O_2 difference is relatively low, only 1 to 2 mL per deciliter of blood. Thus, O_2 consumption by the kidney is about 8 mL of O_2 per minute or 400 μm of O_2/min/100 g, which amounts to 6% to 8% of the whole body O_2 consumption. This level of O_2 use is relatively constant and is not reduced by moderate hypoxemia. Under physiologic conditions, there is a consistent relationship between RBF

3.1 Renal Hemodynamic Function in Humans

	Total	Per Kilogram Body Weight	Per Gram Kidney Weight
Renal blood flow	1,200 mL/min	17 mL/min	4 mL/min
Renal plasma flow	670 mL/min	9.6 mL/min	2.2 mL/min
Glomerular filtration rate	130 mL/min	1.9 mL/min	0.45 mL/min
Number of glomeruli	2 million	28,500	7,000
Oxygen consumption	1,200 μMO_2/min	17 μMO_2/min	4 μMO_2/min

and renal O_2 consumption. However, this relationship is a consequence of the associated changes in GFR and filtered sodium load, reflecting a direct causal relationship between tubular sodium reabsorption and O_2 consumption, as shown in Figure 3.1. The rate of actively transported sodium appears to be the primary determinant of the rate of O_2 consumption. About 20% of the consumption, or approximately 100 μm of O_2/min/100 g of kidney, is used for basal metabolic purposes and continues even in the absence of filtration. Above this basal rate, there is a linear relationship. Approximately 27 to 35 mEq of sodium is reabsorbed per millimole of O_2 consumed, depending on the contribution of passive transport via paracellular pathways, which may be about 40%. In contrast to other organs, the kidneys do not have a hyperemic response to hypoxia, making them more susceptible to hypoxemia.[3,4]

The balance of O_2 consumption for sodium reabsorption and O_2 delivery is reflected by the tissue pressure of O_2 (pO_2). A cortical–medullary gradient of oxygenation exists in the kidney. In the superficial cortex, pO_2 in efferent arterioles is 40 to 45 mm Hg, with values of 40 mm Hg recorded in other cortical structures (proximal tubule, distal tubule, superficial cortical tissue), and 30 mm Hg in the deep cortex. The fact that pO_2 in the renal vein exceeds that of any site in the cortex indicates precapillary shunting of O_2 from artery to vein. Renal medullary pO_2 is about 20 mm Hg, with cells functioning in a climate of constant relative hypoxia. Recent mathematical analysis indicates that arteriovenous O_2 shunting in the cortex is substantial. The shunting contributes to the stabilization of tissue pO_2 levels. Cortical ischemia may exacerbate medullary hypoxia even when medullary perfusion is maintained.[5–8]

In the renal medullary microcirculation, the net amount of O_2 reabsorbed from vasa recta into the interstitium is significantly lower than estimated medullary O_2 requirements based on active sodium reabsorption. Low inner medullary pO_2 results from the countercurrent arrangement of vasa recta and high vascular permeability to O_2, as well as high metabolic needs. Diffusional shunting of O_2 between descending and ascending vasa recta explains why a 20-mm Hg decrease in initial pO_2 at the corticomedullary border only leads to a small drop in pO_2 at the papillary tip (<2 mm Hg with baseline parameter values). In contrast, small decreases in medullary blood flow, hematocrit, and O_2 consumption by tubules markedly reduce interstitial pO_2. Without erythrocytes, papillary tip pO_2 cannot be maintained above 10 mm Hg, even when O_2 consumption is zero. An increase in medullary blood flow during water diuresis improves medullary O_2 delivery.

The renal medulla normally functions in an hypoxic environment. Tissue hypoxia impacts on O_2-regulated genes and leads to the renal production of adrenomedullin and erythropoietin. Hypoxia-inducible factor-1alpha (HIF-1α) is a transcription factor that regulates the O_2-dependent expression of many genes. This transcription factor may contribute to gene expression in renal medullary cells that

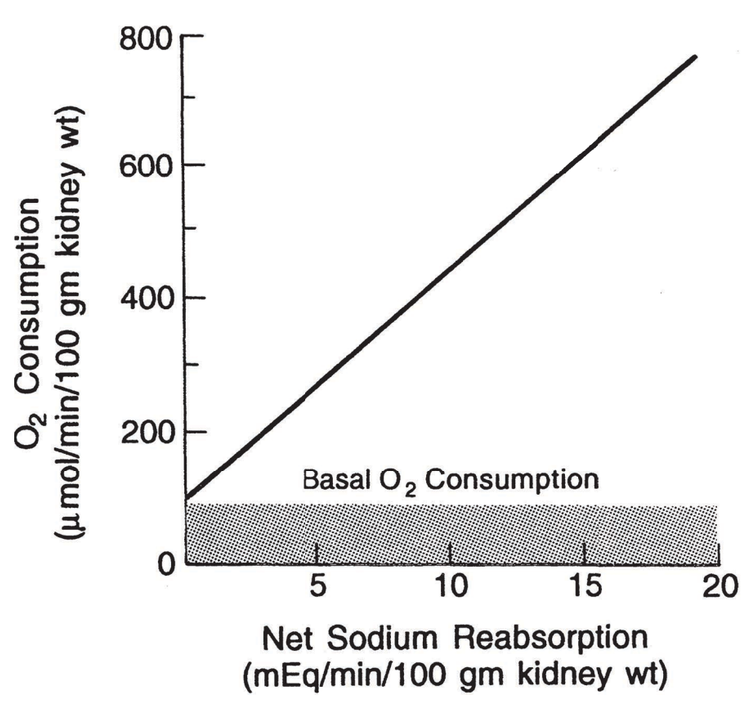

FIGURE 3.1 The relationship between tubular sodium reabsorption and oxygen consumption by the kidney. The primary determinant of oxygen consumption above basal levels is the rate of active sodium transport.

function normally under hypoxic conditions. In this regard, the loop diuretic furosemide markedly increases renal medullary pO_2 levels (20 to 50 mm Hg) in association with the inhibition of reabsorption along the ascending limb of Henle loop and a reduction in HIF-1α.[9,10]

The efficiency of coupling between tubular transport and O_2 consumption is modified by paracrine/autocrine factors. Nitric oxide (NO) normally suppresses O_2 consumption by epithelial mitochondria. The inhibition of NO synthesis increases O_2 extraction and O_2 consumption and reduces the efficiency of sodium transport. The regulation of renal O_2 consumption by NO may become impaired during oxidative stress when superoxide production is excessive. Oxidative stress and decreased availability/activity of NO can lead to reduced intrarenal pO_2 due to enhanced O_2 usage relative to tubular sodium transport. A low sodium intake leads to increased renal medullary oxygenation.[11]

Endothelial-dependent NO plays a role in the regulation of renal O_2 consumption in normal kidneys. Baseline cortical O_2 consumption is about 600 nmol per minute per gram of tissue. Stimulation of NO production by bradykinin or administration of a NO donor reduces O_2 consumption 25% to 30% in the renal cortex and 30% in the medulla. Superoxide scavenging of NO attenuates the stimulatory effect of bradykinin or NO donors.[3,5,8]

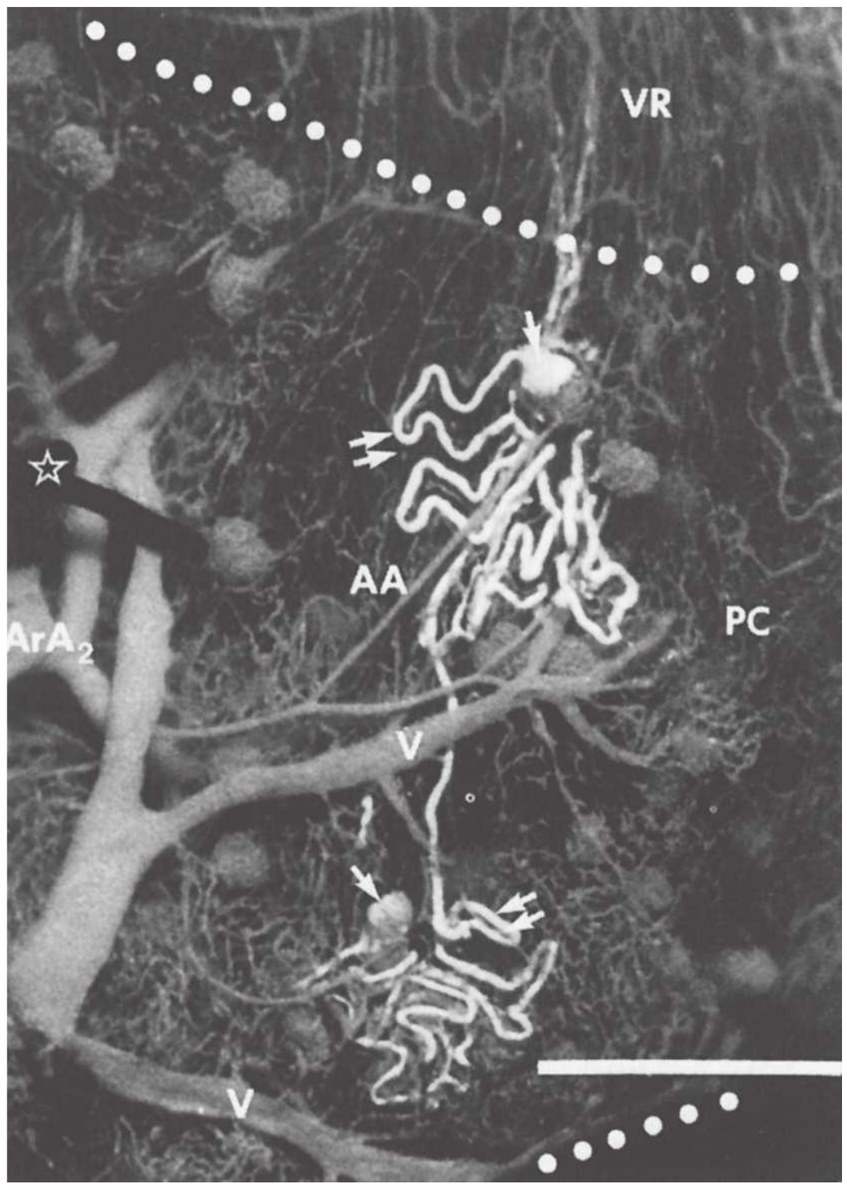

FIGURE 3.2 Renal microcirculation showing branching of afferent arterioles from arcuate arteries, glomerular capillary tufts, efferent arterioles, peritubular capillaries, some initial portions of the vasa recta, and the venous system. The vessels are filled with dark elastic polymer (Microfill), and two tubules are filled with light polymer, showing the Bowman capsule (*single arrow*) and the proximal tubules (*double arrow*) and parts of loop of Henle. *AA*, afferent arteriole; *ArA*, arcuate artery; *PC*, peritubular capillary; *V*, venule; *VR*, vasa recta. (Courtesy of Dr. Daniel Casellas.)

CHARACTERISTICS OF THE CONTRACTILE PROCESS

Structural–Functional Aspects

There are important interactions among various cell types in the microvasculature that determine the caliber of the small diameter resistance vessels. The complex vasculature of the kidney (Fig. 3.2) allows the fine regulation of the intrarenal hemodynamic environment. Smooth muscle cells surround all vascular structures from the main renal artery to the individual afferent and efferent arterioles. The preglomerular vasculature also has extensive innervation and responds to renal nerve stimulation and many different hormonal, paracrine, and physical stimuli. Nevertheless, most of the fine control of preglomerular resistance occurs in the small diameter afferent arterioles. The afferent arterioles vary in length and in the angle at which they branch from the interlobular arteries; those in juxtamedullary portions branch at a much sharper angle. In addition, smooth muscle cells of the afferent arterioles are modified as the vessels approach the glomerulus. The proximal portions of afferent arterioles possess typical elongated smooth muscle cells, whereas cells closer to the glomerulus are more rounded and many possess renin-containing granules.[1,2]

As is shown in Figure 3.3, the magnitude of the hydrostatic pressure drop along the arterial tree is relatively small up to the terminal segments of the afferent arterioles. About 70% of the preglomerular pressure drop occurs in the terminal portion of the afferent arteriole. In rodents, the arteries and the larger arterioles leading to the superficial nephrons contribute more to this pressure drop. Overall, the pressure drop up to the glomerular capillary tuft is much lower than in other vascular beds. This allows for high hydrostatic pressure in the glomerular capillaries, which is much greater than the plasma colloid osmotic pressure and is thus responsible for the ultrafiltration of fluid into the Bowman space.[1]

The terminal portion of an afferent arteriole contains modified granular epithelioid cells that form part of the juxtaglomerular apparatus. The granules contain renin and other components of the renin–angiotensin system; the extent of granulation varies inversely with sodium intake. There is a reciprocal relationship between the amount of renin and actin, and the granular cells may have reduced contractile capability. As is shown in Figure 3.4, the juxtaglomerular granular cells are adjacent to the tubular macula densa segment at the end of the ascending loop of Henle, and they are associated with the nongranular extraglomerular mesangial cells between the afferent and efferent arterioles. The appearance of the macula densa cells with large nuclei along with their close apposition to the glomerular vessels provide the

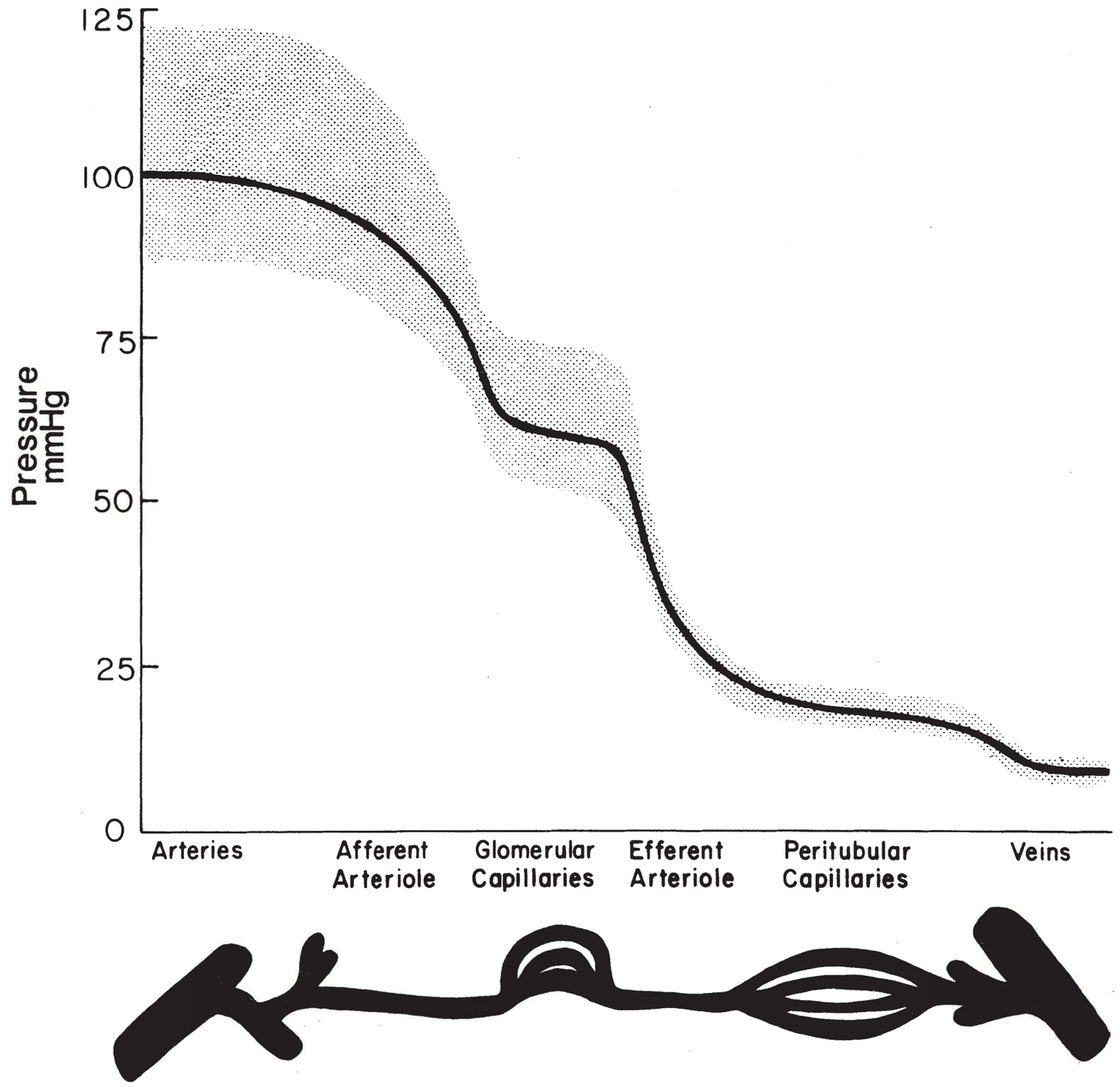

FIGURE 3.3 A representative pressure profile along the renal microvasculature in a normal kidney. The segments are depicted at the bottom of the graph, and the range of ideal pulse pressures is represented by the stippled area.

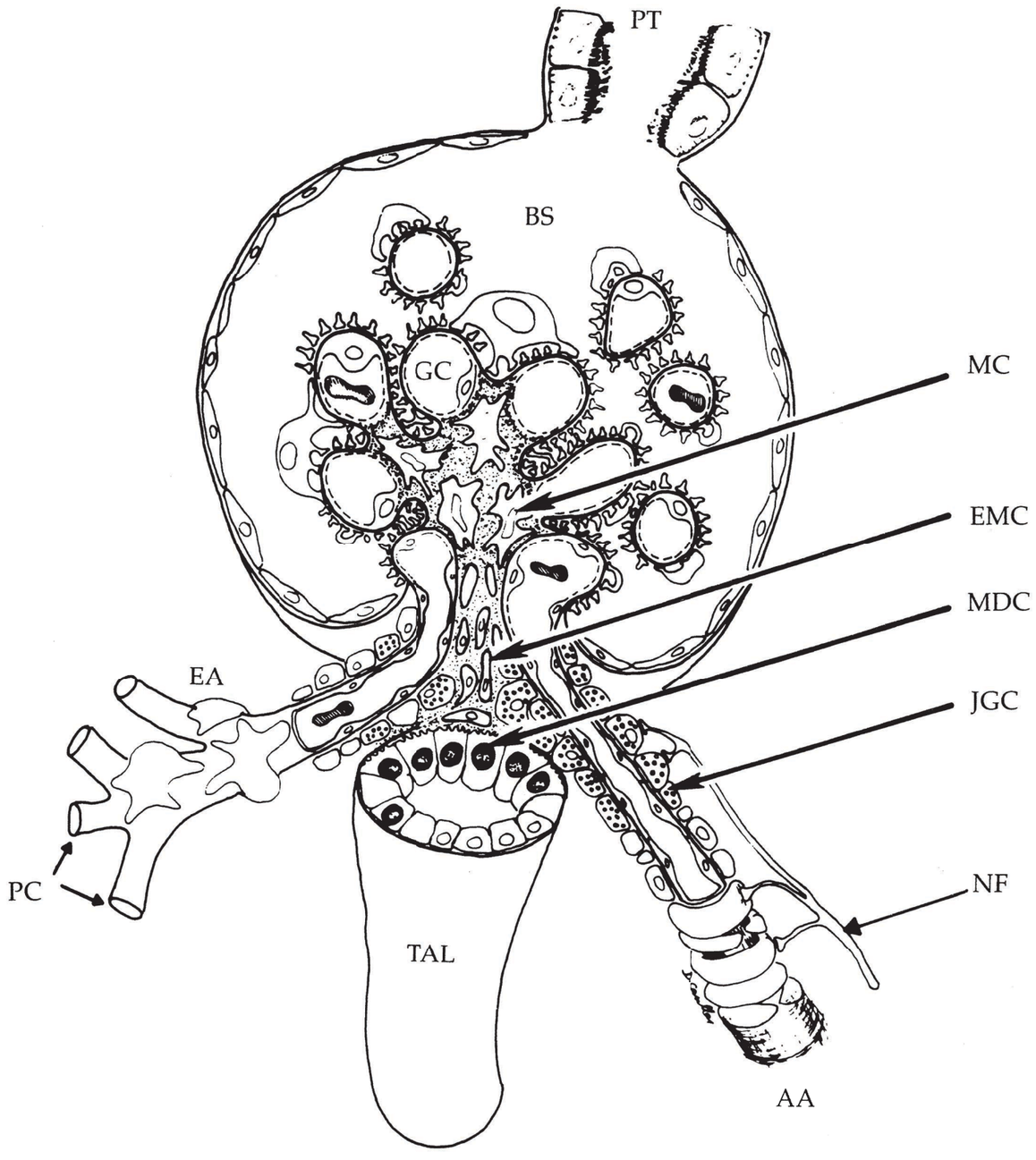

FIGURE 3.4 A drawing of the glomerulus and the juxtaglomerular complex consisting of the afferent arteriole (*AA*) with the granular cells of the juxtaglomerular apparatus (*JGC*), the extraglomerular mesangial cells (*EMC*), the macula densa (*MDC*) segment of the ascending loop of Henle, and the efferent arteriole (*EA*). Also shown are the thick ascending limb (*TAL*), the proximal tubule (*PT*), the Bowman space (*BS*), glomerular capillaries (*GC*), peritubular capillaries (*PC*), mesangial cells (*MC*), and nerve fibers (*NF*). (Drawing by Dr. Daniel Casellas.)

morphologic basis for the influence of alterations in flow or composition of the tubular fluid to generate signals that are transmitted to the afferent arteriole or juxtaglomerular cells to control vascular tone and renin release.[12–14]

As an afferent arteriole approaches a glomerulus, the muscle cells surrounding the vessel intermingle with extraglomerular and intraglomerular mesangial cells. As it enters the glomerulus, the arteriole expands into a manifold lined by endothelial cells, which, in turn, gives rise to a series of glomerular capillary loops. The loops subdivide further into a branching system of exchange channels. Finally, the channels coalesce into a small number of terminal capillaries, which join to form the efferent arteriole. Greater structural detail regarding the glomerular capillaries subserving the filtration process is provided in Chapter 1.

Efferent arterioles originate deep within the glomeruli and vary with regard to length, diameter, and density of smooth muscle cells. In the outer cortex, these vessels are relatively short, have a smaller diameter, and have a less well-developed muscular wall than efferent arterioles in deeper cortical regions. The smooth muscle cells of the superficial efferent arterioles resemble pericytes that often extend onto the peritubular capillaries. In the midcortex, the efferent arterioles are usually longer and have a greater degree of smooth muscle development. In the deeper portion of the cortex, the efferent arterioles are more variable in length. Some efferent arterioles of juxtamedullary nephrons give rise to typical cortical peritubular capillary systems, whereas others are much longer and descend toward the medulla. These two distinct capillary networks subserve the reabsorptive functions of the cortex and medulla, respectively, and may be subject to independent regulation. At the corticomedullary border, the efferent arterioles break up into vascular bundles that branch into numerous descending vasa recta. Vasa recta branch to form a capillary plexus at each level within the medulla. There are three distinct capillary plexuses, with the densest found in the inner stripe of the outer medulla. The ascending vasa recta are morphologically distinct from the descending vasa recta and ascend within vascular bundles to drain into the arcuate veins. The ascending vasa recta are more numerous and have a highly fenestrated, thin endothelium, whereas the descending vasa recta have a continuous thick endothelium. These anatomic differences suggest that the ascending vasa recta have a much greater permeability to macromolecules than the descending vasa recta.[1,2,15,16]

Each glomerular resistance segment contributes to the regulation of glomerular blood flow and pressure in a unique manner because the glomerular capillary is "nested" between the afferent and efferent arterioles. Although a more quantitative analysis of their respective roles is presented in a following section, it should be appreciated that alterations in preglomerular resistance produce changes in glomerular blood flow, pressure, and GFR, which are directionally similar. In contrast, selective changes in efferent arteriolar resistance cause more complex GFR responses because glomerular pressure and blood flow change in opposite directions. The maintenance of appropriate efferent arteriolar tone serves to keep glomerular capillary pressure sufficiently high to provide an adequate hydrostatic pressure for filtration. The efferent arterioles also are responsible for the marked decrease in pressure at the peritubular capillaries, which allows the reabsorptive force of the plasma colloid osmotic pressure to predominate.[1]

There are important regional differences in the circulation within the kidney, which have considerable functional significance. The relative distribution of glomerular and postglomerular blood flow is depicted in Figure 3.5. Glomerular blood flow is proportional to the size of the glomeruli. The larger deep juxtamedullary nephrons have higher flows than the superficial or midcortical glomeruli. These juxtamedullary glomeruli give rise to muscular efferent arterioles that descend toward the medulla and branch into the vasa recta bundles, which are intimately associated with the surrounding concentric rings of loops of Henle and collecting ducts. With regard to the postglomerular blood flow, about 75% to 85% of the total RBF is distributed to the peritubular capillaries in the cortex, whereas 15% to 25% goes to the medullary region. Blood flow throughout the cortex is much higher than in the medulla and is higher in the outer cortex than in the inner cortex. Overall cortical blood flow averages 4 to 6 mL/min/g of tissue. Medullary blood flow ranges from 2.0 to 3.5 mL/min/g in the outer medulla to much lower values of approximately 0.2 to 1.0 mL/min/g of tissue in the inner medulla and papilla. Regional mean transit times of intravascular indicators are 1 to 3 seconds for the cortex,

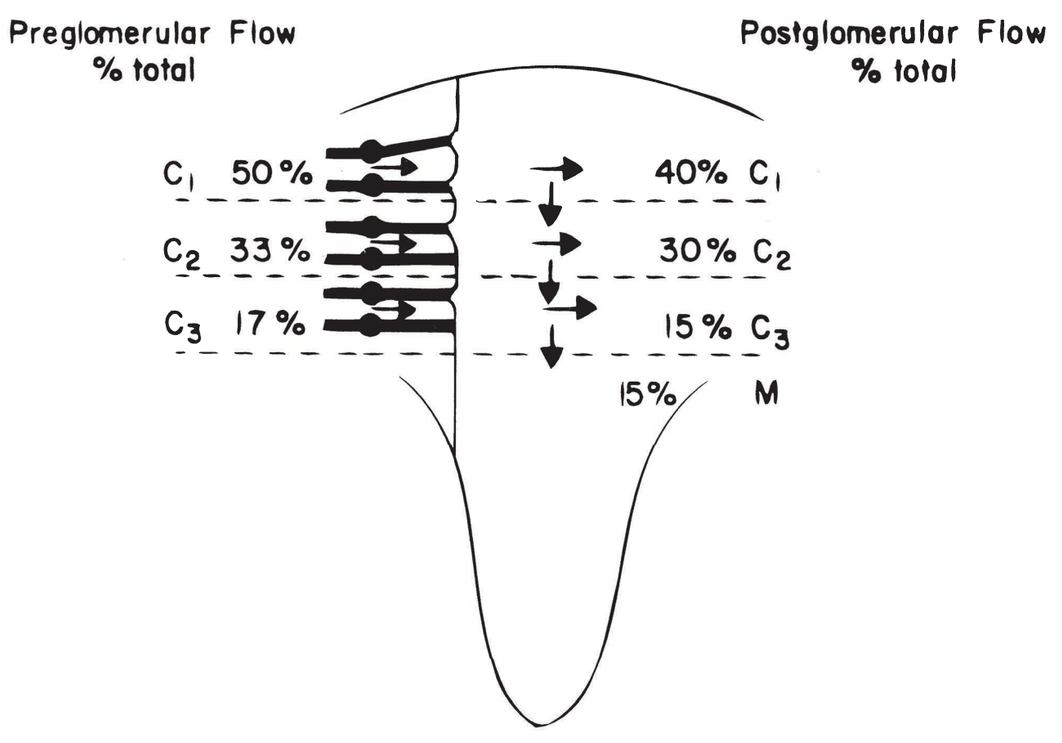

FIGURE 3.5 The distribution of glomerular and postglomerular flow and of cortical (C_1, C_2, C_3) and medullary (M) blood flow. The distribution of glomerular flow is expressed as a percentage of total blood flow; preglomerular flow is presented on the left, and postglomerular flow on the right. The deep cortical flow is subdivided to account for medullary flow distribution. As noted from the *arrows*, there is a general shift of the postglomerular blood flow toward deeper areas.

4 to 6 seconds for the outer medulla, and 10 to 30 seconds for the inner medulla.[15,17–19].

Receptor Activation and Intracellular Signaling

Contractile responses at various sites along the vascular network have different functional characteristics, depending on the expression of receptor populations and/or activation mechanisms. The actions of circulating hormones and neural stimuli combined with local paracrine factors from endothelial and epithelial cells are expressed through different effector mechanisms to provide a highly integrated regulation of the renal microcirculation and the interstitial environment. Many vasoactive agents interact with membrane receptors on the vascular smooth muscle and the endothelial and mesangial cells.

Plasma membrane surface receptors can be subdivided into three main groups in which the receptor is coupled to a guanine nucleotide binding protein (Gα protein), regulates enzyme activity, or serves as part of an ion channel. Examples of the latter two groups are the atrial natriuretic peptide (ANP) receptor, guanylate cyclase, in vascular smooth muscle, and the nicotinic-acetylcholine receptor that directly activates a cation channel at the neuromuscular junction. Almost all known vasoactive agents affect vasomotor tone via receptor coupling to G proteins. G protein-coupled receptors (GPCR) share several common structural features with seven transmembrane domains with three extracellular and three intracellular loops. The extracellular loops act in concert with the transmembrane domains to bind the agonist. The intracellular loops function to activate a G protein. G proteins are heterotrimeric proteins consisting of α, β, and γ subunits. The Gα subunit is unique for each receptor and is responsible for generating a specific intracellular signal. The β and γ subunits share a high degree of homology among G proteins; together they function to modulate the ability of the α subunit to generate the signal.

G proteins undergo a conformational change following agonist binding to a membrane receptor, which in turn enables guanosine triphosphate (GTP) to replace guanosine diphosphate (GDP) on the α subunit. The Gα-GTP complex then dissociates from the β and γ subunits and interacts with an effector such as an enzyme or a channel. The intrinsic GTPase activity of the Gα subunit then hydrolyzes GTP to GDP, and the Gα-GDP complex reassociates with the β and γ subunits, which terminates the response. G proteins linked to adenylate cyclase are classified as $G\alpha_s$ or $G\alpha_i$, depending on whether they stimulate or inhibit adenylate cyclase and cyclic adenosine monophosphate (cAMP) generation. $G\alpha_{q11/12}$ activate membrane-bound phospholipase C (PLC) or activate membrane Ca^{2+} channels, or both. An example of multiple effects an agonist can produce depending on receptor coupling to different G proteins (e.g., $G\alpha q_{11/12}$) is provided by norepinephrine. The binding of norepinephrine to an α_2-adrenoceptor inhibits adenylate cyclase, reduces the formation of cAMP, and attenuates activity of protein kinase A (PKA), whereas binding to a β_1- or β_2-adrenoceptor activates adenylate cyclase to increase cAMP/PKA signaling. Norepinephrine also binds to α_1-adrenoceptors and activates a $G\alpha_q$ protein, which is coupled to PLC, leading to the formation of inositol triphosphate (IP_3) and the release of Ca^{2+} from the sarcoplasmic reticulum. α_1-Adrenoceptors also stimulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to increase superoxide oxide production and adenosine diphosphate (ADP) ribosyl cyclase generation of cyclic ADP ribose that sensitizes ryanodine receptors to release Ca^{2+} from sarcoplasmic reticular stores.[20–24]

GPCRs and/or their immediate signaling partners (G proteins) are concentrated in caveolae, flask-shaped plasma membrane invaginations (50 to 100 nm in diameter) that are subcellular microdomains of lipid rafts and caveolae, enriched in glycosphingolipids and cholesterol and the protein caveolin. Caveolae are complexes with a high concentration of signaling molecules. Caveolin is a scaffolding protein that anchors receptors (e.g., angiotensin II subtype 1 [AT_1], endothelin [ET-1], epidermal growth factor [EGF]) signaling and trafficking proteins ($G\alpha_{q/11}$) as well as ion channels (K^+ and transient receptor potential [TRP] channels) and enzymes (endothelial nitric oxide synthase [eNOS], protein kinase C [PKC], phospholipase C [PLC], NADPH oxidase, ADP ribosyl cyclase) involved in controlling vasomotor tone. Endothelial cells and fibroblasts are rich in caveolins 1 and 2; smooth muscle cells express all three caveolins (Cav-1, -2, and -3). In vascular smooth muscle cells, Cav-1 serves as a scaffold or chaperone to target AT_1 receptors to caveolae to activate downstream signaling; Cav-1 is required for efficient coupling of $G\alpha_{q/ll}$ proteins and PLC_β to promote Ca^{2+} mobilization from the sarcoplasmic reticulum. Cav-1 links AT_1 receptors to NADPH oxidases and promotes recruitment of Rac1 to activate reactive oxygen species (ROS) formation. Cav-1 couples IP_3 receptors and TRPC3 channels for store-operated Ca^{2+} entry. It also suppresses K_{ATP} channel activity and negatively regulates Ang II–induced EGF receptor transactivation. Most caveolae in vascular smooth muscle cells have nanocontacts with the sarcoplasmic reticulum, providing a direct link to intracellular Ca^{2+} mobilization and excitation–contraction coupling.

In endothelial cells, Cav-1 binds eNOS, limiting the translocation and activation of eNOS. Caveolin-deficient animals exhibit unusual endothelial dysfunction in that NO production is unopposed, leading to marked vasodilation. Cav-1 knockout animals display attenuated vasoconstriction to phenylephrine and a lack of myogenic tone, largely due to excessive NO production. Mice lacking caveolin show that caveolae and caveolins play a prominent role in various pathophysiologic conditions, especially those related to the cardiovascular system. These disease phenotypes include atherosclerosis, cardiac hypertrophy, cardiomyopathy, diabetes, and neointimal hyperplasia (smooth muscle cell proliferation).[25–28]

GPCR desensitization reduces receptor downstream signaling and effector response. G-protein coupled receptor kinases (GRKs), a family of serine/threonine protein

kinases, initiate receptor-specific, homologous desensitization that curtails receptor signaling by phosphorylating the C-terminal tail of agonist-bound GPCR to promote docking with inhibitory β-arrestins. β-Arrestins not only uncouple receptors from heterotrimeric G-proteins, they also target GPCRs to clathrin-coated vesicles for internalization. Endocytosed receptors are either resensitized and recycled to the plasma membrane or degraded in lysosomes. GPCRs internalize as a stable complex with β-arrestin with signaling potential and gene transcription.[22,29–31]

Although GRKs regulate GPCR activity, regulators of G protein signaling (RGS) proteins directly control the activity of Gα-protein subunits, functioning as endogenous negative regulators of GPCR signaling by accelerating GTP hydrolysis by Gα-subunits and thereby attenuating signaling. The most well-characterized RGS proteins (e.g., RGS2, PGS4, PGS5 of the R4 family) determine signaling specificity of $G\alpha_q$- and $G\alpha_i$-coupled receptors. RGS2 is a GTPase activating protein that binds to both $G\alpha_{q/11}$ and $G\alpha_i$ subunits of GPCRs to accelerate their intrinsic GTPase activity. As a result, they are deactivated with a reduced stimulation of PLC_β-mediated Ca^{2+} release and subsequent vasoconstriction. RGS2 and/or RGS5 reduce Ca^{2+} signaling initiated by AT_1 and ET-1 and α1-adrenoceptors and subsequent contraction. RGS5 mRNA, a regulator of vascular remodeling, is expressed in medial smooth muscle of afferent arterioles and the main renal artery in nonhuman primates. RGS proteins may play a role in mechanosensation and stretch-induced myogenic responses, as well as coordinating actions of vasoconstrictor hormones and paracrine agents.

RGS2 activity is acutely stimulated by NO and cGMP signaling to promote vascular relaxation by attenuating Ca^{2+} signaling in response to vasoconstrictor agents. RGS2 expression in vascular smooth muscle is upregulated by Ang II and is downregulated in hypertension. RGS2 knockout mice are hypertensive with enhanced vasoconstriction produced by Ang II and α1-adrenoceptor agonists. The exaggerated $G\alpha_{q/11}$ signaling in mutant animals is associated with renovascular abnormalities, exaggerated vasoconstriction, hypertension, thickening of the vascular wall of the aorta and renal interlobular arteries, and cardiac hypertrophy. Kidney cross-transplantation studies demonstrate that a specific loss of RGS2 in the kidney causes hypertension, whereas the absence of RGS2 from all extrarenal tissues, including the peripheral vasculature, does not affect arterial pressure. Isolated perfused kidneys of RGS4 knockout mice show increased renal vasoconstrictor responses to ET-1.[21,32–34]

Regulation of Microvascular Contractility

Changes in vascular perfusion are mediated by smooth muscle cell contraction or relaxation that elicits a change in vessel radius and in vascular resistance. Multiple steps and enzymatic cascades are involved in the contractile process, and many of these mechanisms interact with one another to modulate the contractile response. A pivotal step in mediating the contractile response in vascular smooth muscle cells is an increase in the cytosolic concentration of free ionized calcium ($[Ca^{2+}]_i$) above its very low basal value of 10^{-7} M. (This is approximately 0.01% of the ionic Ca^{2+} levels of plasma and extracellular fluid, 1 mM). As shown in Figure 3.5, cytosolic Ca^{2+} binds with calmodulin. The Ca^{2+}-calmodulin complex activates myosin light chain (MLC) kinase, leading to the phosphorylation of MLC that interacts with actin and adenosine triphosphate (ATP) to elicit tension development. Increased $[Ca^{2+}]_i$ also phosphorylates CPI-17, a phosphoprotein that inhibits MLC. GPCR activation of PKC enhances Ca^{2+} sensitivity of the contractile apparatus by potentiating the efficiency of Ca^{2+} stimulation of CPI-17. In addition, GPCR couples to the $G\alpha_{q/11}$ signal through the monomeric GTPase RhoA/Rho kinase pathway to decrease the activity of MLC phosphatase, resulting in increased Ca^{2+} sensitivity of myofilaments and enhanced vasoconstriction for a given level of cytosolic Ca^{2+} concentration. Relaxation occurs as a consequence of the removal or sequestration of Ca^{2+} from the cytosol and/or MLC dephosphorylation. Decreases in Ca^{2+} signaling occur following dissociation of ligand from GPCR surface receptor, receptor inactivation, or internalization.[2,35]

Various hormones and drugs activate plasma membrane receptors to induce contraction by eliciting an increase in $[Ca^{2+}]_i$. Cytosolic Ca^{2+} is increased through a combination of Ca^{2+} entry from the extracellular environment and mobilization of Ca^{2+} from internal stores. Ca^{2+} can be released from intracellular stores via release channels, activated by IP_3 or by a ryanodine (Ry)-like ligand. Ca^{2+} is released from a common sarcoplasmic reticular pool, with uptake mediated by a common sarcoplasmic–endoplasmic reticular Ca^{2+} ATPase (SERCA). Many vasoconstrictor agents increase intracellular Ca^{2+} by activating a Gα protein that stimulates PLC to break down membrane-bound phosphatidylinositol 4,5-diphosphate (PIP_2) into IP_3 and 1,2-diacylglycerol (DAG). The soluble IP_3 binds to an IP_3 receptor located on the sarcoplasmic reticulum, leading to the activation of Ca^{2+} channels and the release of Ca^{2+} into the cytoplasm. The lipophilic DAG remains within the membrane environment and activates PKC isoform that phosphorylates various regulatory proteins. DAG may also be generated by the actions of phospholipase D on phosphatidylcholine that is not accompanied by concurrent IP_3 generation and the associated increase in $[Ca^{2+}]_i$. Agents that activate PKC, such as phorbol esters, induce slowly developing, sustained contractions or enhance contractile responsiveness to other stimuli.[2,36]

Activation of cell surface GPCRs also leads to the stimulation of plasma membrane-bound ADP-ribosyl cyclase that converts the substrate nicotinamide adenine dinucleotide (NAD+) to adenosine 5′-cyclic diphosphate (cADP)-ribose, a potent Ca^{2+} mobilizing agent that acts on a RyR to trigger Ca^{2+} release from the sarcoplasmic reticulum. cADP-ribose is a calmodulin-dependent Ca^{2+}-mobilizing second messenger system that acts independently of IP_3, with RyR-mediated Ca^{2+} release to amplify IP_3-mediated

Ca^{2+} mobilization. Ryanodine receptors are extremely sensitive to $[Ca^{2+}]_i$ and exhibit Ca^{2+}-induced Ca^{2+} release (CICR), a form of autopotentiation. Concentrations of Ca^{2+} over a wide range (5 to 100 μM) enhance the open probability of RyR, which contrasts with a narrower range (180 to 220 nM) for the IP_3R.[20]

Calcium entry occurs through a variety of pathways, including voltage-operated channels (VOCs) that are activated upon membrane depolarization and voltage-independent receptor-operated channels (ROCs) and store-operated channels (SOCs). Membrane depolarization downstream of GPCR activation results from the activation of Cl^- channels or the inactivation of K^+ channels. Voltage-independent ROCs and SOCs also contribute to agonist-induced Ca^{2+} entry in both preglomerular and efferent arterioles. ROCs are activated downstream of cell surface GPCR, independent of Ca^{2+} mobilization, via signaling mechanisms involving DAG and calmodulin, and other possible intermediates. SOCs are known to contribute to agonist-induced Ca^{2+} entry in renal vascular smooth muscle cells; they are also termed capacitative as they respond to depletion of Ca^{2+} stores.[37,38]

Among the identified families of TRP proteins, the canonical TRP (TRPC) family (TRPC1 through TRPC7) is thought to be involved in Ca^{2+} entry in vascular smooth muscle cells, most likely functioning as voltage-independent SOCs and/or ROCs. TRPC1 proteins in vascular smooth muscle cells form tetrameric channels in association with TRPC4 or TRPC5. TRPC3, -6, and -7 are activated by DAG and thus are attractive possibilities as ROCs. TRPC6 is an essential component of α1-adrenoceptor–activated cation channels in portal venous smooth muscle cells. Preglomerular arterioles have a predominance of TRPC3 and -6 subunits that may comprise SOCs and/or ROCs in these vessels. Evidence to support TRPC channel function is attenuation of Ca^{2+} responses of the afferent arteriole to norepinephrine by the blockers of voltage-insensitive Ca^{2+} entry Gd^{3+} and SKF 96365. Activation of TRPC6 in the afferent arteriole leads to increased $[Ca^{2+}]_i$. The Ca^{2+} permeable TRPC6 is a key signaling component in a functional slit diaphragm formed by podocytes in the glomerular filtration barrier. Gain-of-function mutations in TRPC6 are the cause for progressive kidney failure with urinary protein loss and focal segmental glomerular sclerosis. SOCs are activated by Ca^{2+} mobilization to specifically restore Ca^{2+} content in sarcoplasmic reticulum. Current interest focuses on stromal interaction molecules (STIM) in the sarcoplasmic reticulum, which sense SR Ca^{2+} depletion and then translocate to junctions near the plasma membrane to organize Orai proteins, the Ca^{2+} release-activated Ca^{2+} channels, to form a pore and increase Ca^{2+} entry.[37,39–47]

Na^+/Ca^{2+} exchange (NCX) contributes to the regulation of $[Ca^{2+}]_i$, working reversibly in the exit mode to extrude Ca^{2+} or in the entry mode to facilitate Ca^{2+} entry. Exchanger activity is normally higher in afferent then in efferent arterioles. Ang II stimulates $[Ca^{2+}]_i$ in afferent arterioles by activating NCX to promote Ca^{2+} entry. Consistent with this observation, genetic deletion of Na^+/Ca^{2+} exchanger NCX1 in smooth muscle cells reduces Ang II–induced renal vasoconstriction in vivo, presumably due to less Ca^{2+} entry into smooth muscle cells. Nevertheless, the pharmacologic blockade of NCX or lowering extracellular Na^+ concentration causes renal vasoconstriction and exaggerated Ang II–induced constriction of the isolated perfused kidney. Smooth muscle Na^+/Ca^{2+} activity responds to Na^+/K^+-ATPase inhibition by an endogenous ouabainlike glycoside to extrude the high cellular Na^+ in exchange for Ca^{2+} entry in hypertensive states.[43,48–50]

Several different K^+ channels have been identified; the most prominent are Ca^{2+}-activated and ATP-dependent K^+ channels, which mediate relaxation by hyperpolarizing the cell membrane and reducing Ca^{2+} entry through voltage-gated Ca^{2+} channels. Importantly, increases in intracellular ATP inhibit K^+ channels, thus causing depolarization. Channel activity is reduced by intracellular ATP concentrations normally present, suggesting that the activity of this channel is quite low in normal cells and may serve a primary protective role during the depletion of energy reserves.[2,51–54]

At the whole kidney level, Rho-kinase inhibition dilates the renal vasculature under basal conditions and attenuates renal vasoconstriction produced by intrarenal infusion of Ang II, arginine vasopressin (AVP), or norepinephrine, as well as increased renal perfusion pressure. Frequency analysis of renal vascular admittance indicates that Rho-kinase strengthens the myogenic response. Ca^{2+} sensitivity of the afferent arteriole is increased by adenosine and norepinephrine. Adenosine increases Ca^{2+} sensitivity by PKC, Rho-kinase, and p38 mitogen activated protein (MAP) kinase signaling pathways. This provides a mechanism by which different vasoactive agents can modulate reactivity to other stimuli. Interestingly, adenosine enhances Ang II–induced afferent arteriolar contraction but does not potentiate the contractile responses to ET-1 or norepinephrine. Reactivity to Ang II is enhanced by the ability of norepinephrine to increase Ca^{2+} sensitivity with increased MLC phosphorylation.

Rho-kinase participates in pressure-induced interlobular arterial and afferent arteriolar myogenic behavior and vasoconstrictor responses evoked by Ang II, adenosine A_1, endothelin ET_B, and purinergic P_2X_1 receptor activation as well as membrane depolarization. Ca^{2+} sensitization due to Rho-kinase also contributes to Ang II–induced constriction of efferent arterioles. Rho-kinase inhibition dilates preglomerular and postglomerular arterioles and attenuates vasoconstriction elicited by adenosine A_1 or endothelin ET_B receptor stimulation. Ang II, AVP, and TxA_2 constrict preglomerular arcuate and interlobular arteries in the hydronephrotic kidney in part via Rho-kinase. The afferent arteriolar myogenic response in this preparation is markedly attenuated by Rho-kinase inhibition.[55–59]

As mentioned earlier, cAMP also activates Ca^{2+} translocation mechanisms that increase extrusion of Ca^{2+} out of the cell, return Ca^{2+} to the sarcoplasmic reticulum, or inhibit IP_3-mediated mobilization of Ca^{2+} from sarcoplasmic

Agents that increase cytosolic calcium:

Ang II, AVP, Epinephrine (α), TXA_2, Leukotrienes, Norepinephrine, Endothelin, ATP

Agents that increase cAMP (or cGMP):

Epinephrine (β), PTH, PGI_2, PGE_2, ANP, Dopamine, Bradykinin, Nitric Oxide, Adenosine (A_2)

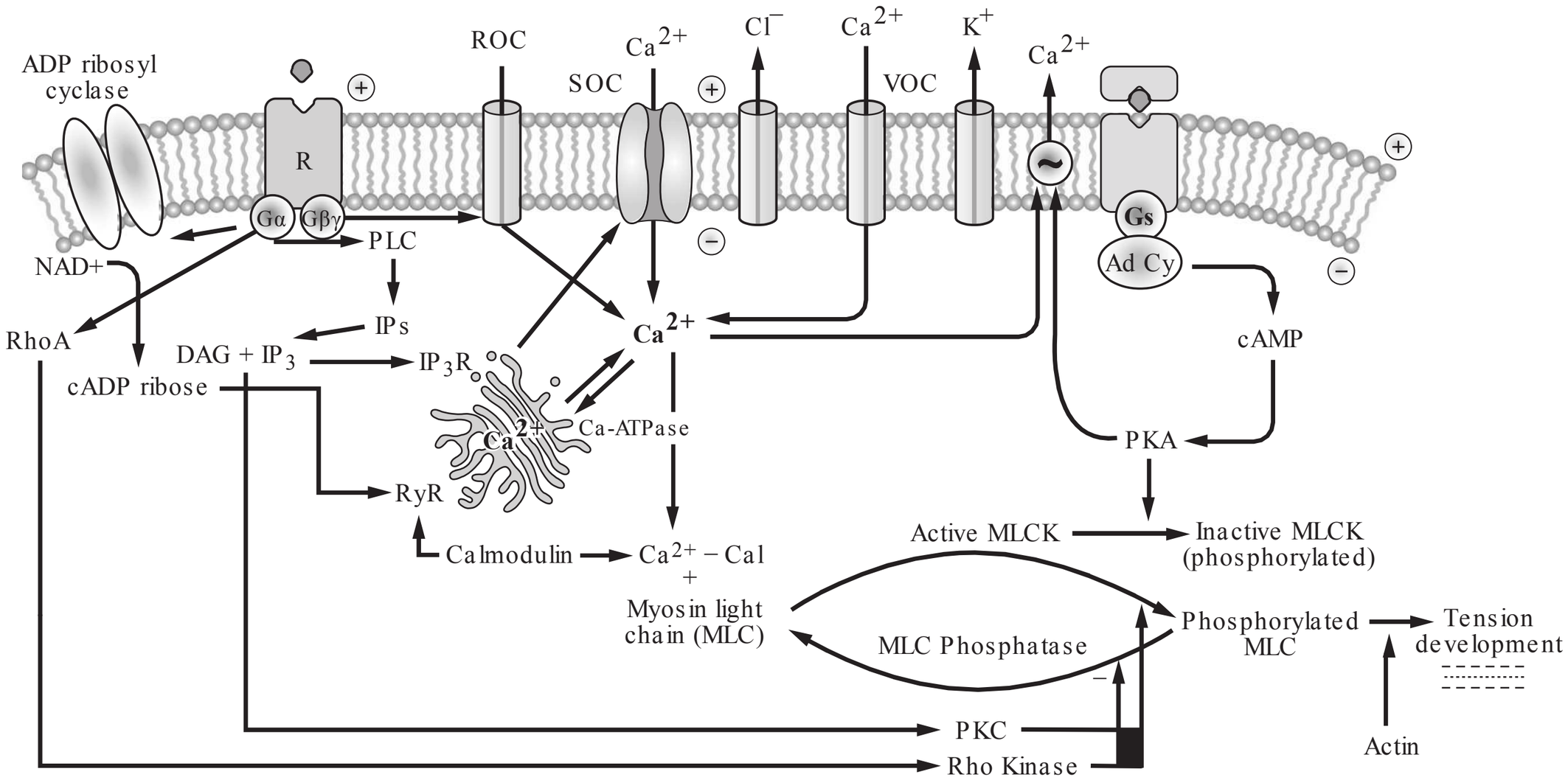

FIGURE 3.6 Primary intracellular signaling systems mediating smooth muscle cell or mesangial cell contraction, with effects of various hormones and vasoactive agents on the two major types of receptor systems. For ease of presentation, only two receptor mechanisms are depicted, but each agent acts on its own receptor system.

reticulum stores (Fig. 3.6). Drugs or agents such as PGE_2 and PGI_2 that increase cAMP via $G\alpha_s$ signaling produce increases in RBF and GFR. Small amounts of such ligands can buffer the action of vasoconstrictor agents without affecting baseline vascular tone. In contrast, ligand-receptor complexes coupled to the inhibitory G protein ($G\alpha_i$) reduce cAMP levels and cause greater contraction for a given level of $[Ca^{2+}]_i$. $G\alpha_i$ proteins are reported to activate PLC and mobilize Ca^{2+} in the afferent arteriole.[60,61]

Another family of receptors operates through the G protein-dependent activation of guanylate cyclase, the generation of cGMP, and the activation of protein kinase C to mediate vasodilation. In addition, two major guanylate cyclase activators are not G protein-dependent. NO derived from endothelial cells directly interacts with soluble guanylate cyclase. Also, ANP directly activates particulate guanylate cyclase in vascular smooth muscle cells. The mechanisms mediating cGMP-dependent vasorelaxation are similar to those used by cAMP. cGMP-dependent kinases lead to the inhibition of voltage-gated L-type Ca^{2+} channels, activation of a Na^+/Ca^{2+} exchanger, stimulation of Ca^{2+}-ATPase, inhibition of IP_3 formation, and phosphorylation of phospholamban, resulting in increased Ca^{2+}-ATPase activity in the sarcoplasmic reticulum. Ca^{2+} desensitization of the contractile machinery, independent of $[Ca^{2+}]_i$ changes, takes place because cGMP/PKG can block RhoA activation and can reduce MLC activity by inhibiting CPI-17 phosphorylation and activating MLC phosphatase. cGMP also may stimulate Ca^{2+}-activated K^+ channels, which leads to hyperpolarization.[33,62]

The arachidonic acid pathways constitute another intracellular signaling system. Increased $[Ca^{2+}]_i$ can activate phospholipase A_2 and can release arachidonic acid from membrane phospholipids, resulting in the production of various metabolites that lead to vasodilation or vasoconstriction. Arachidonic acid metabolites exert effects through multiple pathways, including cAMP, cytosolic Ca^{2+}, and inhibition of K^+ channels. Arachidonic acid itself may increase Ca^{2+} entry via a noncapacitative Ca^{2+} entry channel. These actions will be discussed in detail later in this chapter.

Differences in cellular sites and mechanisms of smooth muscle activation may be partially responsible for the large variety of renal hemodynamic responses produced by different vasoactive agents. There is a major difference in the mechanisms leading to Ca^{2+} activation in the vascular smooth muscle cells of the afferent and efferent arterioles. Preglomerular vessels have a strong dependence on L-type voltage-gated Ca^{2+} channels, whereas their influence is not readily apparent in efferent arterioles. Antagonists of Ca^{2+} influx through L-type, dihydropyridine-sensitive Ca^{2+} channels selectively block agonist-induced constriction of

the preglomerular arterioles, including the afferent arteriole, without affecting efferent arteriolar contraction. This is the case for Ang II, ET-1, norepinephrine, and potassium chloride–induced depolarization. Agents that block L-type Ca^{2+} channels such as nifedipine, diltiazem, and verapamil primarily cause afferent vasodilation and impair autoregulatory responses to changes in renal perfusion pressure, affecting both myogenic and tubuloglomerular feedback (TGF responses). In contrast, T-type Ca^{2+} channels are active at both afferent and efferent arteriolar sites and influence vascular responsiveness. T-type Ca^{2+} channel blockers vasodilate afferent and efferent arterioles and prevent contractile responses to various stimuli at both sites. In afferent arterioles, T-type channels may act cooperatively with L-type channels to bring about membrane depolarization and Ca^{2+} entry. A primary action on the preglomerular vasculature also explains the increases in GFR and glomerular capillary pressure produced by Ca^{2+} entry blockers as well as inhibition of the TGF system. An example of a hormone that exerts its effects through different mechanisms is Ang II. Its effects are mediated by at least two mechanisms. Afferent arteriolar responses are highly dependent on Ca^{2+} entry via L-type channels, whereas the efferent arteriolar response is influenced by Ca^{2+} mobilization and Ca^{2+} entry via T-type channels and through store-operated channels in the absence of any entry through voltage-gated L-type channels. NO may suppress voltage-sensitive Ca^{2+} entry in both afferent and efferent arterioles. Ca^{2+} entry in outer medullary vasa recta is mediated by a combination of L- and T-type Ca^{2+} channels as well as store-operated cation channels.[63–67]

In addition to the smooth muscle cells of the resistance vessels, the mesangial cells within the glomerular tufts possess contractile capability, which may contribute not only to the regulation of blood flow through the glomerulus but also to the filtering capacity. Mesangial contraction is postulated to reduce the glomerular filtration coefficient (K_f) by decreasing the radius of capillaries or the surface area available for filtration, or both, but the precise mechanism is not clear. Many agents, including Ang II and vasopressin, reduce K_f. Low K_f values have also been observed during sodium depletion when plasma and local concentrations of endogenous Ang II are elevated. These responses reflect specific receptor-mediated effects on mesangial cells, because the response can be reversed by selective receptor antagonists in vivo and in cultured mesangial cells. However, podocytes are closely associated with mesangial cells in vivo and also respond to Ang II, suggesting that part of the actions of Ang II on K_f could be due to responses by podocytes. Some mesangial cell receptors exert stabilizing effects on mesangial cell contraction and counteract the influence of excessive levels of vasoconstrictor agents or serve metabolic functions. β-Adrenergic agonists and vasodilator prostaglandins (PGE_2 and PGI_2) increase cAMP in isolated glomeruli and mesangial cells and they directly oppose the apparent contractile effects of Ang II. Nevertheless, the structural mechanism by which mesangial cell contraction actually alters K_f remains unclear.[2,68–70]

Endothelial Interactions with Vascular Smooth Muscle

The vasculature is lined with a continuous layer of endothelial cells, which has many functions including serving as a diffusion barrier and preventing vascular thrombosis. Endothelial cells are dynamic metabolic units having membrane receptors and membrane-bound enzymes, which allow them to respond to and contribute to changes in the concentration of humoral agents. Membrane-bound ectoenzymes form or degrade, circulating vasoactive substances such as Ang II (angiotensin converting enzyme [ACE]), ET-1 (endothelin converting enzyme and metallopeptidase), bradykinin (kininase II), and adenonucleotides (three ectonucleotidases convert ATP, ADP, and AMP). Localization of ACE in preglomerular vessels allows the conversion of systemically delivered Ang I, an inactive decapeptide, to the biologically active octapeptide Ang II, that can then induce vasoconstriction locally or in downstream segments.[2]

The vascular endothelium serves an important paracrine role (Fig. 3.7). Endothelial cells participate in contractile and dilator mechanisms by responding to a variety of stimuli and increasing or decreasing formation of potent vasoactive substances that act locally to modulate the tone of adjacent smooth muscle cells. General classes consist of endothelium-derived relaxing factors (EDRF) and endothelium-derived contracting factors (EDCF). Specific examples of relaxing factors that cause renal vasodilation are NO, PGE_2, and PGI_2 (prostacyclin), carbon monoxide (CO), and an epoxyeicosatrienoic acid (EET), a hyperpolarizing factor that is a metabolite of the cytochrome P450 pathway. Examples of EDCF include Ang II, ET-1, thromboxane (TxA_2), and oxygen-free radicals. These paracrine factors act on smooth muscle cells to modify vasomotor tone, proliferative state, and provide a balance between antioxidant defense mechanisms and excess generation of O_2-derived free radicals. Thus, the endothelial cells are intimately involved in controlling the renal microcirculation.[71,72]

One of the most studied interactions between endothelial cells and smooth muscle cells involves the ability of the endothelium to modify the vascular responses to acetylcholine and other agents. Acetylcholine is a powerful vasodilator in vivo and also in isolated smooth muscle preparations that have an intact endothelium because it stimulates endothelial cells to produce vasodilatory paracrine substances. However, when applied to vascular preparations whose endothelium has been removed, acetylcholine induces vasoconstriction by acting directly on muscarinic receptors on smooth muscle cells. Many other substances have now been shown to stimulate the release of endothelium-derived vasoactive factors.

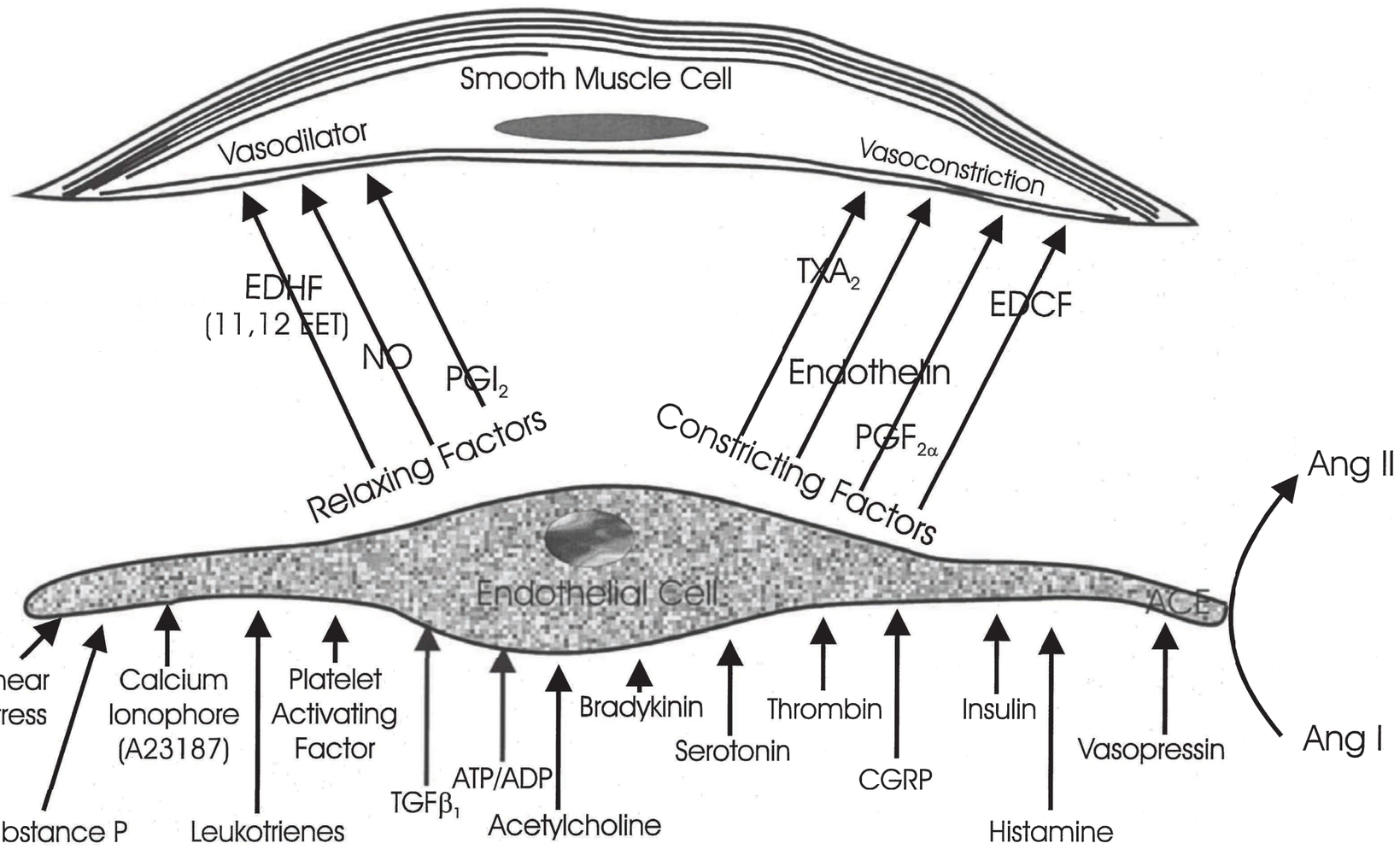

FIGURE 3.7 The interaction of endothelial cells with smooth muscle or mesangial cells. Agents that are known to influence endothelial-derived relaxing factors or nitric oxide (*NO*) and endothelial-derived constricting factors (EDCF) production by endothelial cells are shown. Endothelial cells also produce several vasoconstrictor and vasodilator agents, as shown in the figure and described in the text.

One of the major relaxing factors is NO derived from L-arginine. These paracrine systems will be discussed in later sections.[2,73,74]

Endothelial cells have a remarkable capability to transport substances across their layers through a variety of mechanisms. One of the most impressive features of endothelial cells lining the vasculature is their ability to form fenestrations that serve as extracellular channels. This feature occurs predominantly in capillary structures having large rates of transcapillary volume flux. Both glomerular and peritubular capillary systems have fenestrations. The glomerular capillaries have abundant, well-rounded fenestrations that are 50 to 100 nm in diameter and lack a diaphragm. These fenestrations constitute highly permeable pathways for the large volume of plasma filtrate that continuously traverses from the glomerular capillaries into the Bowman space. Although it is not clear whether subtle changes in the size of the fenestrations contribute to the regulation of the hydraulic conductivity of the glomerular capillary barrier, it is apparent that their integrity is essential for the maintenance of glomerular filtration.[2,75]

The fenestrations of the peritubular capillaries are bridged by a thin diaphragm and are smaller in diameter (20 nm). Considering the total number of capillaries, there is much more peritubular than glomerular capillary surface area. However, because the overall reabsorptive rate by the peritubular capillaries is nearly equal to the GFR, the average hydraulic conductivity of the peritubular capillaries per unit of surface area is estimated to be less than that of glomerular capillaries. Fenestrations also exist in the terminal segments of afferent arterioles, and they may provide a pathway for renin entry into the circulation from juxtaglomerular granular cells.[2,76]

TRANSCAPILLARY EXCHANGE IN RENAL MICROCIRCULATION

Forces Governing Ultrafiltration at the Glomerulus

Bulk movement of fluid across capillary membranes of the renal microcirculation is passive in nature, driven by physical forces. As blood flows from the afferent arterioles into the glomerular capillary tufts, the high hydrostatic pressure predominates over the counteracting forces caused by Bowman space hydrostatic pressure and plasma colloid osmotic pressure. Therefore, fluid is driven from the glomerular capillaries through the endothelial fenestrations, across the basement membrane, and between the podocyte foot processes into the Bowman space. This movement of fluid can be described quantitatively by the Starling filtration-reabsorption principle, which is based on the premises that (1) water and solutes flow through extracellular channels or pathways and (2) the diameters of these channels are large with respect to water molecules, hydrated ions, and solutes of low-molecular weight, such as urea, glucose, and amino acids. Thus, except for the larger solutes, mainly plasma proteins that approach or exceed the size of the channels, the filtrate is translocated without substantive compositional alterations. Detailed consideration of the structure and biochemical composition of the glomerular filtration barrier is provided in Chapter 1.

The physical forces acting across the glomerular membrane are glomerular capillary pressure (P_g), Bowman space

pressure (P_B), glomerular plasma colloid osmotic pressure (π_g), and colloid osmotic pressure of filtrate in the Bowman space (π_B). The filtering capacity of the filtration barrier is expressed as the glomerular filtration coefficient (K_f), which is the product of the hydraulic conductivity of the glomerular membrane (L_p) and the total filtering surface area (S_f). Because the net forces change as fluid is filtered along the length of the glomerular capillaries, total GFR can be expressed by the equation:

$$GFR = K_f \int_0^1 [(P_{g(x)} - P_B) - \sigma (\pi_{g(x)} - \pi_B)]\, dx \qquad (1)$$

where x represents the normalized length of the glomerular capillaries, with 0 designating the afferent end and 1 designating the efferent end; σ (sigma) is the reflection coefficient, which has a range of 0 to 1. When sigma is 1, proteins are completely "reflected" by the capillary wall, and the colloid osmotic pressure is maximally effective. Normal glomerular capillaries are extremely efficient in restricting the passage of macromolecules, and the amount of protein present in the normal filtrate in the Bowman space is less than 0.1% of the plasma protein. For practical considerations, the effective colloid osmotic pressure is considered equivalent to that of the plasma in the glomerular capillaries (π_g). As is shown in Figure 3.8, this value increases progressively along the length of the capillaries as a function of the relative volume of protein-free fluid that is filtered. Because colloid osmotic pressure is the major force retarding glomerular filtration, filtration is greatest in the initial segments of the glomerular capillaries and decreases progressively along the length of the capillaries.[1,2,77]

The exact hydrostatic pressure drop along the glomerular capillaries is uncertain because experimental assessment is not possible. Nevertheless, there are abundant parallel capillaries that collectively have a large cross-sectional area relative to that of the afferent and efferent arterioles; thus, the hydrostatic pressure drop along the glomerular capillaries is small as compared with the pressure drops across the afferent and efferent arterioles. Computations based on the number and dimensions of the glomerular capillaries yield estimates that are in the range of 1 to 4 mm Hg. Thus, P_g is usually treated as a constant value. With these simplifying assumptions and the use of average values for hydrostatic and colloid osmotic pressures in glomerular capillaries, the more commonly used formulation for GFR results:

$$GFR = K_f (P_g - P_B - \pi_g) \qquad (2)$$

The net, or mean, effective filtration pressure (EFP) is calculated as

$$EFP = P_g - P_B - \pi_g \qquad (3)$$

The increase in plasma protein concentration is a direct function of the filtration fraction, defined as the quotient of GFR and renal plasma flow. Because of the nonlinear relationship between plasma protein concentration and colloid osmotic pressure, the rate of increase in colloid osmotic pressure from the afferent to the efferent arteriole increases progressively (Fig. 3.9). Empirically derived relationships allow the prediction of colloid osmotic pressure (π) from the total plasma protein concentration (C) when the albumin-to-globulin (A/G) ratio is known. The commonly used Landis-Pappenheimer relationship

$$\pi = 2.1\, C + 0.16\, C^2 + 0.009\, C^3 \qquad (4)$$

applies to an A/G ratio of about 1.2, which is considered normal for humans.[2]

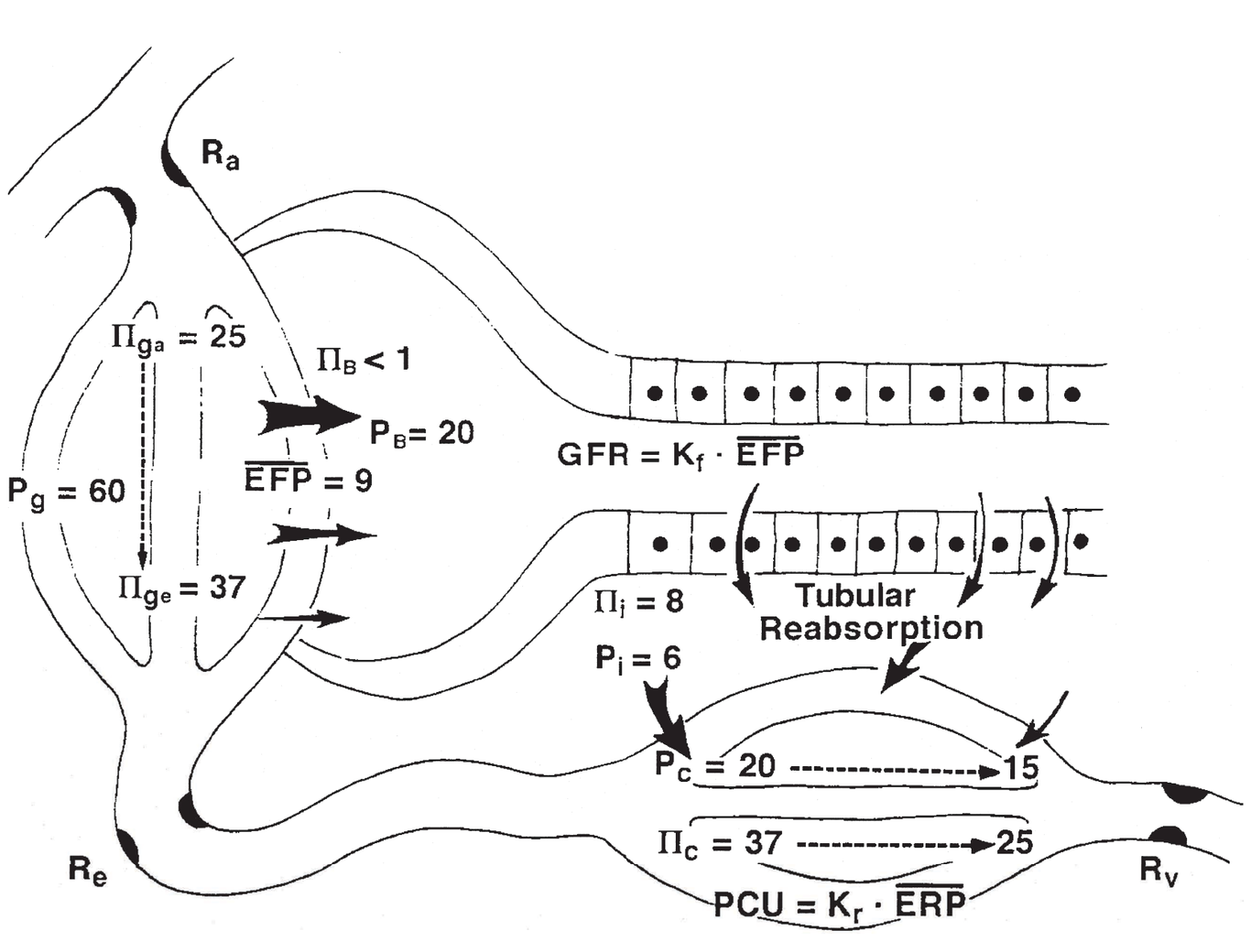

FIGURE 3.8 A schematic diagram of the forces responsible for the filtration of fluid from the glomerular capillaries and the reabsorption of fluid into the peritubular capillaries. The values are considered representative of forces in humans. R_a, afferent arteriole resistance; R_e, efferent arteriole resistance; $P_{g,B,c,i}$, pressure in glomerular capillaries, Bowman's space, peritubular capillaries, and renal interstitium; $\Pi_{g,B,C,i}$, colloid osmotic pressure in glomerular capillaries, Bowman's space, peritubular capillaries, and renal interstitium; EFP, effective filtration pressure; K_f, filtration coefficient; GFR, glomerular filtration rate.

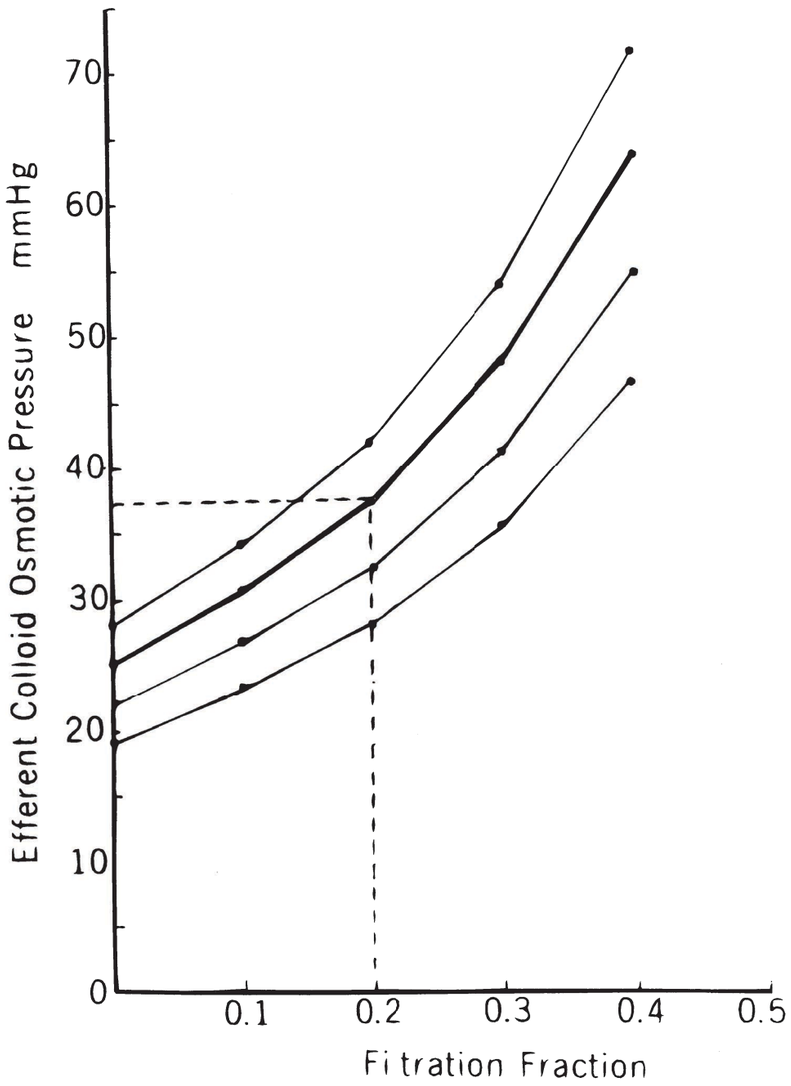

FIGURE 3.9 Anomogram relating the efferent arteriolar colloid osmotic pressure to the initial plasma colloid osmotic pressure and the filtration fraction. Normal afferent arteriolar colloid osmotic pressure, 25 mm Hg, is indicated by the *thicker curve*. An example of how to estimate efferent arteriolar colloid osmotic pressure for any given filtration fraction and plasma colloid osmotic pressure is shown by the *dashed lines*.

The efferent arteriolar colloid osmotic pressure is determined by the initial plasma value and the filtration fraction. The nomogram in Figure 3.9 allows for the estimation of the efferent arteriolar colloid osmotic pressure and is independent of A/G ratios. For example, at a normal filtration fraction of 0.20 and normal plasma colloid osmotic pressure of 25 mm Hg, the predicted value for efferent colloid osmotic pressure is 37 mm Hg.

The hydrostatic pressure in Bowman space (P_B) in humans has not been measured directly. In laboratory animals, P_B is equal to proximal tubular pressure, which ranges from 11 to 15 mm Hg in rats and from 18 to 22 mm Hg in dogs. Also, proximal tubular pressure is slightly higher than the pressure in adjacent peritubular capillaries. Peritubular capillary pressure has also not been measured directly in humans, but it can be estimated from intrarenal venous pressure measurements obtained by retrograde passage of a renal vein catheter. Values obtained in humans are 20 to 25 mm Hg and provide reasonable estimates of proximal tubular pressure. This pressure plus an average efferent colloid osmotic pressure of 37 mm Hg provides a minimal glomerular pressure in humans in the range of 57 to 62 mm Hg; actual values are higher to the extent that there is net filtration pressure at the terminal end of the glomerular capillaries.

Micropuncture studies in animals also indicate that glomerular pressure is 50 to 60 mm Hg and approximately 40 mm Hg greater than the opposing hydrostatic pressure in the Bowman space. From this difference in transglomerular capillary hydrostatic pressure, it can be calculated that EFP ranges from 15 mm Hg at the afferent end of the glomerular capillaries to about 3 mm Hg at the efferent end, yielding an average EFP of 9 mm Hg (Fig. 3.8). Using this value and one of 120 mL per minute for total GFR, a K_f of 13 mL/min/mm Hg for the total nephron population is calculated. Assuming there are 2 million nephrons in both human kidneys, the K_f for a single glomerulus is approximately 6 to 7 nL/min/mm Hg. This value generally agrees with micropuncture measurements, which indicates that K_f for an individual glomerulus is 4 to 5 nL/min/mm Hg in dogs and 2 to 5 nL/min/mm Hg in rats. The large variation in K_f among rats is due in part to differences observed among different strains.[2,77]

The filtration process can operate under one of two conditions. The first condition is the case described previously, in which filtration continues throughout the entire length of the glomerular capillaries and a finite positive EFP remains at the efferent end of the glomerular capillaries. This pattern of disequilibrium is shown by the solid lines in the left panel of Figure 3.10. The second condition occurs when the increase in colloid osmotic pressure is so rapid that the forces favoring and opposing filtration become equal at some point within the capillary system, a condition termed *filtration pressure equilibrium* (Fig. 3.10, solid lines in right panel). Under equilibrium conditions, the latter part of the available filtering surface area is not used and becomes a functional reserve. Studies in some strains of rats have suggested that the normal condition is one of filtration equilibrium. Data from other strains of rats and dogs indicate that, under normal circumstances, glomerular capillary hydrostatic pressure is sufficiently high and the K_f is sufficiently low to prevent the achievement of filtration equilibrium within the glomerular capillaries, and thus filtration occurs along the entire length of the glomerular capillaries.[2]

A physiologic consequence of the equilibrium or disequilibrium of filtration pressures is the influence of plasma flow on GFR. Using a mathematical model presented later, the specific effect of plasma flow can be predicted for both conditions when the transcapillary hydrostatic pressure gradient is kept constant. As shown by the dashed line in Figure 3.10 (right panel), an increase in plasma flow to a system in filtration equilibrium diminishes the rate of increase of colloid osmotic pressure along the length of the glomerular capillaries. The EFP is not dissipated as quickly, and the point of equilibration of hydrostatic and colloid osmotic forces is moved distally, which, in effect, results in the recruitment of additional filtering surface area (S_f) and an increase in the functional K_f. Consequently, increases in plasma flow can increase the GFR proportionately even when glomerular capillary pressure is unchanged. In the case of filtration

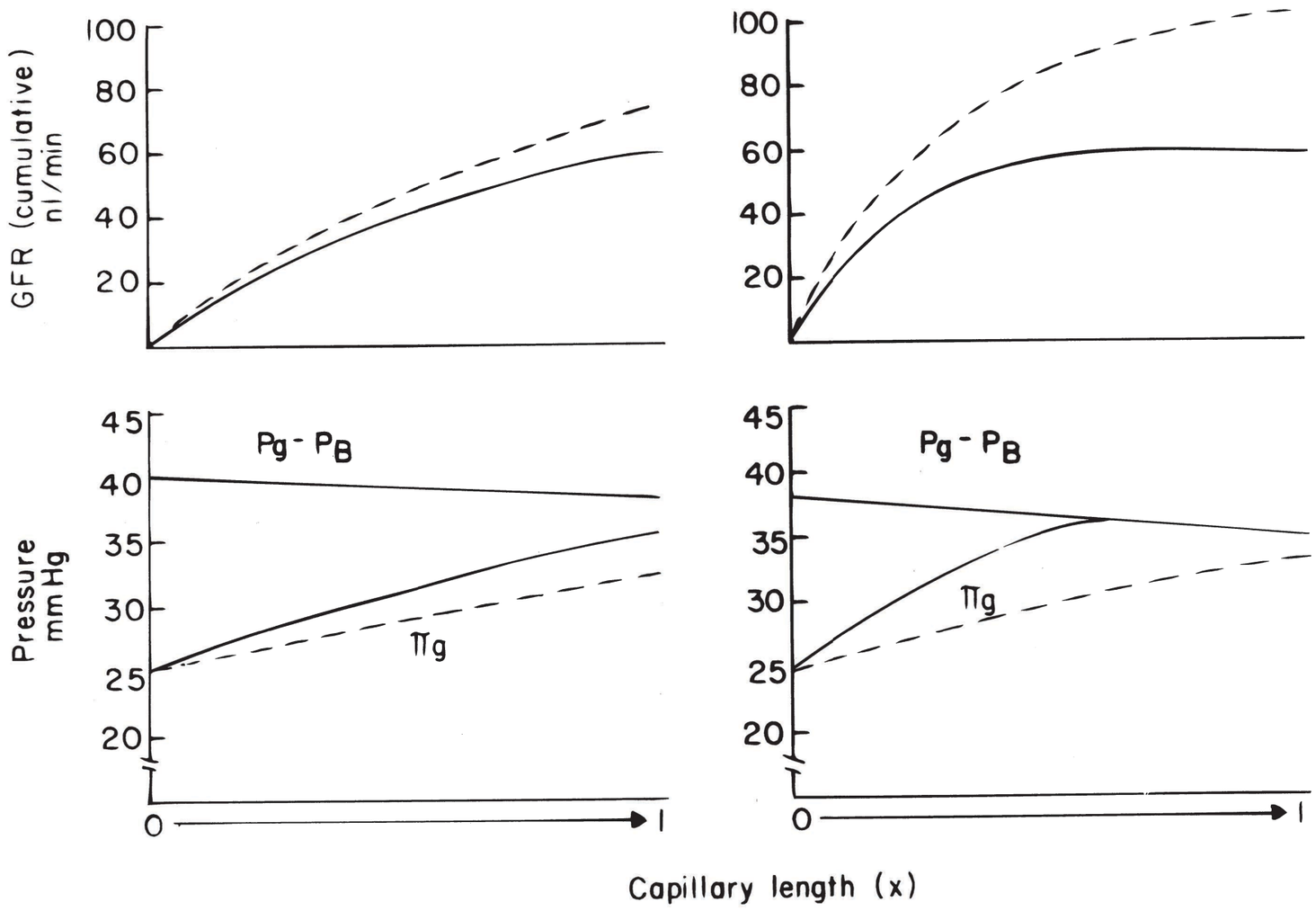

FIGURE 3.10 A comparison of filtration dynamics in conditions of filtration equilibrium (*right*) and disequilibrium when filtration occurs throughout the length of capillary (*left*). The lower panels represent the changes in the transcapillary hydrostatic pressure gradient ($P_g - P_B$) and the glomerular plasma colloid osmotic pressure (π_g), and the upper panels represent the cumulative GFR along the length of the glomerular capillary. The *dashed lines* indicate the changes occurring in response to doubling of plasma flow under both conditions. P_g, glomerular capillary hydrostatic pressure; P_B, hydrostatic pressure in Bowman's space.

pressure disequilibrium, increases in plasma flow increase GFR only modestly as a consequence of a reduced colloid osmotic pressure profile, and there is no net recruitment of previously unused surface area (see Fig. 3.10, dashed lines in left panel). Thus, the magnitude of a selective plasma flow effect is smaller during filtration pressure disequilibrium than during equilibrium. In humans, the low filtration fraction and the relative lack of plasma flow dependence of GFR suggest that the filtration process continues throughout the entire length of the glomerular capillaries (i.e., disequilibrium, as shown in the left panel of Fig. 3.9).[1,2,77]

Glomerular Permeability to Macromolecules

Experiments examining the filterability of test molecules of different sizes, shapes, and charges have been used to characterize the hydrodynamic properties of the filtration barrier. A sieving coefficient (Φ), or fractional clearance of a test molecule, is obtained relative to that of a freely filtered reference molecule such as inulin. Accurate determinations can be made when both substances enter the urine by means of filtration and are not subjected to tubular reabsorption or secretion. Such data have been fitted to various theoretic models based on limiting membrane structures, consisting of an impermeable matrix that is perforated with cylindrical pores, rectangular slitlike openings, or a meshwork of fibrous or granular gel-like structures. An evaluation of molecular sieving or steric restriction in each model, however, is based on the principle of geometric exclusion of large solute molecules from a portion of the membrane that is accessible to water and small solutes. In essence, the larger molecules that approach or exceed the effective size of the channels are restricted or "sieved." Conceptually, the simplest model that is applicable to the glomerular barrier consists of a size-discriminating membrane with a large population of fluid-filled cylindrical pores of about 5 nm in radius, which totals approximately 5% of the total surface area. There may also be a very small population of much larger pores.[2,75]

Studies involving quantitative consideration of macromolecular passage through capillary membranes have relied on the thermodynamic approach developed by Kedem and Katchelsky. Derivations for solute flux (J_s) across a constraining membrane include a convection term, which is the solute flux that occurs as a consequence of the bulk volume flow (J_v), and a diffusion flux, which is a function of the concentration gradient of the solute. In its most elementary form, solute flux due to convection is

$$J_s = J_v C_s (1 - \sigma) \tag{5}$$

and solute flux due to diffusion is

$$J_s = PS (\Delta C_s) \tag{6}$$

where J_v is the volume flow (in this case the GFR), and C_s is the average concentration across the membrane; sigma (σ) is the reflection coefficient previously discussed. ΔC_s is the concentration difference across the capillary wall, and PS is the diffusional, permeability surface-area product coefficient. With small uncharged molecules, such as glucose, sigma approaches zero and thus glucose flux is simply defined by the product of GFR and the plasma glucose concentration. For very large molecules that are restricted with almost complete efficiency, sigma approaches 1 and thus solute flux due to convection is negligible. The most relevant example is for plasma albumin. Using a value of 1 to 3 mg per deciliter for albumin concentration in early tubular fluid and a systemic plasma albumin concentration

of 3,600 mg per deciliter, sigma is greater than 0.99. Furthermore, the PS coefficient is so low (0.001 mL per minute) that solute flux due to diffusion also approaches zero. These quantitative considerations also highlight the difficulty in attempting to evaluate mechanisms of proteinuria. Theoretically, protein passage across the glomerular membrane could increase more than 100-fold, which could be accounted for by a change in sigma from 0.99 to 0.95. Such small changes in membrane permeability would not be expected to be associated with discernible morphologic changes.[2,75]

Passage of macromolecules across capillary membranes is dependent on several factors in addition to the effective radius. These factors include the electrical charge and the structural conformation and rigidity of the molecule. As shown in Figure 3.10, the glomerular sieving coefficient or fractional clearance (usually determined as C_D/C_{IN}) of graded sizes of electrically neutral dextran molecules declines progressively as effective radius and molecular weight increase. Water, electrolytes, and other small, uncharged solute molecules with an effective Stokes-Einstein radius of less than 1.8 nm freely permeate. As the effective radius increases, there is a progressive restriction. The fractional clearance of macromolecules the size of immunoglobulin G (IgG) (5 nm) is essentially zero. For the same equivalent radius, the fractional clearances of albumin (3.6 nm) and negatively charged dextran sulfate are considerably lower than the clearances of uncharged molecules. In addition, polycationic macromolecules are filtered more readily than neutral molecules. These differences in transport of electrically charged macromolecules are due to the membrane-bound polyanionic glycoproteins that are rich in sialic acid and heparin sulfate residues, which set up a negative electrostatic field that repels polyanions. These are associated with the glycoprotein coat that covers the endothelial fenestrations, the basement membrane, and the podocytes. Partial loss of these anionic sites on the glomerular capillary wall can lead to albuminuria in the absence of any gross structural abnormalities and in cases of mild glomerulonephritis. Such a loss has been induced experimentally by neutralization of the electrostatic barrier with the polycation protamine. In more severe glomerular injury–associated proteinuria, a larger fraction of the filtrate appears to pass through a population of large diameter, nonselective pores.

In addition to size and charge, molecular configuration influences the sieving coefficient (Fig. 3.11). Rigid or globular molecules such as horseradish peroxidase or ficoll have lower sieving coefficients for any given molecular size than neutral dextran polymers with highly deformable linear structures. Thus, it is likely that the curve for neutral dextrans in Figure 3.11 overestimates the true permeability characteristics of more rigid, globular-structured macromolecules such as plasma proteins. Because shape, flexibility, and deformability contribute to the quantitative relationship between molecular size and transglomerular solute flux, it is difficult to establish the true dimensions of the extracellular

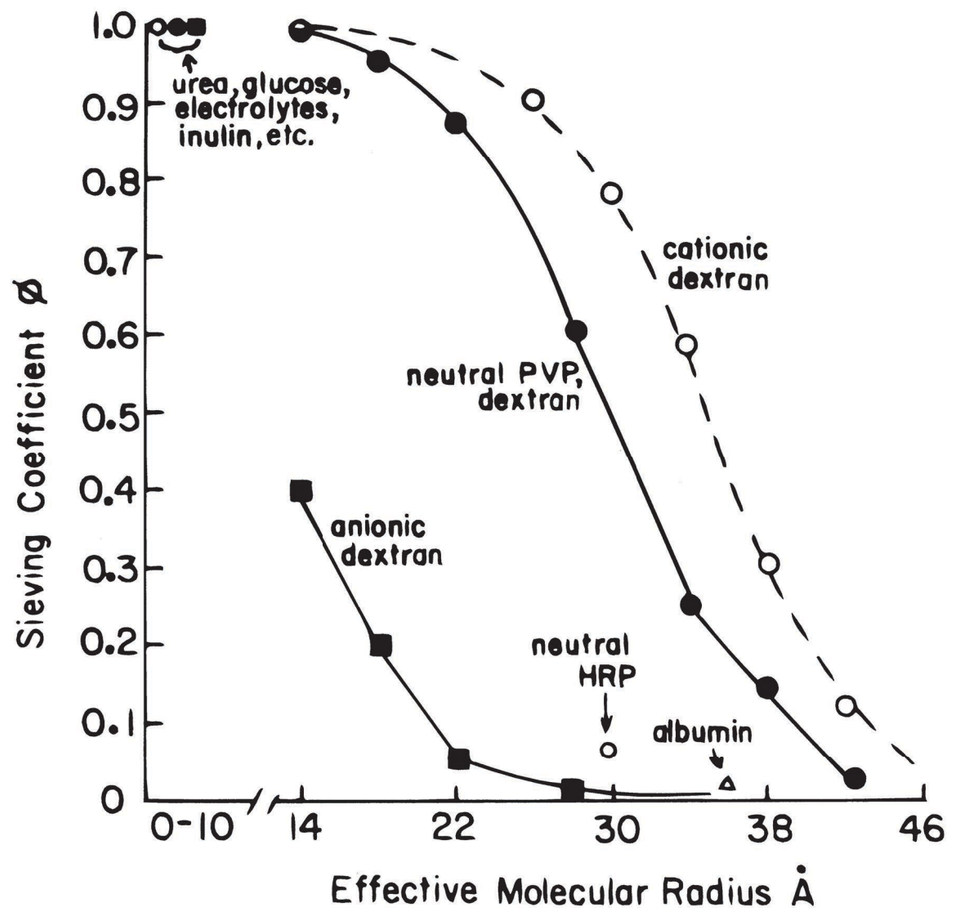

FIGURE 3.11 Representative sieving curves for several test molecules in the glomerular circulation. The curve representing neutral molecules is based on data obtained with the use of polyvinylpyrrolidone (PVP) and neutral dextran. The curves for anionic and cationic molecules are based on studies with charged dextrans. Also shown are the sieving values for neutral horseradish peroxidase (*neutral HRP*) and for albumin. The smaller molecules are shown to have a sieving coefficient of 1.0. See text for details.

channels. Data currently available indicate that the effective radius of the channels in the glomerular membrane is in the range of 4.5 to 6 nm.[2,75]

Recent studies have challenged the generally held notions described previously regarding glomerular permeability and have suggested that much greater amounts of albumin are filtered across the glomerular capillaries and that most of it is then reabsorbed in the proximal tubules by megalin and other macromolecule transporters. Studies based on measurements of glomerular permeability of fluorescent labeled albumin suggest that even normal glomerular capillaries allow passage of albumin that is avidly bound and retrieved by proximal tubules. This concept has been met with substantial resistance and alternative studies have failed to indicate such high levels of albumin passage across normal glomerular capillaries. Advanced multiphoton determinations have yielded a glomerular sieving coefficient of 0.001, a value consistent with the view that the primary determinant of albuminuria is of glomerular origin and that charge does play a significant role.[78–83]

The controversy about glomerular permselectivity highlights important recent findings regarding the extremely complex nature of the glomerular barrier because it consists of three distinct layers in series; each having its own selectivity characteristics. Gene targeted mouse models illustrate the

important role that podocytes have in restricting macromolecules. In particular, studies of the structural and molecular characteristics of the slit diaphragms between adjacent podocytes reveal an extremely complex network of molecular interactions that serve to maintain the normal structure of the podocytes and their close relationship with the basement membranes. The structure of the glomerular membrane is described in detail in Chapter 1. With regard to macromolecular permeability, the podocyte slit-diaphragm layer and its complex array of proteins, including nephrin, podocin, cadherin, and integrins, are clearly identified as the final barrier responsible for the extremely high degree of restriction. Nevertheless, the emerging consensus is that all of the three major restriction sites make a contribution. In essence, when the endothelial glycocalyx is compromised, proteinuria develops. Disorganization of the basement membrane also leads to proteinuria. Furthermore, disruption of the nephrin-actin cytoskeleton complex of the podocyte layer and foot process effacement are associated with the greatest degree of proteinuria. Conceptually, it is attractive to attribute a progressively greater reflection coefficient (sigma) to the three layers in series.[81,84]

Hemodynamics in Peritubular Capillaries and Its Role in Fluid Reabsorption

Virtually all of the peritubular capillary network stems from efferent arterioles. About 85% of the postglomerular blood flow is distributed to peritubular capillaries in the cortex, and the remaining 15% goes to the medulla and papilla (Fig. 3.5). The overall density of peritubular capillaries and the total surface area are considerably greater than those of glomerular capillaries. The peritubular capillary wall consists of a fenestrated endothelial layer covered by a thin basement membrane. Per unit of surface area, it has a lower hydraulic conductivity and a slightly higher permeability to large molecules than the glomerular wall.

In a manner analogous to the process of filtration, the peritubular capillary reabsorption (PR) of interstitial fluid that is reabsorbed by the renal tubules is determined by the imbalance of hydrostatic and osmotic forces between the interstitial space and adjacent peritubular capillaries. If one considers the forces responsible for reabsorption into the capillaries, then

$$PR = K_r [(\pi_c - \pi_i) - (P_c - P_i)] \quad (7)$$

where K_r is the reabsorptive coefficient, π_c and π_i represent the average colloid osmotic pressures in the capillaries and in the interstitial fluid, and P_c and P_i represent the corresponding hydrostatic pressures.

As plasma emerges from the glomerular capillaries, it has a colloid osmotic pressure of 35 to 37 mm Hg (Fig. 3.8). Furthermore, the hydrostatic pressure drops about 40 mm Hg along the efferent arteriole (Fig. 3.3), yielding an initial peritubular capillary pressure of about 20 mm Hg. With regard to the interstitial compartment, π_i and P_i are about 6 to 8 mm Hg and tend to cancel each other out. Thus, the mean effective reabsorption force is 15 mm Hg at the beginning of the peritubular capillary bed. As fluid is reabsorbed into the capillaries, plasma proteins are diluted and the colloid osmotic pressure progressively decreases to the original value entering the kidney. There is also a small decline in capillary hydrostatic pressure along the peritubular capillaries. Thus, there is an effective reabsorptive force over the entire length of the peritubular capillaries, which falls from about 15 mm Hg to about 8 mm Hg (Fig. 3.8). The hydraulic reabsorptive coefficient, K_r, for the peritubular capillaries is about 9 to 10 mL/min/mm Hg, which is slightly lower, overall, than the glomerular K_f. This suggests a lower hydraulic conductivity, which is compensated by the larger surface area of the peritubular capillaries.

With regard to macromolecular permeability, the situation existing in the peritubular circulation contrasts with that in the glomerulus because the convective component is directed inward in association with the continuous fluid reabsorption. Thus, the loss of macromolecules from the postglomerular capillaries occurs only as a consequence of diffusion of macromolecules from the plasma into the interstitial compartment. Although significant amounts of protein accumulate in the interstitium, the actual permeability is still quite low because of the low removal rate by the lymphatics in the renal cortex. Some studies indicate that the postglomerular circulation constrains molecules that can readily pass through the glomerular membrane. There also may be a small population of pores with diameters greater than 5 nm. Nevertheless, most of the channels have a high degree of efficiency in restricting albumin and other plasma proteins, so their reflection coefficients are very close to 1. This occurrence is due, in part, to an electrostatic barrier similar to that found in the glomerular capillaries such that negatively charged macromolecules permeate more slowly than neutral molecules of the same size. Thus, plasma proteins exert almost their full osmotic pressure across the peritubular capillaries. In spite of these high reflection coefficients, the concentration of albumin in the renal lymph, and presumably in the interstitial fluid, is about one-fourth that in systemic plasma. Although this concentration seems rather high, it should be noted that lymph flow is very low and less than 1% of net protein is lost from the plasma flowing through the peritubular capillaries.[2]

Maintaining the high density of peritubular capillaries is of critical importance in providing adequate oxygenation to the surrounding tubules. Renal interstitial inflammation may lead to reduced peritubular capillary density and the impairment of renal function, resulting in salt-sensitive hypertension. Peritubular capillary loss in renal transplant patients is associated with interstitial fibrosis and tubular atrophy, leading to reduced renal function.[85,86]

Lymphatic capillaries, primarily distributed throughout the cortex, are very permeable to protein and fluid. They

serve to return the proteins that leak out of the peritubular capillaries back to the circulation, and it is usually assumed that the protein concentration in the lymph reflects the protein concentration in the interstitial fluid. The normal renal lymph flow in humans is estimated to be about 2 to 5 mL per minute, or less than 1% of the plasma flow. Lymph flow is increased by elevations in interstitial hydrostatic pressure, such as those accompanying diuretic states, ureteral obstruction, or increases in renal venous pressure.[87,88]

Capillary Uptake by the Vasa Recta

Efferent arterioles of juxtamedullary nephrons provide the vascular supply to the medulla. These efferent arterioles branch into long-looped capillaries, termed the vasa recta, which descend into the medulla in vascular bundles. The vasa recta bundles are intimately associated with and are surrounded by concentric rings of loops of Henle and collecting ducts. The medullary circulation has the important function of removing water and solutes reabsorbed from descending and ascending loops of Henle and collecting ducts without disrupting the large longitudinal osmotic gradients that exist in the inner medulla during water conserving states. This delicate balance is achieved by virtue of the low blood flow and an efficient countercurrent diffusion of fluid and small molecular-weight solutes, which occur because of the specialized structures of the hairpin-shaped parallel loops of the descending and ascending vasa recta. The end result is passive equilibration and shunting of fluid across the vasa recta loops, from the descending to the ascending limbs, and the trapping of solute at the bends. The descending vasa recta have a continuous thick endothelium but aquaporin-1 channels allow for water efflux. Because protein permeability is low, the high plasma protein concentration of the efferent arteriolar blood is preserved. In contrast, the ascending vasa recta have a highly fenestrated thin endothelium, which greatly facilitates passive reabsorption. The ascending vasa recta also have a higher permeability to protein. However, the hydrostatic pressure in the ascending vasa recta is relatively low, about 10 mm Hg, and probably not much higher than interstitial hydrostatic pressure. In the face of a very small outward hydrostatic pressure gradient, the transcapillary colloid osmotic pressure gradient provides an important reabsorptive force, favoring capillary fluid uptake throughout these specialized capillaries. Importantly, the outer medullary descending vasa recta are encircled at points by contractile pericytes that provide a means to locally regulate blood flow.[2,15,19,89,90]

A Quantitative Analysis of Filtration and Reabsorption Dynamics

The mechanisms regulating GFR involve complex interactions among the individual determinants. To achieve a better understanding of the singular effects of each determinant, one can examine the theoretical influence of selective changes in an idealized situation where the other determinants are held constant. Such theoretical predictions can be made from the simple mathematical model shown in Figure 3.12, which analyzes fluid flow dynamics along the length of a single filtering capillary and the resistances of the afferent and efferent arterioles.

This model can be used to analyze the effects on GFR of singular perturbations, such as changes in the transcapillary hydrostatic pressure gradient, the systemic plasma protein concentration, the glomerular plasma flow, and the filtration coefficient. As shown in Figure 3.13 (panel A), changes in the transcapillary hydrostatic pressure difference produce striking responses in GFR. An increase of 10% causes a greater relative increase in EFP leading to an increase in GFR of 19%. GFR is inversely related to plasma colloid osmotic pressure; as can be seen in panel B, a 10% increase of the plasma protein concentration reduces GFR by 25%. The influence of changes in K_f and in plasma flow on GFR are more complex because they affect the rate of rise of plasma colloid osmotic pressure along the capillary bed and thus EFP. Panel C in Figure 3.13 shows that GFR is affected more by decreases than by increases in K_f. The reduced effect of increases in K_f above the normal values reflects the achievement of filtration–pressure equilibrium. Once filtration equilibrium is reached, further increases in K_f enhance ultrafiltration in early portions of the capillary, which causes protein concentration to increase more rapidly. However, this effect is offset because the colloid osmotic pressure equilibrates with the

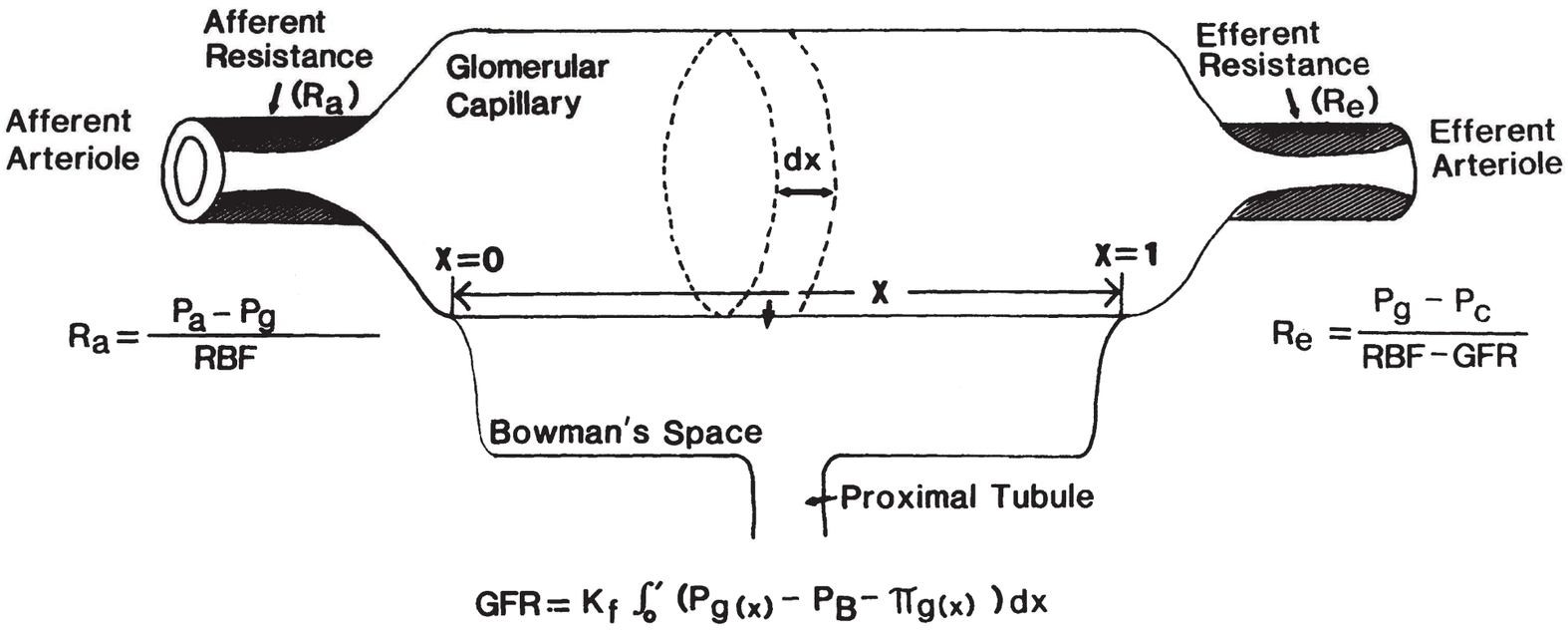

FIGURE 3.12 A "single capillary" model of glomerular filtration dynamics and afferent arteriolar (*R*a) and efferent arteriolar (*R*e) resistances. P_a, arterial pressure; P_g, glomerular capillary pressure; P_c, peritubular capillary pressure; P_B, Bowman's space pressure; *RBF*, renal blood flow; *GFR*, glomerular filtration rate.

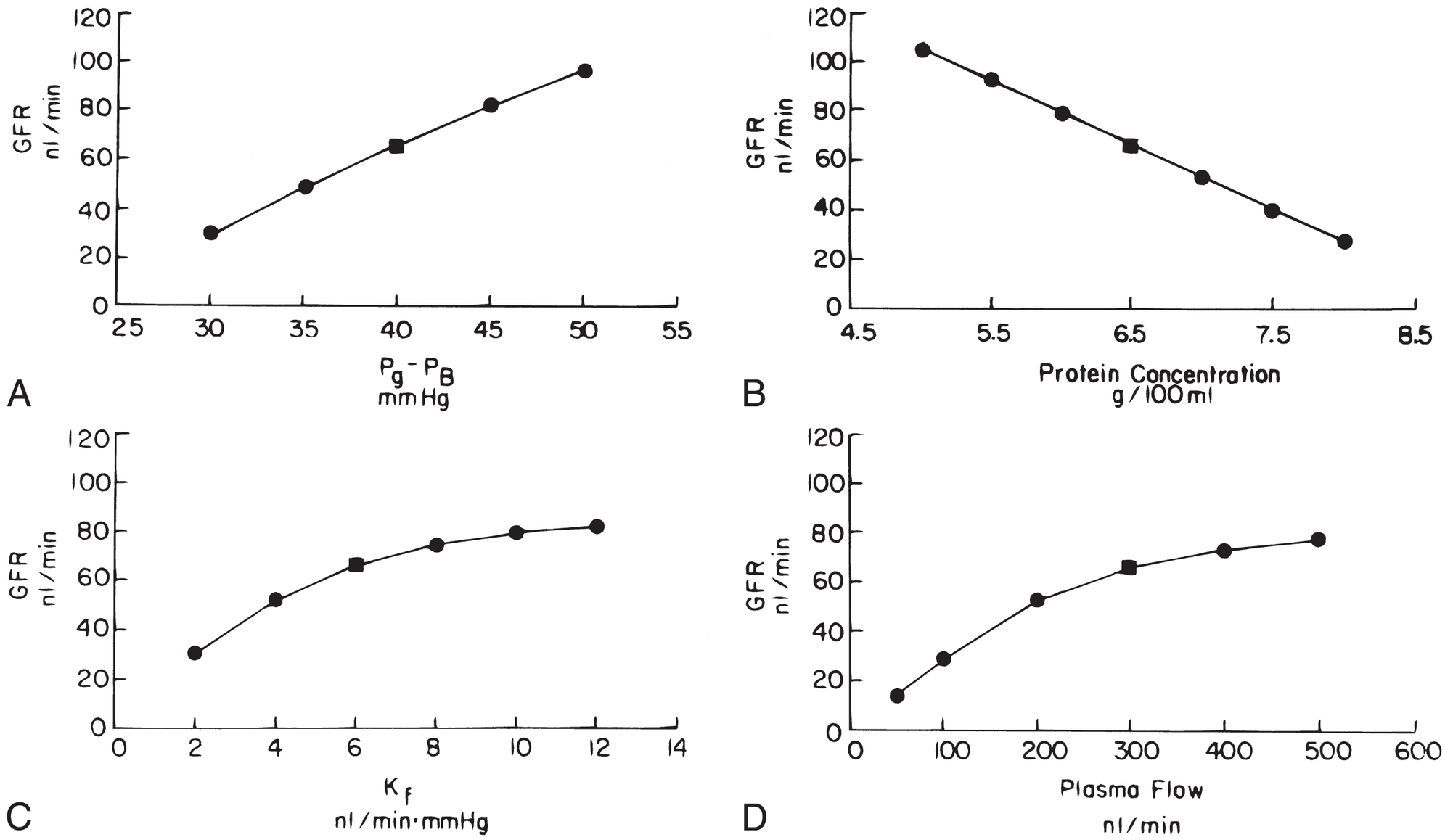

FIGURE 3.13 Theoretical effects of singular perturbations in **(A)** the transcapillary hydrostatic pressure gradient ($P_g - P_B$), **(B)** the plasma protein concentration, **(C)** the filtration coefficient (K_f), and **(D)** the plasma flow at entry to glomeruli. For these simulations, control values (*squares*) estimated to be representative of single nephron function in humans were used: GFR = 65 nL/min; plasma flow = 300 nL/min; ($P_g - P_B$) = 40 mm Hg; plasma protein concentration = 6.5 g/dL. *GFR*, glomerular filtration rate.

hydrostatic pressure difference at a more proximal site along the capillary. Thus, the mean EFP and the total GFR remain the same, although the locus of equilibrium is shifted to an earlier site.

Panel D of Figure 3.13 demonstrates that the effects of glomerular plasma flow on GFR are also nonlinear. In the absence of changes in the other determinants, GFR increases only modestly with increases in plasma flow. On the other hand, decreases in plasma flow below 200 nL per minute produce roughly proportional decrements in GFR. This increase in sensitivity is again due to the attainment of filtration equilibrium at the lower values of plasma flow. Glomerular plasma flow exerts these effects by modifying the intraglomerular profile of colloid osmotic pressure and thus mean EFP. The effects of increases in plasma flow during filtration equilibrium and not in equilibrium were discussed earlier and are shown in Figure 3.10. Changes in plasma flow have a relatively small effect on the colloid osmotic pressure profile during filtration disequilibrium (Fig. 3.10). A decrease in plasma flow increases the fraction of plasma being filtered per unit of capillary length in proximal segments. As a result, the rate of rise of colloid osmotic pressure is increased progressively. During filtration pressure equilibrium, GFR is highly plasma flow dependent. Thus, there are two major functional consequences of filtration pressure equilibrium. GFR is insensitive to increases in K_f and is strongly influenced by changes in plasma flow. In contrast, GFR is directly responsive to K_f and is less plasma flow dependent under disequilibrium conditions. In either situation, glomerular capillary pressure is quantitatively a much more powerful determinant of GFR than plasma flow.

A mathematical analysis that is of more physiologic relevance involves an integrated consideration of changes in preglomerular and efferent arteriolar resistance on glomerular dynamics. The predicted effects of constriction and dilation of either afferent or efferent arterioles under idealized conditions are presented in Figure 3.14, when the other resistances as well as other inputs are maintained at normal values. A selective increase in afferent resistance reduces plasma flow and hydrostatic pressure in glomerular and peritubular capillaries. GFR decreases proportionately more than plasma flow and thus the filtration fraction falls. In contrast, an increase in efferent arteriolar resistance reduces plasma flow but increases glomerular pressure. GFR initially increases slightly but then reaches a plateau. The plateau region of mean EFP is due to the counteracting effects of the increases in glomerular capillary and colloid osmotic pressures. From these quantitative considerations, it is apparent that the preglomerular resistance is ideally suited to control GFR. Efferent arteriolar resistance contributes to subtle alterations in GFR but exerts major effects on the dynamics of the postglomerular circulation. It is important to

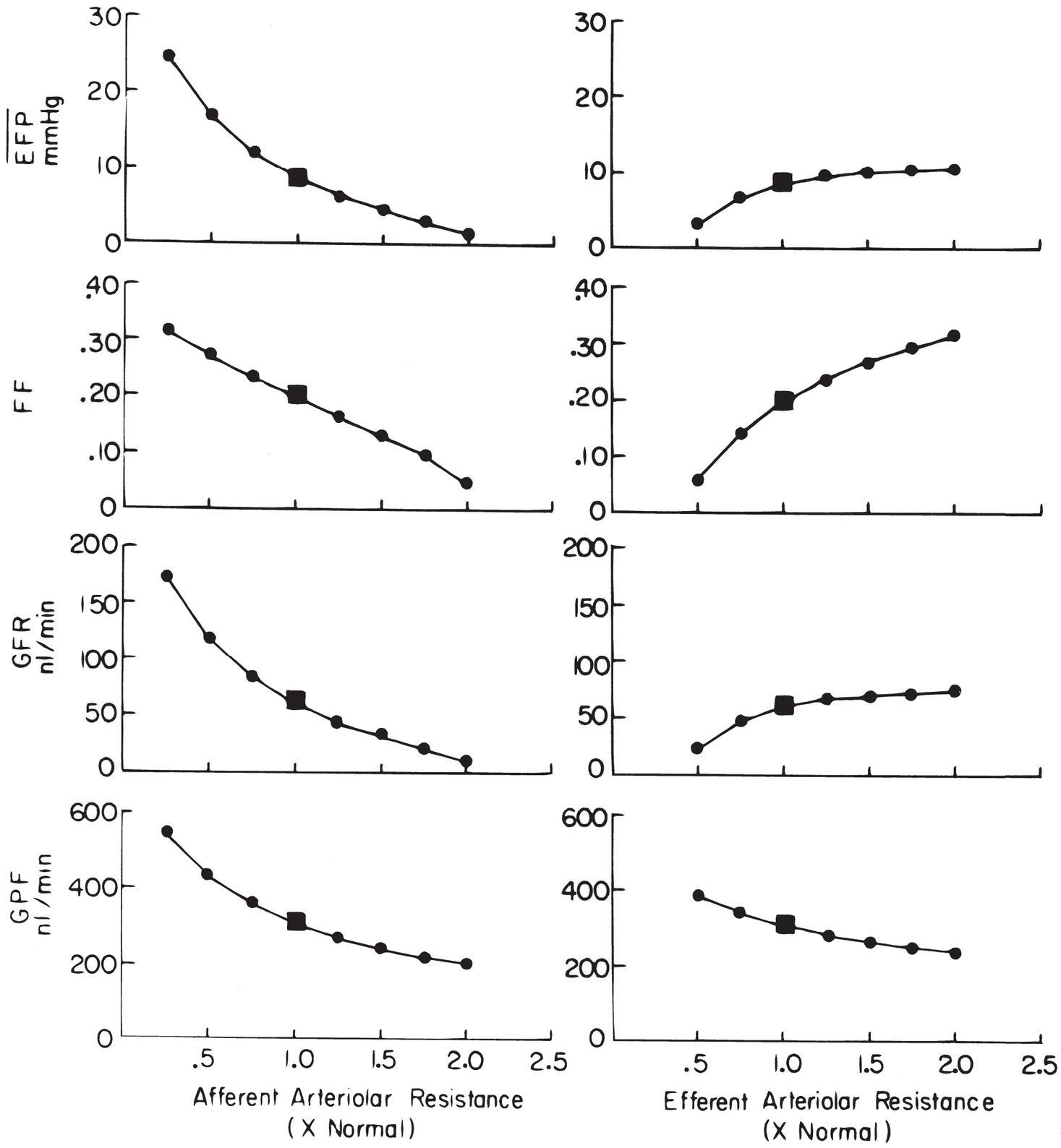

FIGURE 3.14 Effects of increases and decreases in afferent and efferent arteriolar resistance on the glomerular filtration rate (*GFR*), plasma flow (*GPF*), the filtration fraction (*FF*), and the mean effective filtration pressure (*EFP*).

emphasize that changes in filtration fraction alone cannot be used to determine the localization of resistance changes to a specific manipulation, condition, or drug. For example, combined increases in afferent and efferent resistances reduce the plasma flow proportionately more than the GFR, and the filtration fraction increases. Likewise, combined decreases in afferent and efferent arteriolar resistances increases blood flow proportionately more than GFR and filtration fraction falls. These changes in the filtration fraction have often been interpreted incorrectly as being indicative of selective change in efferent arteriolar resistance.

Control of Glomerular Dynamics by the Filtration Coefficient

In addition to the regulation of capillary flow and pressure by resistance changes of the preglomerular and postglomerular vascular segments, K_f and K_r may be influenced by many factors. Alterations in the size of the capillaries or closure of a fraction of the capillaries may reduce the available filtering surface area and thus influence K_f. The hydraulic conductivity may be altered by adjustments in the size and number of endothelial fenestrations, the thickness or permeability of the basement membrane, and the number or structural configuration of the slit pores between the foot processes. Recent attention has been focused on the role of the podocytes in control of GFR. Changes in any of these properties could be manifested as changes in K_f.[91]

Animal studies suggest that vasoconstrictor hormones and some vasodilators are capable of reducing K_f. Also, K_f may be reduced drastically in disease states that involve sclerosis of glomerular capillaries or thickening of the basement membrane. K_f may be increased slightly in certain circumstances, such as increased plasma colloid osmotic pressure. Differences in basal K_f are reported among different species and different strains and colonies of rats. Agents such as Ang II and catecholamines decrease K_f. Blockade of the vascular effects of Ang II negates the K_f lowering effect of prostaglandins E_2 and I_2 and parathyroid hormone (PTH). In addition, several vasodilators (acetylcholine, bradykinin, and histamine) decrease K_f in rats through a mechanism that is not clear. Although the vasodilator actions are primarily due to NO release, it is not apparent how this would decrease K_f. In contrast to the effects observed in rats, vasodilator

agents do not affect K_f appreciably in dogs. The reason for this apparent species difference is not known. Nevertheless, it seems clear that a variety of paracrine agents, including NO and ET-1, can influence K_f and filtration dynamics.[1,36,77]

With regard to the postglomerular vasculature, the major regulator of hydrostatic pressure in the peritubular capillaries is the efferent arteriolar tone. For a given flow, an increase in efferent arteriolar resistance increases the pressure drop along this vessel and thus reduces the pressure in peritubular capillaries. An increase in downstream resistance due to venous obstruction or elevated tubular pressure increases hydrostatic pressure in the capillaries. Interstitial hydrostatic pressure changes in the same direction as pressure in the peritubular capillaries. Colloid osmotic pressure of blood entering the peritubular capillaries is primarily regulated by the filtration fraction. A higher colloid osmotic pressure exerts a greater reabsorptive force in the postglomerular circulation. The colloid osmotic pressure in interstitial fluid is determined by a balance of protein entry from circulating plasma and protein exit by means of the lymphatic circulation. In general, stimuli promoting efferent arteriolar constriction reduce hydrostatic pressure in peritubular capillaries and increase efferent arteriolar colloid osmotic pressure, changes that favor increased fluid reabsorption from the renal interstitium into the peritubular capillaries. Vasodilating stimuli have the opposite effects and are often accompanied by natriuretic and diuretic responses. In all cases, however, there is a very intimate coupling between the rate of fluid reabsorption from the tubules into the interstitium and fluid reabsorption from the interstitial compartment into peritubular capillaries.[87]

THE REGULATION OF RENAL HEMODYNAMICS

The high sensitivity of glomerular and peritubular capillary dynamics to variations in the intrarenal pressures and flows emphasizes the importance of regulatory mechanisms that maintain the intrarenal hemodynamic environment. Overall control is shared by several mechanisms that exert specific effects on various segments of the renal vasculature. Some of these mechanisms are intrinsic to the kidney, whereas others depend on extrarenal signals mediated by neural or hormonal stimuli.

Mechanisms of Autoregulation

Intrinsic paracrine signals can adjust intrarenal vascular resistance in response to a variety of extrarenal perturbations. Alterations in vascular resistance serve to counter the effect of the extrarenal disturbance to stabilize RBF and GFR. The most widely studied manifestation of these intrinsic mechanisms is the phenomenon of renal autoregulation. In response to alterations in renal arterial pressure over a wide range, the kidney adjusts its vascular resistance to maintain, or "autoregulate," RBF. This range encompasses the physiologically relevant arterial pressures, both above and below normal. In response to reductions in arterial pressure, which may occur during situations such as sleep or recumbence, intrarenal mechanisms decrease renal vascular resistance (RVR) to maintain RBF and GFR at optimum levels. Likewise, increases in arterial pressure, which might occur during exercise or acute episodes of stress, elicit intrarenal signals that increase vascular resistance and thus maintain RBF and GFR at or near control levels. In addition to RBF and GFR, the microvascular and tubular pressures exhibit autoregulatory behavior. Because glomerular pressure and GFR are autoregulated, the predominant adjustments of vascular resistance are localized to the preglomerular arterioles. Figure 3.15 shows representative relationships between the renal arterial pressure and RBF, GFR, and segmental vascular resistances. The responses of vascular resistance to changes in perfusion pressure represent the most commonly investigated aspect of the renal autoregulatory mechanism, but other stimuli, such as increases in ureteral or renal venous pressure or changes in plasma colloid osmotic pressure, also elicit autoregulatory responses. The response serves a negative feedback function to counteract the effect of the disturbance and restore RBF or GFR back toward normal.[1,2,92–94]

Although complex interactions with multiple signaling pathways are involved in the total response, the autoregulatory component of the vasculature requires activation of voltage-dependent Ca^{2+} channels and is prevented by L-type Ca^{2+} channel blockers. Much of the research oriented toward understanding this basic response is focused on the mechanisms by which messages are initiated, transmitted to, and received by the smooth muscle cells to affect the requisite alterations in vascular resistance. The two basic mechanisms that contribute to the autoregulation phenomenon are the myogenic and the TGF mechanisms. A third possible contributor has been identified recently, but the underlying mechanism is not known.[2,92–94]

The myogenic mechanism responds to a distending force on the vessel wall caused by an increase in arterial pressure. The actual distending force can be calculated from the law of Laplace that relates tangential wall tension (T) to the inner radius of the vessel (r) and the transmural pressure difference:

$$T = r\,(P_a - P_i) \tag{8}$$

where P_a is intra-arteriolar hydrostatic pressure, and P_i is the interstitial fluid pressure. When the transmural pressure difference increases, wall tension is increased, which activates Ca^{2+} channels and leads to constriction and a reduction of the radius allowing the tension to return to normal. A myogenic response occurs in preglomerular vessels but not postglomerular efferent arterioles. This may be due to the differential activating mechanisms in these vessels because efferent arterioles do not normally have functional L-type Ca^{2+} channels. In addition to the afferent arteriole, the arcuate and interlobular arteries also display myogenic responses.

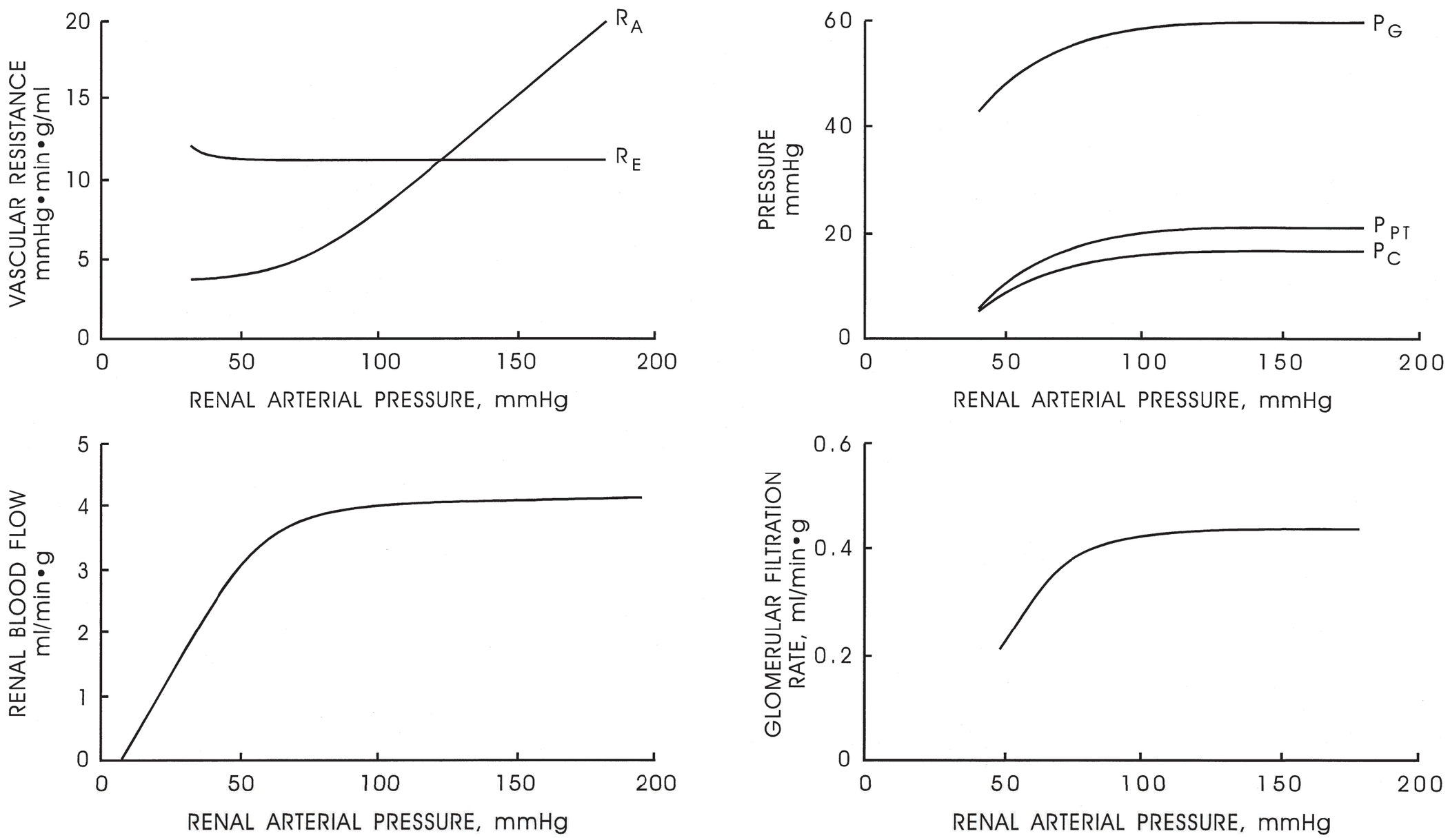

FIGURE 3.15 Representative relationships between renal arterial pressure and renal blood flow (*lower left panel*), glomerular filtration rate (*lower right panel*), and segmental vascular resistance (*upper left panel*) for afferent arteriolar resistance (R_A), efferent arteriolar resistance (R_E), and hydrostatic pressure (*upper right panel*) in glomerular capillary (P_g), proximal tubule (P_{PT}), and peritubular capillary (P_C).

Although a large vessel such as the arcuate artery responds to pressure, its contribution to total resistance is quite small. Thus, the preglomerular arteriolar network has the ability to respond to extrinsic physical or mechanical disturbances by an intrinsic myogenic response of vascular smooth muscle. The initial autoregulatory adjustments in vascular resistance occur rapidly (a few seconds) as a result of a direct local vascular mechanism. Such a fast response is thought to buffer the glomerular capillaries and the tubular network from sudden changes in arterial pressure and protect from high systolic pressures, especially at higher frequencies. Long-standing glomerular hypertension is associated with proteinuria and the development of glomerular sclerosis.[1,93,95–97]

A contraction caused by an increase in intraluminal pressure is elicited by cell membrane depolarization and increased Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels. L-type channel blockers inhibit the myogenic response and the stretch-activated Ca^{2+} channels in the preglomerular microcirculation. T-type Ca^{2+} channels contribute to the myogenic response as a blockade of T-type channels also attenuate autoregulatory responses. In afferent arterioles, the T-type channels may sense relatively small stimuli, which then cause sufficient Ca^{2+} entry and membrane depolarization to activate voltage-gated L-type channels to activate intracellular signaling pathways and to elicit contraction. The increased $[Ca^{2+}]_i$ activates the inositol phosphate cascade to increase IP_3 and DAG and activate PKC. Inhibition of PKC attenuates the autoregulatory constrictor response to a pressure increase. Subconstrictor concentrations of either Ang II or ET-1 potentiate myogenic contraction of afferent arterioles. NO attenuates the rate and strength of the myogenic response. Although endothelial cells play a role in the rate of autoregulation, stretch-induced, steady-state myogenic tone is observed in vessels without a functional endothelium. The actual mechanosensitive transducer on vascular smooth muscle cell membranes has not been completely characterized. Cell surface integrins have been postulated to serve as mechanotransducers by responding to shear, stretch, or tension. Sodium channels have also been shown to respond to mechanical deformation via a degenerin/epithelial sodium channel complex.[2,14,92,93,98–101]

Additional theories to explain the autoregulatory phenomenon evolved because of the recognition that slower acting hemodynamic mechanisms are responsive to the metabolic demands of tubular transport. The existence of structures within the kidney that seem ideally suited to act as communication links between the distal tubular segments and the vasculature provide the morphologic basis for the TGF hypothesis. Indeed, the unique configuration of the juxtaglomerular apparatus (Fig. 3.4) allows the macula densa to sense some aspect of fluid composition at the end of the ascending loop of Henle and transmit a signal(s) to the afferent arteriole of the parent glomerulus. Thus, the juxtaglomerular apparatus provides the anatomic basis for a negative feedback mechanism, operating in each nephron,

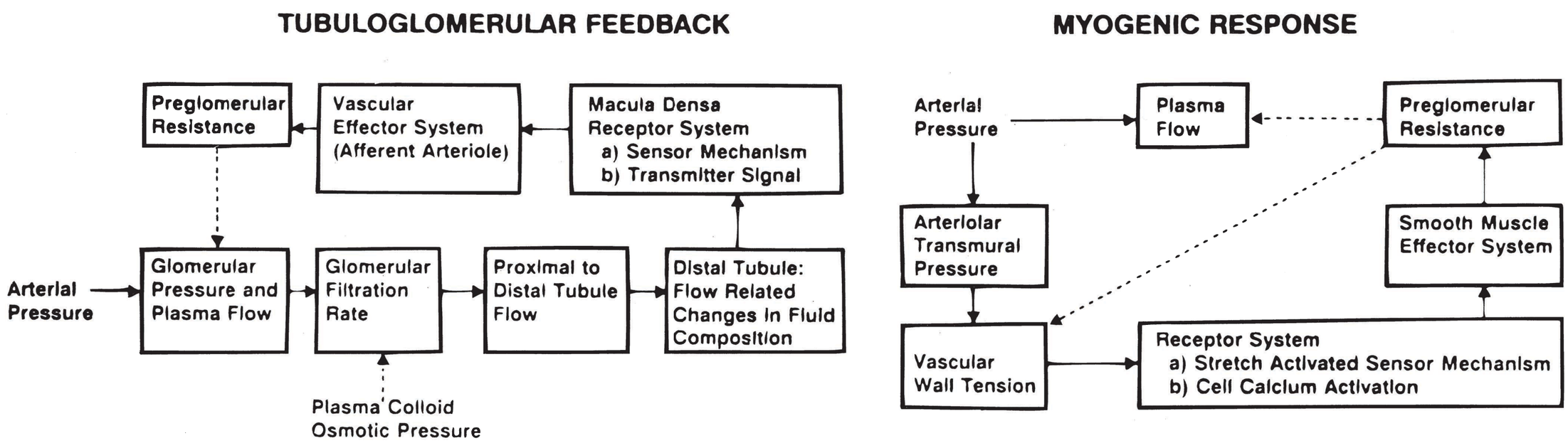

FIGURE 3.16 The macula densa tubuloglomerular feedback hypothesis (*left panel*) and the myogenic response (*right panel*) as mechanisms to explain renal autoregulation. *Solid lines* indicate direct relations; *dashed lines* indicate inverse effects.

that maintains balance between the hemodynamic inputs that control GFR and the filtered load and the metabolically determined reabsorptive function of the tubules.[1,2,102]

Autoregulation mediated by the TGF mechanism is shown in the left panel of Figure 3.16. For example, an increase in arterial pressure initially increases RBF, glomerular pressure, and GFR. The increased filtered load increases fluid and solute delivery from the proximal convoluted tubule into the loop of Henle. Such an effect leads to flow-dependent increases in NaCl concentration and osmolality of the tubular fluid in the ascending loop of Henle. The macula densa cells sense the increased tubular fluid NaCl or total solute concentration and transmit a vasoconstrictor signal(s) to the afferent arteriole and thus restore RBF and GFR to preexisting levels. Conversely, a decrease in arterial pressure causes a reduction in tubular fluid flow that elicits dilation of the afferent arterioles. The presence of primary cilia on the lumen of macula densa cells provides a flow sensing mechanism such that increased flow, per se, activates macula densa signals that elicit afferent arteriolar constriction. The TGF mechanism helps explain vascular responses that occur when the solute load to the distal nephron changes as a consequence of changes in tubular reabsorptive function such as those pharmacologically induced in the proximal reabsorption rate.[2,12,14,103–105]

Studies at the single nephron level indicate that the maintenance of flow to the distal nephron is a requisite for the full manifestation of autoregulation of GFR. As is shown in Figure 3.17, autoregulation of single nephron GFR in

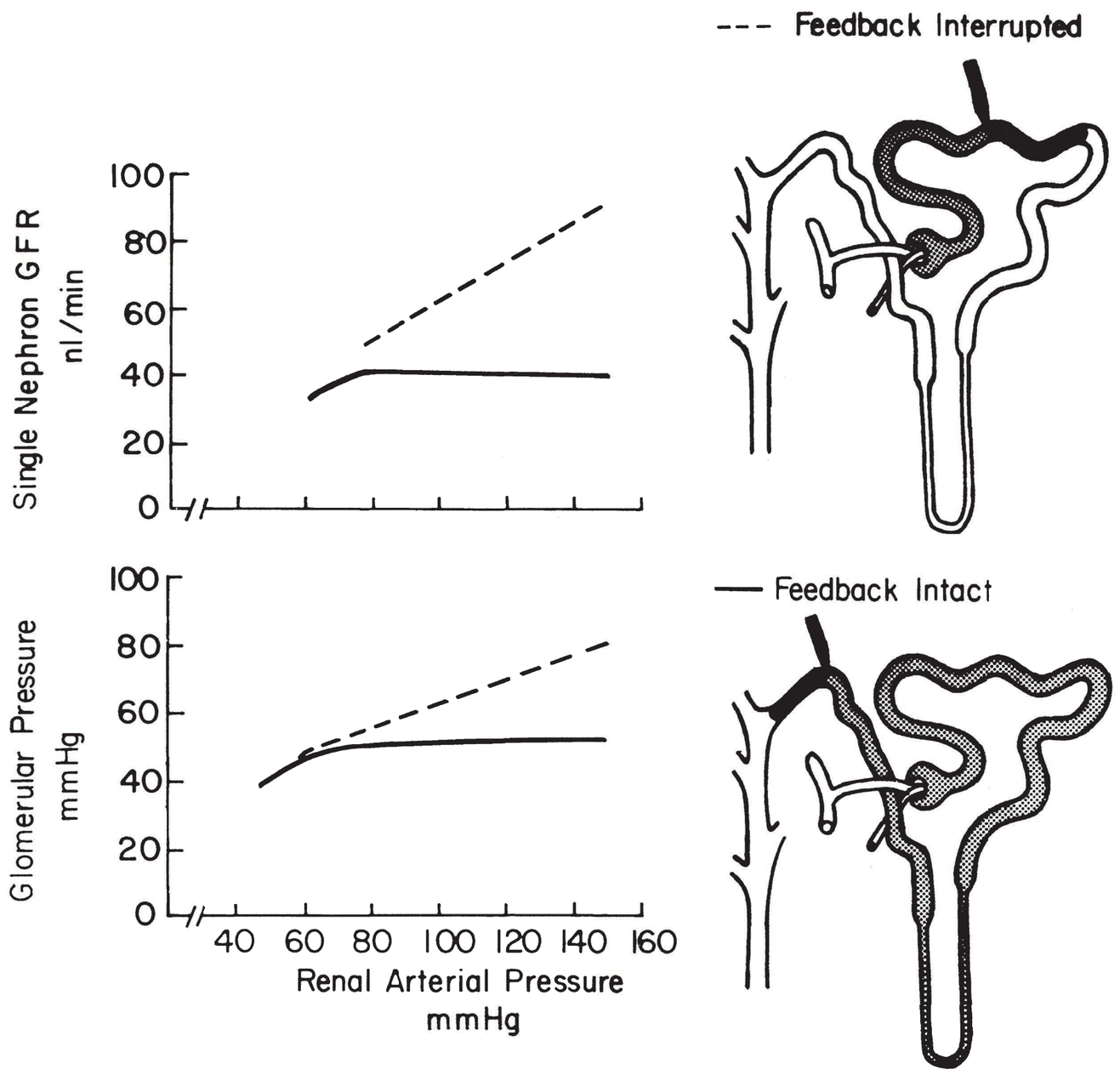

FIGURE 3.17 Responses of single nephron glomerular filtration rate (*GFR*) and glomerular pressure to changes in arterial pressure during conditions of intact flow to the macula densa (*solid lines*) and during interrupted flow conditions (*dashed lines*).

response to acute changes in arterial pressure is highly efficient when tubular fluid flow to the distal nephron is maintained, but it is significantly impaired when flow past macula densa cells is interrupted. Similar responses have been reported for glomerular capillary pressure. Nevertheless, the impairment in GFR autoregulation is not as great as would be predicted for a fully passive mechanism, indicating that the TGF mechanism works in concert with the myogenic mechanism to yield the highly efficient autoregulation characteristic of renal circulation. Recent studies suggest that the interactions are synergistic, in that the presence of active macula densa signals augments the sensitivity of the myogenic response. The afferent arteriole is the effector limb of both mechanisms, and the blockade of Ca^{2+} entry through L-type channels inhibits both the myogenic and TGF responses. The highly localized myogenic component can respond very quickly to a pressure stimulus. The TGF loop is more complex, involving multiple structures and cell types, and its response to a pressure change being transmitted along the tubule is slower, on the order of 15 seconds. The relative importance of these two systems may vary according to experimental conditions. Normally each contributes about 45% to near perfect autoregulation initiated by an acute, single step change in renal perfusion pressure. A putative third component contributes roughly 10% to the final adjustments in RVR that occurs between 25 and 100 seconds.[92,93,96,97,106]

Increases in flow through the loop of Henle elicit the constriction of the parent afferent arteriole with consequent reductions in glomerular pressure and the filtration rate of the same nephron. These responses are represented in Figure 3.18. Note that the response is nonlinear, with the most sensitive region in the physiologic range of tubular flow. Another important feature is that the reactivity or sensitivity of the TGF mechanism can be modified by a variety of paracrine agents, hormones, and pharmacologic agents. Increased sensitivity is generally associated with extracellular fluid volume contraction, and reduced responsiveness has been observed during expansion of extracellular fluid volume.[107]

The macula densa sensing segment is located at the end of the ascending loop of Henle, a nephron segment that is virtually impermeable to water and has a powerful NaCl cotransport mechanism. Such transport characteristics result in the delivery of a hypotonic fluid to the macula densa cells. The nature of the transport processes of the thick ascending limb is discussed in detail in Chapter 4. In essence, increases in fluid delivery from the proximal tubule lead to progressive increases in distal flow, sodium chloride concentration, and osmolality. This coupling between fluid flow through the ascending limb and tubular fluid solute concentration at the macula densa provides the means by which volume delivery out of the proximal tubule is sensed and regulated. The signal sensed by macula densa cells may be a specific constituent of tubular fluid such as sodium or chloride or total solute concentration.[1,107,108]

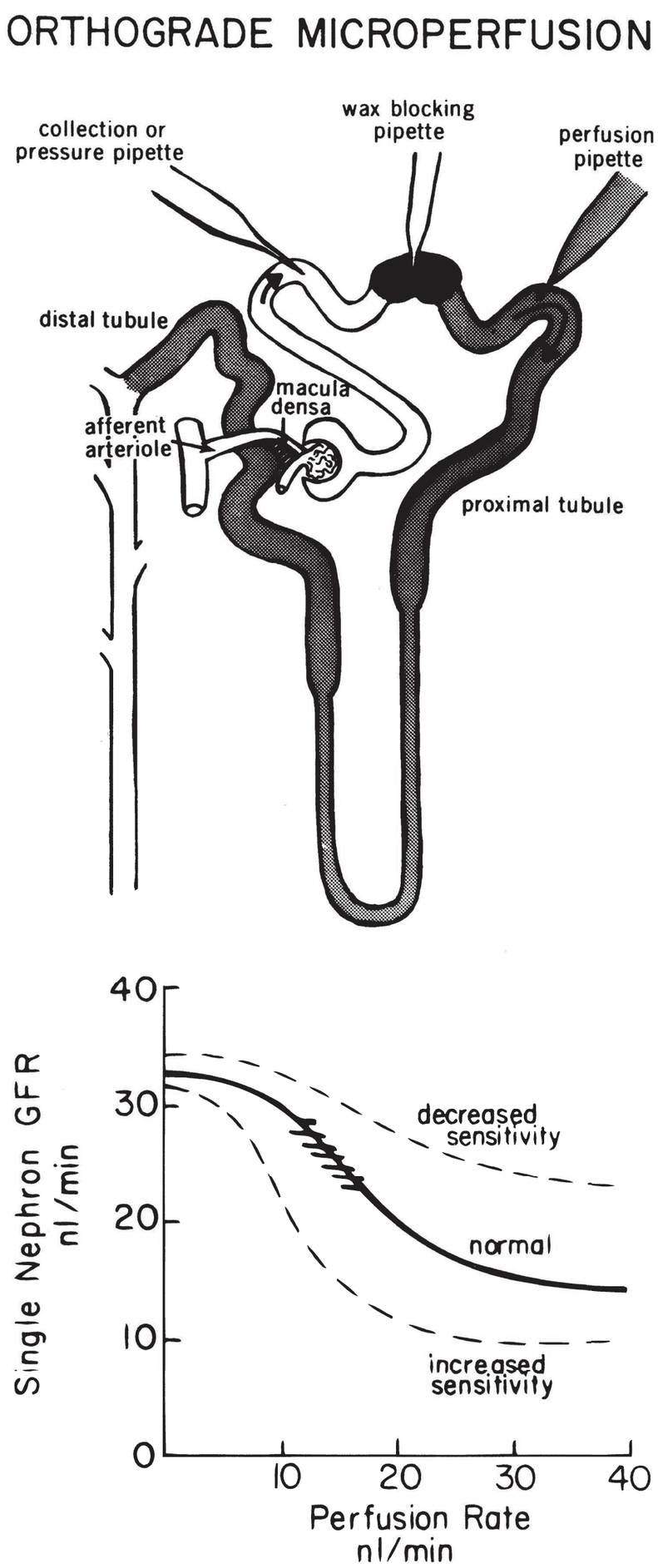

FIGURE 3.18 The relationships between the perfusion rate into a late proximal tubule and a single nephron glomerular filtration rate (GFR). *Dashed lines* show responses during conditions of decreased and increased feedback sensitivity. The *stippled area* shows the physiologic range of single nephron GFR as a function of late proximal flow rate. The technique used to obtain the feedback responses is illustrated in the *top panel*.

The cellular mechanisms responsible for transmitting signals have remained controversial and intriguing. Macula densa cells, like those of the thick ascending limb of Henle, possess a Na-K-2Cl cotransporter, which is sensitive to the diuretic furosemide. This cotransporter must be functional for TGF signals to be transmitted, but it may not be the actual mechanism that activates intracellular signals, which appear to involve increases in Ca^{2+} entry from the basolateral side of the macula densa cells and increased $[Ca^{2+}]_i$ in response to increases in tubular fluid osmolarity or NaCl concentration

of the luminal fluid. The mechanisms by which $[Ca^{2+}]_i$ is related to the formation and release of vasoactive mediators of TGF signals remain unclear. Calcium changes can be counteracted by elevations in cAMP. These intracellular ionic changes signal the macula densa cells to form and secrete constrictor and dilator substances that influence vascular contraction as a function of macula densa transport. The actual final effector messenger between the macula densa cells and the afferent arteriole(s) remain under investigation. Early studies fostered the idea that the effector signal was locally formed Ang II. However, it is now clearly established that the changes in the activity of the renin–angiotensin system modulate the sensitivity of the feedback response but do not directly mediate TGF signaling. The effector agent is thought to operate primarily by activating voltage-dependent Ca^{2+} channels in the afferent arteriole and perhaps the interlobular artery. Attractive candidates that have been considered recently include purinergic agents such as adenosine and ATP or arachidonic acid metabolites. Several recent studies link the secretion of ATP by macula densa cells to afferent arteriolar constriction via the activation of P_2 receptors and associated increases in Ca^{2+} entry via voltage-dependent Ca^{2+} channels. In support of this notion, a defective P_2X receptor function is associated with impaired TGF function of juxtamedullary nephrons.

An alternative view is that the ATP is metabolized to adenosine, which elicits afferent arteriolar vasoconstriction. Consistent with this proposal, mice lacking adenosine A_1 receptors do not exhibit TGF responses in superficial nephrons. Also, mice lacking the nucleotidase involved in degrading ATP to adenosine have impaired TGF. Another possibility is that an arachidonic acid metabolite such as 20-HETE participates in mediating TGF-dependent vasoconstriction. Studies show that increased luminal NaCl concentration leads to NO release, which counteracts the constrictor response. In contrast, PGE_2 release is increased by reduced NaCl concentration.[2,12,103,107,109–112]

The Modulation of Tubuloglomerular Feedback Activity by Vasoactive Agents

Macula densa cells also signal the juxtaglomerular cells to regulate renin synthesis and release. Mechanisms of renin release are discussed in the section on the renin–angiotensin system. In brief, the directional changes in renin release and Ang II formation are opposite to those that are required for Ang II to mediate TGF. For example, high tubular flows and elevated luminal NaCl concentrations are associated with reduced renin release but TGF-mediated afferent arteriolar constriction. Nevertheless, Ang II exerts an important role in modulating TGF activity during changes in salt diet, extracellular fluid volume, and renal perfusion pressure. Tubuloglomerular feedback is absent in mice lacking AT_{1A} receptors, although the renal vasculature is capable of responding to Ang II. Tubuloglomerular feedback is nonresponsive in animals unable to produce Ang II when ACE is mutated.[2,14,104]

The neuronal NO synthase (NOS) isoform localized in macula densa cells produces NO in response to increased tubular flow above the normal range, which modulates TGF activity. A blockade of NO synthesis augments the strength of TGF responses, whereas enhanced NOS levels attenuate the vasoconstrictor response to increased distal nephron flow. Salt uptake across the luminal membrane by a furosemide-sensitive Na-K-2Cl transporter may link increases in cellular cAMP and $[Ca^{2+}]_i$ to NO production. O_2 radicals generated in the vicinity of the juxtaglomerular apparatus can act to scavenge NO, limiting macula densa NO signaling and thereby producing vasoconstriction and enhancing TGF activity. Normal TGF is found in gene knockout animals lacking neuronal NOS.[2,113–115]

Arachidonic acid metabolites also modulate TGF activity and interact with other vasoactive modulators. Cyclooxygenase 2 (COX-2) has been localized to macula densa cells and the surrounding ascending loop of Henle cells; the release of PGE_2 from macula densa cells occurs in response to reduced luminal NaCl. A COX-2 metabolite attenuates the vasoconstrictor autoregulatory and TGF-mediated response of the afferent arteriole to an increase in arterial pressure. Such a dilator agent also appears to contribute to the macula densa production of NO, which inhibits afferent arteriolar responses to pressure. Thromboxane A_2 and a cytochrome P450 metabolite such as 20-HETE are also involved in the constrictor limb of TGF. However, gene targeting rendering the thromboxane receptor nonfunctional has no effect on TGF activity.[104,116–118]

Recent evidence supports the existence of a second TGF loop that links increases in connecting tubule sodium reabsorption via epithelial sodium channels to dilation of the afferent arteriole of the parent glomerulus. It is noteworthy that the response of this positive feedback loop is opposite to the constrictor signal arising from macula densa cells in response to increased salt delivery to the end of the thick ascending limb of Henle. The connecting tubule signal transmitted to the afferent arteriole to increase GFR involves COX-derived prostaglandins and epoxygenase-derived EETs. The magnitude of this feedback response is enhanced by Ang II acting on AT_1 receptors to stimulate epithelial sodium channel activity and sodium reabsorption. The connecting tubule–glomerular feedback circuit may function to dampen the effects of vasoconstrictor stimuli on the afferent arteriole.[119,120]

The Renin–Angiotensin System

The Formation of Ang II

The renin–angiotensin system exerts control of renal hemodynamics via its major vasoactive metabolite Ang II, which acts as both a circulating hormone and a locally generated paracrine agent. Renin is a proteolytic enzyme that cleaves Ang I from renin substrate (angiotensinogen) that is formed primarily by the liver but also in the kidney. Renin is synthesized primarily in epithelioid granular cells of the

juxtaglomerular apparatus and is secreted into the surrounding interstitium; renin is also formed in proximal tubule cells and principal cells of connecting tubule and collecting duct segments. Angiotensinogen availability in the plasma and intrarenal compartments is less than is required to produce maximum reaction velocity, so alterations in substrate levels contribute to the regulation of Ang I production. The inactive decapeptide Ang I is then cleaved by ACE to form the active octapeptide Ang II. There are abundant amounts of endothelium-bound ACE in the lungs and in almost all other tissues. Most of the Ang II in the systemic arterial blood is formed in the lungs. The major sites of ACE localization in the kidney are on the luminal surface of endothelial cells lining arteries and arterioles (in particular, the afferent arterioles), but also the efferent arterioles and the glomerular capillaries, and on the brush border and basolateral membranes of the proximal tubule; extravascular ACE is also present in the interstitial compartment and on the lumen of collecting duct segments.[2,13,121–125]

Several peptidases act on angiotensinogen to form biologically active peptides other than Ang II. Angiotensin with amino acids 1 to 7 is formed by neutral endopeptidase, but appears to have only slight effects on the renal vasculature under physiologic conditions. Elevated levels of Ang 1 to 7 occur during ACE inhibition and may dilate renal and nonrenal vascular beds. Ang III (angiotensin with amino acids 2 to 8) has actions similar to that of Ang II, which can be blocked by Ang II receptor antagonists. Ang IV (angiotensin with amino acids 3 to 8), a hexapeptide, is reported to produce vasodilation by the release of NO or prostanoids from endothelial cells by acting on a specific AT_4 receptor. The recently described enzyme ACE-2 forms Ang (1 to 9) from Ang I and Ang (1 to 7) from Ang II. Thus, ACE-2 can produce more Ang (1 to 7) from Ang II and, in this manner, reduces the available Ang II.[124,126–128]

Ang II is delivered to renal vascular receptors as a circulating hormone or may be formed locally from systemically delivered Ang I by endothelial ACE. About 20% of the circulating Ang I is converted to vasoactive Ang II in the kidney. Ang II is also formed in the interstitial fluid from Ang I, generated as a consequence of enhanced renin release or from Ang I that diffuses from peritubular capillaries into the interstitium. The renal tissue Ang II levels are higher than that of arterial blood, indicating significant amounts of Ang II are generated intrarenally. In addition, Ang I and II may be formed within juxtaglomerular granular cells and coreleased with renin to act on adjacent glomerular arterioles. High concentrations of Ang II also exist in proximal and distal tubular fluid as a result of secretion by proximal tubular cells. Ang II derived from proximal tubular cells also may have vascular effects after traversing the interstitium.[123,129,130]

Renin Production and Release

As Figure 3.19 shows, renin is released in response to several stimuli, including decreases in sodium intake, the contraction of extracellular fluid volume or blood volume, increased sympathetic renal nerve activity, decreased sodium load to the macula densa, and decreased renal arterial perfusion pressure. Ang II, ET-1, NO, vasopressin, prostaglandins, and potassium also influence renin release, acting directly on juxtaglomerular cells. The final effector mechanisms center on changes in the Ca^{2+} and cAMP concentrations

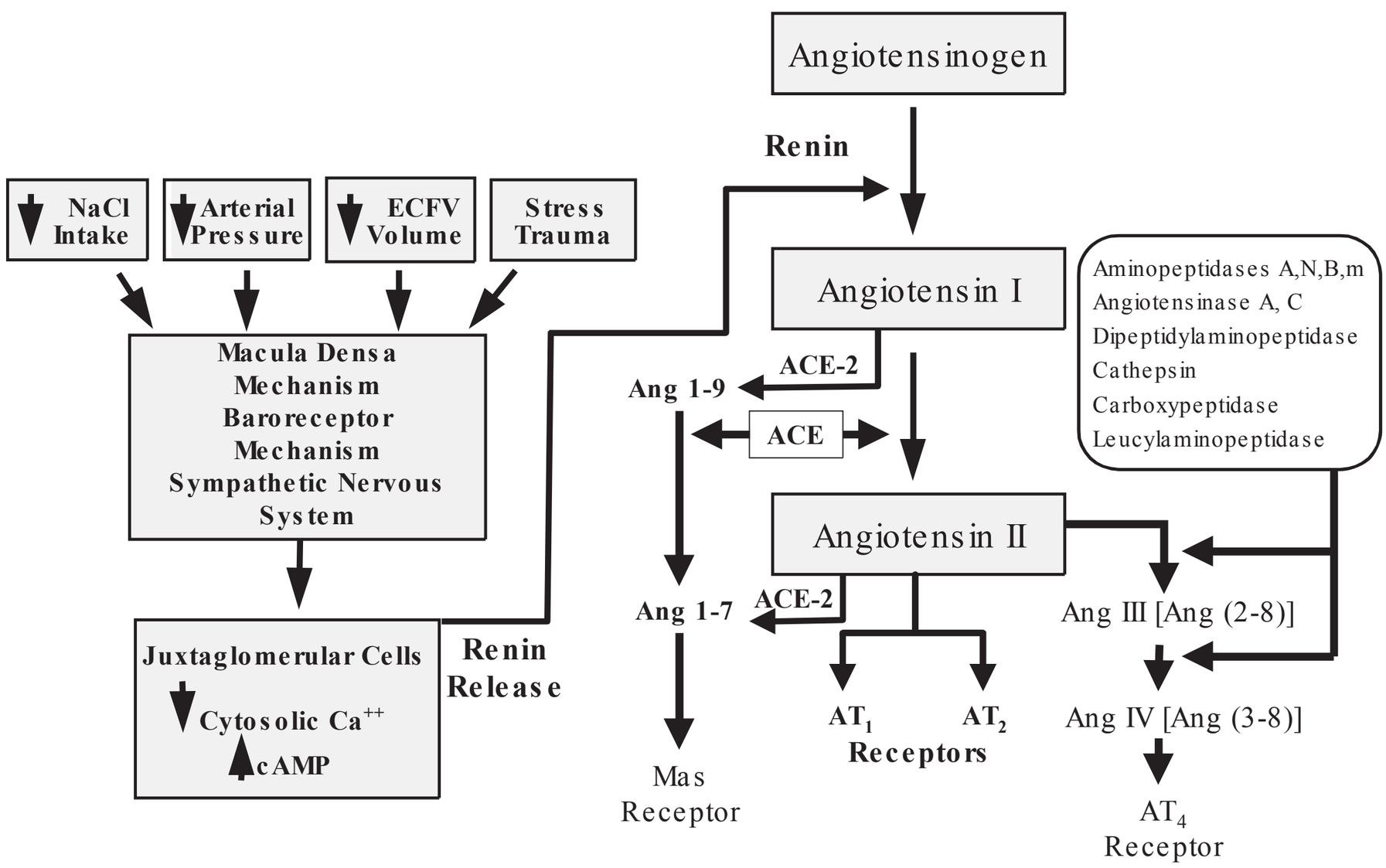

FIGURE 3.19 A schematic representation of the renin–angiotensin system and the mechanisms of renin release.

in juxtaglomerular granular cells. The Ca^{2+} mechanism is unusual for exocytosis of secretory renin granules in that a decrease in $[Ca^{2+}]_i$ functions as a stimulator of renin release, in contrast to the opposite in most other secretory cell types. Renin exocytotic release is elicited by increases in cellular cAMP levels. Cytosolic cAMP concentration is controlled by synthesis via adenylate cyclase and hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). Intracellular Ca^{2+} inhibits PDE to modulate the magnitude of cAMP-mediated renin release. PDE_3 is the major isoform localized to afferent arterioles, and recent evidence indicates that the pharmacologic inhibition of PDE_3 increases cAMP and basal renin secretion and also enhances the renin secretory response to β-adrenergic and PGE_2/EP_4 receptor stimulation.

Exocytosis of secretory granules in the juxtaglomerular cells is also stimulated by cell shrinkage due to high extracellular osmolality. Juxtaglomerular cells have Ca^{2+}-sensitive voltage-gated K channels (BK_{Ca}) that are activated by cAMP to hyperpolarize cells from -32 to -48 mV. Vasoconstrictor G-protein coupled receptor agonists such as Ang II and ET-1 inhibit renin release by activating PLC and mobilizing Ca^{2+} that activates Ca^{2+} entry through store-operated cation channels.[2,13,121,131]

There are several first messenger systems that impact on cAMP or $[Ca^{2+}]_i$ in juxtaglomerular cells to regulate renin release. These are discussed briefly in the following section and in more detail in Chapter 9.

Sympathetic Nervous System and Catecholamines. Juxtaglomerular granular cells are richly innervated and respond to renal sympathetic nerve stimulation and to circulating catecholamines. There are both direct and indirect effects on renin release. Subtle increases in renal nerve traffic or circulating epinephrine activate β_1-adrenoreceptors on juxtaglomerular cells, which activate $G\alpha_s$ proteins to increase cAMP, thereby enhancing renin release. Strong renal nerve activity reduces RBF and GFR through activation of α_1-adrenergic receptors, with subsequent indirect stimulation of renin release secondary to afferent arteriolar vasoconstriction that reduces intrarenal baroreceptor and macula densa stimuli.[13,132]

Renal Vascular Baroreceptor. Decreases in renal afferent arteriolar pressure directly increase renin release independent of the renal nerves and the macula densa mechanism. The juxtaglomerular cells appear to be directly sensitive to intraluminal pressure and stretch such that decreased wall tension decreases cell Ca^{2+} entry and $[Ca^{2+}]_i$, whereas the opposite occurs at elevated arterial pressures. Under some circumstances, the same extrinsic disturbance may influence both the macula densa and the vascular baroreceptor mechanism to increase renin release, but the vascular receptor system can act independently.[2]

Macula Densa. Macula densa cells detect decreases in the NaCl load or concentration emerging from the ascending loop of Henle and send a signal(s) to the juxtaglomerular cells to increase renin secretion. Although the sensing mechanism involving furosemide-sensitive luminal uptake of NaCl is not completely understood, evidence suggests that adenosine can inhibit renin release, and its precursor, ATP, is secreted by macula densa cells in response to increases in tubular NaCl concentration and is converted to adenosine via ectonucleotidases. Circumstances that result in extracellular volume depletion or sodium deprivation stimulate renin release, at least in part, by the macula densa mechanism. Reduced tubular fluid flow to the macula densa leads to increased COX-2 activity and PGE_2 release that directly stimulates renin release via activation of EP_4 receptors and increased cAMP production in juxtaglomerular granular cells.[12,14,116,133]

Other Factors. Renin secretion is inhibited by elevated plasma or local concentrations of Ang II, vasopressin, adenosine, thromboxane A_2, and potassium. The effects of Ang II and other vasoconstrictors appear to be a consequence of an end-product inhibition due to increased $[Ca^{2+}]_i$ in juxtaglomerular cells. PGE_2 and PGI_2 can stimulate renin release through direct effects, which may be related to the stimulation of cellular cAMP levels. The atrial natriuretic peptide increases cellular cGMP production and inhibits renin release. Endothelium-derived factors also modulate renin release, with NO stimulating renin release.[13,116,121,134]

COX-2, nNOS, and renin synthesis often change parallel with changes in salt diet and alterations in tubular fluid NaCl concentration, and their products may mutually determine synthesis and activity of these enzymes. nNOS and COX-2 are coexpressed in macula densa cells and the expression of both enzymes is stimulated in volume contraction and high renin states. Parallel changes are observed during chronic changes in salt in the diet, with low salt stimulating nNOS, COX-2, and renin secretion as compared to the inhibition occurring during a chronic high salt diet. NO derived from nNOS exerts a stimulatory role on COX-2 expression to produce PGE_2 / PGI_2 and to stimulate renin release by acting on EP_4 and IP receptors, respectively. PGE_2 exerts short-loop feedback to inhibit nNOS expression. In some situations, NO appears to play a more indirect permissive role, permitting the macula densa pathway of renin secretion to function normally. NO stimulates renin release by cGMP, inhibiting PDE_3 to increase cell cAMP concentration by reducing cAMP breakdown in juxtaglomerular cells, with the activation of cAMP sensitive PKA.[13,116,121,135]

Although many stimuli increase activity of the renin–angiotensin system, most are related to circumstances that compromise body fluid volume homeostasis. Thus, the stimulation of renin release and the activation of Ang II–dependent mechanisms help to minimize renal fluid and sodium losses and to maintain extracellular fluid volume and arterial blood pressure. The myriad of actions exerted by Ang II all seem to be homeostatically appropriate to achieve this end. Only the renal vascular actions of Ang II will be covered in this chapter, but it should be pointed out

that Ang II is also a potent stimulator of aldosterone release and can directly enhance salt reabsorption by the proximal tubule, the Henle loop, and the distal nephron segments. It also has important effects on the central nervous system such as stimulating thirst, vasopressin release, and sympathetic nerve activity.[2,129]

The macula densa plaque has distinct mechanisms for renin release and the TGF system. The TGF mechanism involves the macula densa by sending a vasoconstrictor signal to the afferent arteriole in response to increases in tubular flow and the accompanying increases in solute and sodium concentration from the Henle loop. Under these conditions, the macula densa signals to decrease renin release and local Ang II activity, which is opposite to that required for Ang II to mediate a TGF-induced contraction of the afferent arteriole. Thus, Ang II clearly does not mediate TGF responses; it does, however, modulate the sensitivity of the TGF mechanism to the macula densa vasoconstrictor signal(s). When tissue Ang II levels are increased, smooth muscle responsiveness is augmented. In contrast, when Ang II levels are suppressed, whether in response to physiologic manipulations or as a consequence of a pharmacologic blockade, the sensitivity of the TGF system is attenuated. Interestingly, sensitivity can be restored by the administration of Ang II but not norepinephrine. In addition to directly affecting the afferent arteriole, Ang II affects TGF activity by altering tubular reabsorption and fluid delivery to the macula densa. The vascular and tubular effects work in concert to reduce GFR when sodium excretion is reduced as observed during volume contraction. As previously mentioned (Fig. 3.18), changes in TGF responsiveness during altered volume states may be largely due to changes in tissue levels of Ang II. The interactions between the renin–angiotensin system and the TGF mechanism are illustrated in Figure 3.20.[1,2]

The precursor of renin, prorenin, is also released by juxtaglomerular granular cells and renin-producing tubular cells. Although prorenin is inactive, it can bind to the prorenin receptor that has been recently discovered and characterized. Binding of prorenin to the prorenin receptor activates prorenin and increases its catalytic efficiency to generate Ang I from angiotensinogen. Thus, increased tissue levels of prorenin or the prorenin receptor may influence the local generation of Ang I, leading to increased Ang II formation. Prorenin also appears to signal via the MAP kinases extracellular signal regulated kinase (ERK) 1/2 to upregulate profibrotic and COX-2 genes independent of Ang II. Prorenin receptors are localized in vascular endothelial and smooth muscle cells, glomerular mesangial cells and podocytes, and collecting duct segments of the nephron.[136,137]

Angiotensin Receptors

There are two major subtypes of receptors for Ang II in the renal circulation. In utero, animals have a larger population of AT_2 than AT_1 receptors, which decreases progressively after birth; in adult life, the major subtype on vascular smooth muscle cells is the AT_1 receptor. The AT_1 receptor is present on preglomerular arteries and arterioles, juxtaglomerular granular cells, glomerular mesangial cells, efferent arterioles, and vasa recta bundles of the inner medullary stripe. Humans have one AT_1 receptor, whereas rodents have two, termed AT_{1A} and AT_{1B}, which are 94% homologous. Currently available pharmacologic antagonists of AT_1 receptors, such as candesartan, losartan, and valsartan, do not distinguish between the two AT_1 subtypes in rodents. Almost all of the vasoconstrictor actions of Ang II on the renal vasculature under physiologic conditions are mediated by AT_1. Both

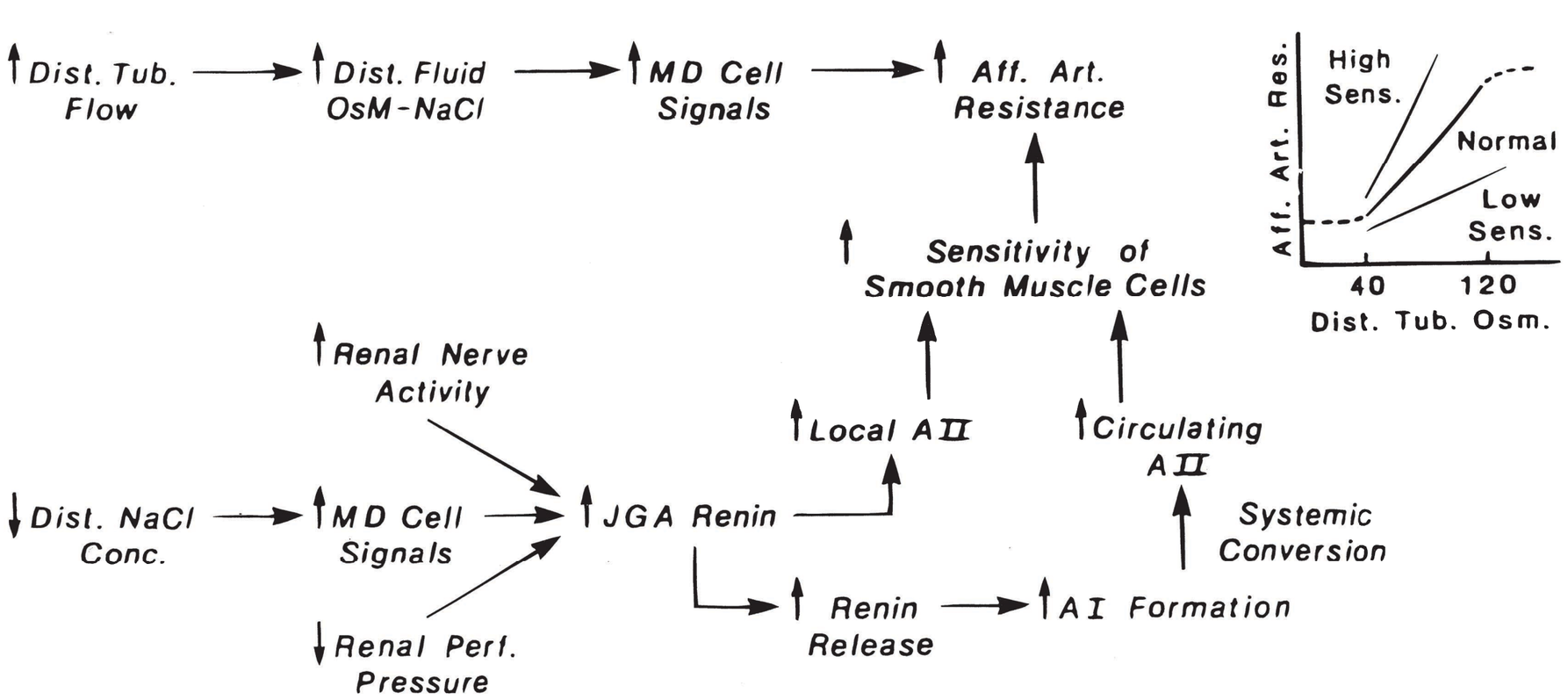

FIGURE 3.20 The modulator hypothesis for postulated interactions between the tubuloglomerular feedback mechanism and the intrarenal renin–angiotensin system. Flow-related changes in the tubular fluid concentration can elicit signals from macula densa (*MD*) cells to vascular contractile cells independent of angiotensin levels. Changes in the angiotensin II (*A II*) concentration influence the sensitivity or responsiveness of macula densa cells or of vascular smooth muscle cells to the signals coming from the macula densa cells. *A I*, angiotensin I; *A II*, angiotensin II; *Aff. Art.*, afferent arteriole; *JGA*, juxtaglomerular apparatus.

the AT_{1A} and AT_{1B} receptor mediate Ang II–induced Ca^{2+} signaling in smooth muscle cells and renal vasoconstriction. Evidence for the role of AT_{1B} receptors derives from mice lacking a functional AT_{1A} receptor.[36,138–141]

The AT_1 receptor is primarily coupled to the GTP-binding protein $G\alpha_{q11/12}$, whose activation leads to the triggering of several signaling pathways, including the stimulation of PLC_β. The receptor activation of protein tyrosine kinases leads to somewhat slower stimulation of phospholipase $C\gamma$. Phospholipases D and A_2 may also be activated, favoring the release of DAG and phosphatidic acid from phosphatidylcholine and arachidonic acid, respectively. The PLCs act on membrane-bound phosphoinositides to yield DAG and IP_3. DAG stimulates protein kinase C, whereas IP_3 diffuses through the cytosol to activate IP_3-sensitive receptors/release channels on membranes of the sarcoplasmic reticulum, triggering Ca^{2+} release to the cytosol. The mobilized Ca^{2+} triggers a complex array of events. Calcium-sensitive chloride channels may be stimulated to promote chloride efflux and depolarization of the plasma membrane. Such a signal will activate voltage-gated L-type calcium channels to allow for influx down a very steep gradient, from 1 to 2 mM in the extracellular fluid to 100 to 200 nM in the cytosol. Calcium release from internal stores also signals store-operated cation channels to open and allow further Ca^{2+} entry. AT_1 receptor activation may also trigger voltage-dependent Ca^{2+} channels independent of Ca^{2+} release from intracellular stores. As discussed earlier, afferent arteriolar contractions induced by Ang II is dependent on Ca^{2+} entry through voltage-gated channels, whereas efferent arterioles are not affected by L-type channel blockers but are influenced by T-type channel blockers. Recent studies indicate that AT_1 receptors rapidly activate NADPH oxidase to produce superoxide anion by the afferent arteriole. This leads to Ca^{2+} mobilization mediated by RyR through a direct action and/or one mediated by ADP ribosyl cyclase and the production of cADP ribose that sensitizes RyR/release channels on the sarcoplasmic reticulum to increase $[Ca^{2+}]_i$.[1,2,36,107,142,143]

The AT_2 receptor is characterized by a high affinity to the nonpeptide antagonists PD123319, CGP 42112, and Compound 21, and has a sequence 33% identical to that of the AT_1 receptor. Its high expression in fetal tissue suggests a role in embryonic development. This receptor has a typical pattern of seven transmembrane domains and is coupled to a G protein. Recent studies suggest that AT_2 receptors exert modulatory actions to partially counteract the effects caused by AT_1 receptor activation. AT_2 receptor activation increases bradykinin and NO levels, leading to increases in cGMP and vasodilation. AT_2 receptor activity may be upregulated during chronic salt deprivation. AT_3 and AT_4 receptors may be selective for angiotensin with amino acids 1 to 7 and Ang IV (angiotensin with amino acids 3 to 8), although they appear to play a minor role in regulating renal hemodynamics.[107,126,138,144]

Ang II receptors are regulated in response to different physiologic conditions. It is noteworthy that glomerular and vascular receptors are regulated differently from proximal tubular receptors. A low salt diet and high levels of Ang II lead to the downregulation of arteriolar and mesangial cell AT_1 receptors and the upregulation of tubular receptors.[140,145,146]

Actions of Ang II on the Renal Microvasculature

Ang II elicits dose-dependent AT_1-mediated decreases in RBF and GFR. The decreases in GFR are often smaller than the decreases in RBF such that filtration fraction increases. The increased vascular resistance is due to both afferent and efferent arteriolar constriction, and glomerular capillary pressure is well maintained. At high concentrations the glomerular filtration coefficient is reduced. Ang II produces more pronounced vasoconstriction when endogenous levels are low, presumably because of receptor upregulation and when vasodilator prostaglandins are blocked by COX inhibitors. Larger Ang II effects are also noted after the endothelial production of NO is blocked. As mentioned earlier, Ang II can potentiate TGF-mediated changes in preglomerular vascular tone. The multiple effects of Ang II are illustrated in Figure 3.21. In addition

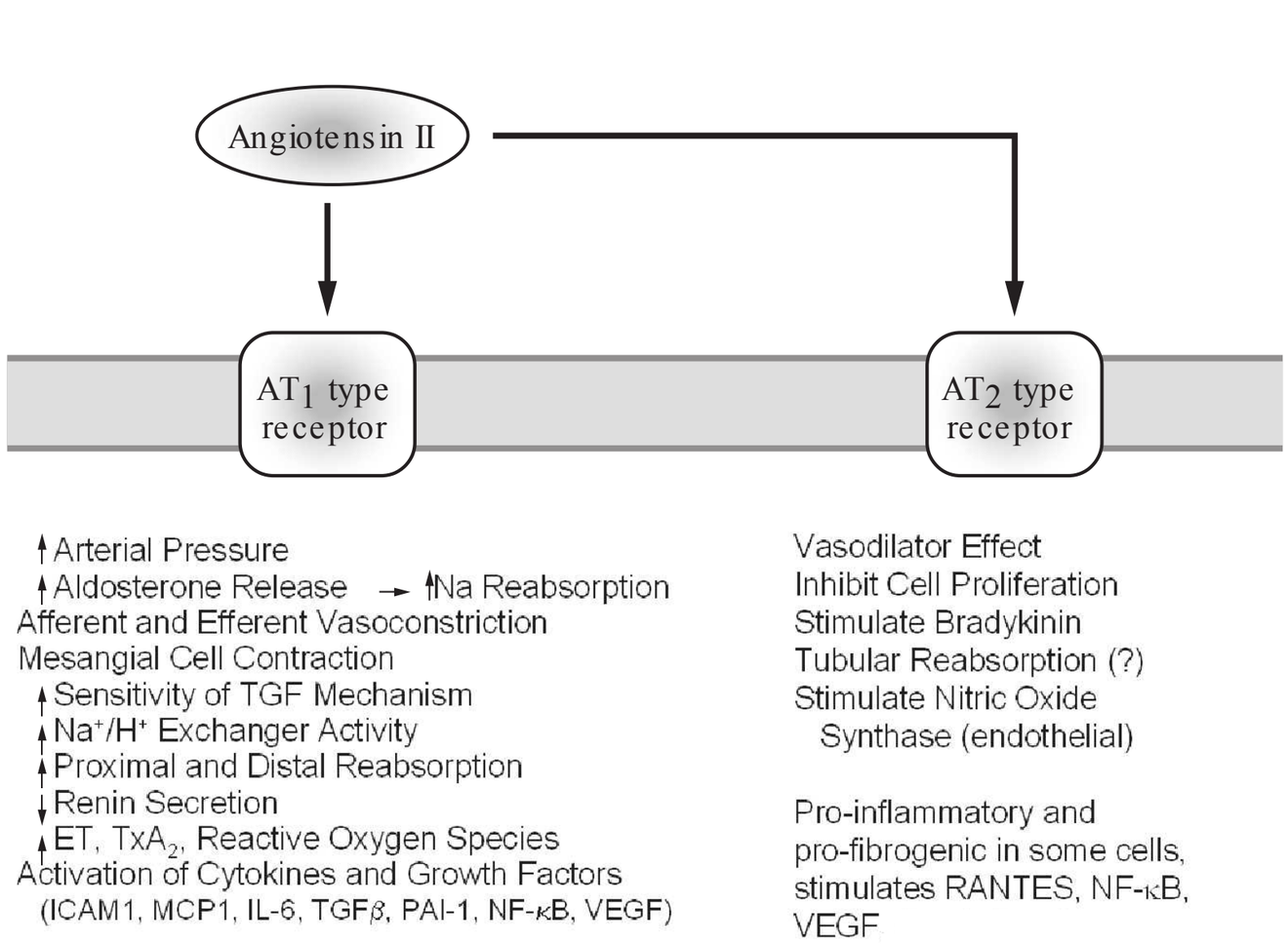

FIGURE 3.21 Multiple actions of angiotensin II on renal function mediated by AT_1 and AT_2 receptors. *ET*, endothelin; T_XA_2, thromboxane; *ICAM1*, intercellular adhesion molecule 1; *MCP1*, monocyte chemotactic protein 1; *IL-6*, interleukin 6; *TGFβ*, transforming growth factor β; *PAI-1*, plasminogen activator inhibitor 1; *NF-κB*, nuclear factor kappa light chain enhancer of activated B cells; *VEGF*, vascular endothelial growth factor.

to these effects, Ang II influences the medullary circulation, notably at concentrations lower than those required to elicit overall vasoconstriction in the cortex. This notion is supported by the finding that the Ang II receptor density is much higher in the medullary vessels and in the interstitial cells than in the postglomerular cortical vasculature.[1,2,36]

The effects of endogenous Ang II are observed using angiotensin receptor antagonists or ACE inhibitors in states when the prevailing Ang II levels are high. During sodium restriction, RBF is increased after the AT_1 receptor blockade, whereas the GFR responses are smaller and more variable; filtration fraction usually declines. Renin inhibition produces similar results, and combined renin and angiotensin inhibition causes decreases in RVR and increases in GFR. Activation of the AT_1 receptor is primarily responsible for the renal vascular effects of the endogenous Ang II on afferent and efferent arterioles and on K_f. Whole-kidney autoregulation is not affected by AT_1 receptor blockade or ACE inhibition, although the contribution of the TGF mechanism may be reduced and the plateau of the autoregulation relationship is elevated.[1,2,36]

Endothelium-Derived Vasoactive Factors

Nitric Oxide

Endothelial cells release NO in response to many stimuli, including mechanical shear stress and hormone, paracrine, and autocrine factors, that increase $[Ca^{2+}]_i$. NO is formed by vascular endothelial cells from the amino acid L-arginine via NOS, which is a soluble $NADPH^-$, and the Ca^{2+}-calmodulin–dependent citrulline-forming enzyme that requires two cosubstrates (O_2 and NADPH) and four cofactors (heme, flavin mononucleotide, flavin adenine dinucleotide, and tetrahydrobiopterin). As shown in Figure 3.7, several agents induce their vascular effects via endothelial cell eNOS activation and the release of NO. Examples include factors that stimulate the M_1-muscarinic receptor (acetylcholine), the B_2-kinin receptor, the α_2-adrenoceptor, the purinergic receptors (ATP, ADP), and the ET_B receptor. The rate of renal NO production is higher in the medulla than in the cortex. NO and nitrosamines have short biologic half-lives of less than 10 seconds, which is especially shortened by hemoglobin scavenging and in oxygenated solutions in the presence of the superoxide anion ($\cdot O_2^-$), by a mechanism involving the production of peroxynitrite (NO_3^-) from NO. In the same manner, NO acts to scavenge $\cdot O_2^-$.[147–149]

The basal release of NO tonically maintains a low RVR at rest, acting to buffer vasoconstriction produced by Ang II, ET-1, catecholamines, and other endogenous factors. Acute inhibition of basal NO production causes renal vasoconstriction, with decreases of 25% to 35% in RBF and reductions in cGMP levels in the interstitium and the urine. GFR responses are smaller and tend to be unchanged or decrease about 10%. Thus, the filtration fraction is increased. Basal NO dilates both the preglomerular vasculature (arcuate and interlobular arteries, afferent arterioles) and the efferent arterioles. NO also appears to contribute to the increased or maintained glomerular filtration coefficient. Endothelial factors mediate or modulate pressure-dependent responses of vascular segments exhibiting autoregulatory behavior. Note however, that NO does not fit a mediator role in this scheme because increased pressure and shear stress cause increased production of NO, a vasodilator, at the same time the responsive vascular elements exhibit vasoconstriction. NO inhibition results in renal vasoconstriction, but the steady-state autoregulatory RBF responses to changes in perfusion pressure are unaffected and remain highly efficient.[2,149,150]

NO-induced renal vasodilation is mediated by cGMP-dependent and perhaps a cGMP-independent system. The latter appears to involve cGMP cross-talk with the cAMP pathway, with cGMP inhibiting cAMP breakdown by PDE_3, more so than the activation of cGMP-protein kinase activity. Part of the dilatory response to NO may be mediated through increased K^+ channel activity and reduced production of 20-HETE.[62,118,151]

Although NO does not affect overall steady-state autoregulatory adjustments in RVR, NO attenuates the strength and rapidity of the myogenic adjustments in resistance. NO also attenuates the strength of TGF responses to increased distal tubular flow. The inhibition of NOS leads to greater relative decreases in medullary blood flow than in cortical blood flow. A NO blockade usually reduces renin release and responsiveness to reductions in perfusion pressure.[92,93,152,153]

Endothelial NO synthase is localized to the endothelial cells all along the renal vasculature (the interlobular arteries, the afferent and efferent arterioles, the glomerular capillaries, and the vasa recta) and the thick ascending limb of the Henle loop. Targeting of eNOS to specialized plasma membrane invaginations termed caveolae is required for maximal eNOS activity. Neuronal NO synthase (nNOS) is present primarily in epithelial cells (thick ascending limb of the Henle loop, the macula densa, the collecting duct). NO inhibits tubular transport along the nephron. An inducible form of NO synthase is normally quiescent but is capable of producing large amounts of NO in vascular smooth cells and mesangial cells during inflammation. An endogenous inhibitor of L-arginine cellular uptake and NOS activity is asymmetric dimethylarginine (ADMA) that is normally inactivated by NG-NG-dimethylarginine dimethylaminohydrolase (DDAH) to form L-citrulline. DDAH expression is found at the same sites as NO synthase. ADMA concentrations are elevated during states of oxidative stress and disease.[154–156]

Endothelin

Endothelin refers to a family of long lasting vasoconstrictor peptides that act locally as paracrine hormones. ET-1, -2, and -3, each a 21 amino-acid peptide, are constitutively released. ET-1 is the major form synthesized and secreted by endothelial cells of the preglomerular vasculature and the vasa recta

and by the medullary collecting ducts. Endothelin-converting enzyme (ECE) is the main enzyme responsible for the genesis of ET-1 from prepro-ET-1 (<200 amino acids); chymase and matrix metalloproteinase II are also involved in the production of ET intermediates. Ang II is a potent stimulus for ET-1 production. Other simulants include bradykinin, ATP, platelet activating factor, thrombin, and shear stress. Neutral endopeptidase 24-11 degrades and inactivates ET-1. Cytokines such as interleukin-1-beta (IL-1β) stimulate ET-1 production. The two known receptor types, ET_A and ET_B, are G-protein coupled and lead to IP_3 and cyclic ADP ribose formation, PKC activation, and Ca^{2+} mobilization, in addition to Ca^{2+} entry. ET_A receptors are predominantly found on vascular smooth muscle cells. ET_B receptors are localized on endothelial and tubular cells as well as vascular smooth muscle cells. The afferent arteriolar constriction produced by ET-1, like that caused by Ang II, is dependent in part on Ca^{2+} entry through voltage-dependent L-type channels. The action of ET-1 on efferent arterioles appears to depend exclusively on the mobilization of intracellular Ca^{2+} and/or entry through voltage-independent cation channels. The highest concentration of ET-1 in the body exists in the renal medulla, where it is synthesized by collecting duct cells and acts in a paracrine/autocrine manner to cause natriuretic and diuretic effects through ET_B receptors via the stimulation of NO. In addition, ET-1 has inotropic, chemotactic, and mitogenic properties. Overall, ET-1 increases blood pressure and vascular tone.[156–158]

The vascular actions of ET-1 reflect a combination of vasoconstrictor ET_A and ET_B receptors on smooth muscle cells and ET_B receptors on endothelial cells, which cause vasodilation mediated by NO. Concurrent ET-1 stimulation of ET_A + ET_B receptors causes net renal vasoconstriction, and the inhibition of ET_B receptor activation leads to more pronounced vasoconstriction. Thus, endothelial ET_B receptors buffer the constriction caused by the stimulation of both ET_A and ET_B receptors on the smooth muscle cells. Nevertheless, selective stimulation of vascular ET_B receptors using a pharmacologic agonist elicits renal vasoconstriction. Thus, there appears to be a complex interaction between receptor signaling. Endothelial ET_B receptors in the kidney and lung also seem to function as nonsignaling clearance receptors, effectively reducing the local concentration of ET-1, and thereby attenuating vasoconstriction.[150,159]

Exogenous ET-1 reduces RBF by stimulating both ET_A and ET_B receptors to cause the constriction of the arcuate and interlobular arteries and the afferent and efferent arterioles. Glomerular capillary pressure is relatively well maintained in the presence of reduced RBF. The decrease in GFR is primarily mediated by reductions in plasma flow and K_f. The reduction in K_f appears to be mediated by a secondary release of Ang II, eicosanoids, or neurotransmitters. The preglomerular vasculature has equal proportions of ET_A and ET_B receptors as compared to a ratio of 2:1 on their smooth muscle cells devoid of endothelial cells. Under basal conditions, ET-1 exerts dual actions on the vasculature that are equal and opposite. The ET_A receptor blockade produces renal vasodilation due to a 5% to 10% increase in RBF, whereas ET_B receptor antagonism leads to constriction of 5% to 10%. Although ET-1 contributes to basal vascular tone, it does not interfere with renal autoregulatory mechanisms.[2,150,160,161]

ET_A and ET_B receptors stimulate $[Ca^{2+}]_i$ in smooth muscle cells of preglomerular arteries/arterioles through a combination of mobilization and entry pathways. Low concentrations of ET-1 activate Ca^{2+} entry channels, with higher concentrations mobilizing intracellular Ca^{2+} via the PLC-IP_3R and NADPH oxidase/cADPR/RyR pathways. Calcium channel blockers reduce the magnitude and duration of ET-1–induced renal vasoconstriction. The afferent arteriolar constriction produced by ET-1, similar to that caused by Ang II, is dependent in part on Ca^{2+} entry through voltage-dependent L-type channels as well as mobilization in intracellular Ca^{2+} stores. The action of ET-1 on efferent arterioles appears to depend exclusively on the mobilization of intracellular Ca^{2+}. ET-1 inhibits renin synthesis and release by ET_A and ET_B receptor stimulation of $[Ca^{2+}]_i$ in juxtaglomerular granular cells.[13,20,162]

ET-1 acts on glomerular mesangial cells to cause the contraction and stimulation of mitogenesis. High concentrations of ET-1 that reduce sodium excretion increase plasma renin activity, presumably via the macula densa mechanism. In addition, ET-1 may affect neurotransmission, eicosanoid synthesis, and ANP synthesis and release. The activation of endothelin receptors causes the release of eicosanoids and NO from endothelial cells and ANP from myocytes.[157,158,163]

Heme Oxygenase and Carbon Monoxide

Heme oxygenases (HO) are microsomal enzymes that catalyze the degradation of heme to form iron, biliverdin, and carbon monoxide (CO). The vascular actions of CO include the direct relaxation of vascular smooth muscle cells and the indirect contraction through the inhibition of NOS. Similar to NO, CO produces vasodilation by stimulating soluble guanylyl cyclase in smooth muscle cells to signal through the cGMP/PKG pathway. CO also may bind directly to Ca^{2+}-activated BK channels to depolarize the plasma membrane. A primary action of CO is to attenuate vasoconstriction produced by agents such as Ang II and catecholamines, with greater buffering effects in the absence rather than in the presence of NO. Nevertheless, CO can dose-dependently increase NADPH oxidase-dependent O_2^- production and constrict interlobar and interlobular arteries via thromboxane thromboxane prostanoid (TP) receptor activation, perhaps involving isoprostanes, effects inhibited by the O_2^- scavenger biliverdin. Thus, the vascular response to CO is mixed in that CO can elicit signaling leading to vasodilation and vasoconstriction, the net effect depending on experimental conditions.[164,165]

HO-2 is constitutively expressed in the kidney, mainly in proximal tubules with relatively weak presence in the renal vasculature. HO-1 is inducible during inflammation

and oxidative stress. The influence of endogenous HO-2 metabolites on renal hemodynamics appears to be minor under physiologic conditions. Pharmacologic inhibition of HO (chromium mesoporphyrin [CrMP]) reduces urinary excretion of sodium and water even under conditions where there are no effects on arterial pressure, RBF, GFR, or plasma renin activity, both in control animals and after NOS inhibition, suggesting a primary tubular effect of endogenous CO. Likewise, HO inhibition with CrMP does not alter afferent arteriolar diameter of juxtamedullary nephrons of normal rats. However, other studies using SnMP to inhibit HO report decreases in RBF with variable responses in GFR. Another study shows that acute inhibition of renal medullary HO activity and CO production reduces medullary blood flow and sodium excretion and blunts pressure natriuresis. Chronic HO inhibition produces hypertension that is salt sensitive. Mice deficient in HO-2 are normotensive with normal RBF during basal conditions or during NOS inhibition, whereas Ang II paradoxically produces less pronounced renal vasoconstriction in the absence of HO-2. Other investigators report that the pressor and renal vasoconstrictor responses to low levels of Ang II are magnified by inhibition of HO activity (tin mesoporphyrin) in normal euvolemic animals.[164,166–169]

Both HO-1 and HO-2 mRNA are expressed in macula densa cells, and HO metabolites inhibit TGF. Increased HO activity attenuates TGF-induced vasoconstriction through macula densa release of CO and biliverdin during increased salt reabsorption. The CO effect on TGF is linked to cGMP pathway and biliverdin to reduce O_2^- levels. Renal HO-1 induction (hemin, $SnCl_2$) dilates afferent arteriolar diameter and attenuates juxtamedullary afferent arteriolar autoregulatory responses to increases in renal perfusion pressure, an effect mimicked by CO acting through cGMP/PKG signaling but not biliverdin. These results are consistent with findings that increasing CO levels directly or with hemin administration increase RBF, urine flow, and sodium excretion. Whether the effects involve myogenic and/or TGF mechanisms await investigation.[164,170,171]

Hypoxia-induced HO upregulation protects renal tissue from acute and chronic injury. Activation of HO has an antioxidant effect by degrading the heme moiety of heme-containing enzymes such as NOS, COX-2, and cytochrome P450 monooxygenase. HO can attenuate the production of reactive oxygen species through its heme degradation and production of CO, biliverdin/bilirubin, and free iron. Excess free heme catalyzes ROS formation, which may lead to endothelial cell dysfunction, vasoconstriction, and tissue damage commonly associated with pathologic cardiovascular–renal conditions. Increased HO-1 activity and the metabolite bilirubin suppress NADPH oxidase activity and O_2^- production in vascular smooth muscle cells.[164,172]

Hydrogen Sulfide

Endogenous hydrogen sulfide (H_2S) is a recently discovered vasoactive gas transmitter (joining NO and CO in this classification). Cystathionine-γ-lyase and -β-synthetase are the main enzymes forming H_2S from L-cysteine or L-homocysteine in endothelial and smooth muscle cells of the vasculature wall and erythrocytes, physiologically activated by Ca^{2+}-calmodulin. H_2S is inactivated by binding to hemoglobin to form sulfhemoglobin, As an endothelial-derived relaxing factor, H_2S produces concentration-dependent dilation of large conduit arteries and small resistance arterioles, primarily acting directly to open K_{ATP} channels to hyperpolarize vascular smooth muscle cells. In contrast to NO and CO, H_2S does not stimulate soluble guanylate cyclase. Being a reducing agent, H_2S appears to alter cellular redox status. Low concentrations of H_2S may cause vasoconstriction reducing NO availability by reacting with NO to form a nitrosothiol compound and inhibit eNOS. H_2S and the H_2S donor NaHS downregulate cAMP production in vascular smooth muscle cells, producing vasoconstriction, and in juxtaglomerular granular cells, inhibiting renin release. Physiologic levels of H_2S are also angiogenic, antiproliferative, and anti-inflammatory. Mice deficient in a synthetic enzyme have reduced H_2S levels, reduced endothelium-mediated vasodilation, and develop hypertension.[173–176]

Little is known about H_2S effects on the renal microcirculation. H_2S is produced in the kidney and the exogenous H_2S donor NaHS exerts diuretic, natriuretic, and kaliuretic effects in association with increases in GFR and RBF. Conversely, acute intrarenal inhibition of H_2S production reduces GFR and sodium excretion without affecting RBF. Chronic inhibition of H_2S synthesis reduces RBF but not GFR because sodium excretion decreases in association with the development of hypertension. The actions of H_2S on afferent and efferent arterioles and TGF activity await investigation.[177,178]

Because H_2S is oxidized in mitochondria in pO_2-dependent manner and ambient pO_2 is lower in the renal medulla than the cortex, H_2S accumulates in medullary regions. High H_2S concentrations in the relatively hypoxic renal medulla function as an oxygen sensor that acts to increase local O_2 by increasing medullary blood flow in combination with the direct inhibition of mitochondrial respiration.[179]

Arachidonic Acid Metabolites

Renal prostaglandins, or eicosanoids, are biologically active fatty acid products of arachidonic acid that contribute to the regulation of renal hemodynamics. They are synthesized intracellularly and immediately released to act locally on the renal vasculature as paracrine/autocrine agents. Free intracellular arachidonic acid can be metabolized on demand via one of three major enzymatic pathways: cyclooxygenase, lipoxygenase, or cytochrome P-450 monooxygenase. Depending on cell types, the net production of the various metabolites may cause vasoconstriction under some conditions and vasodilation during others. The multiple products of this cascade are depicted in Figure 3.22. Phospholipase A_2 (PLA_2) catalyzes the formation of arachidonic

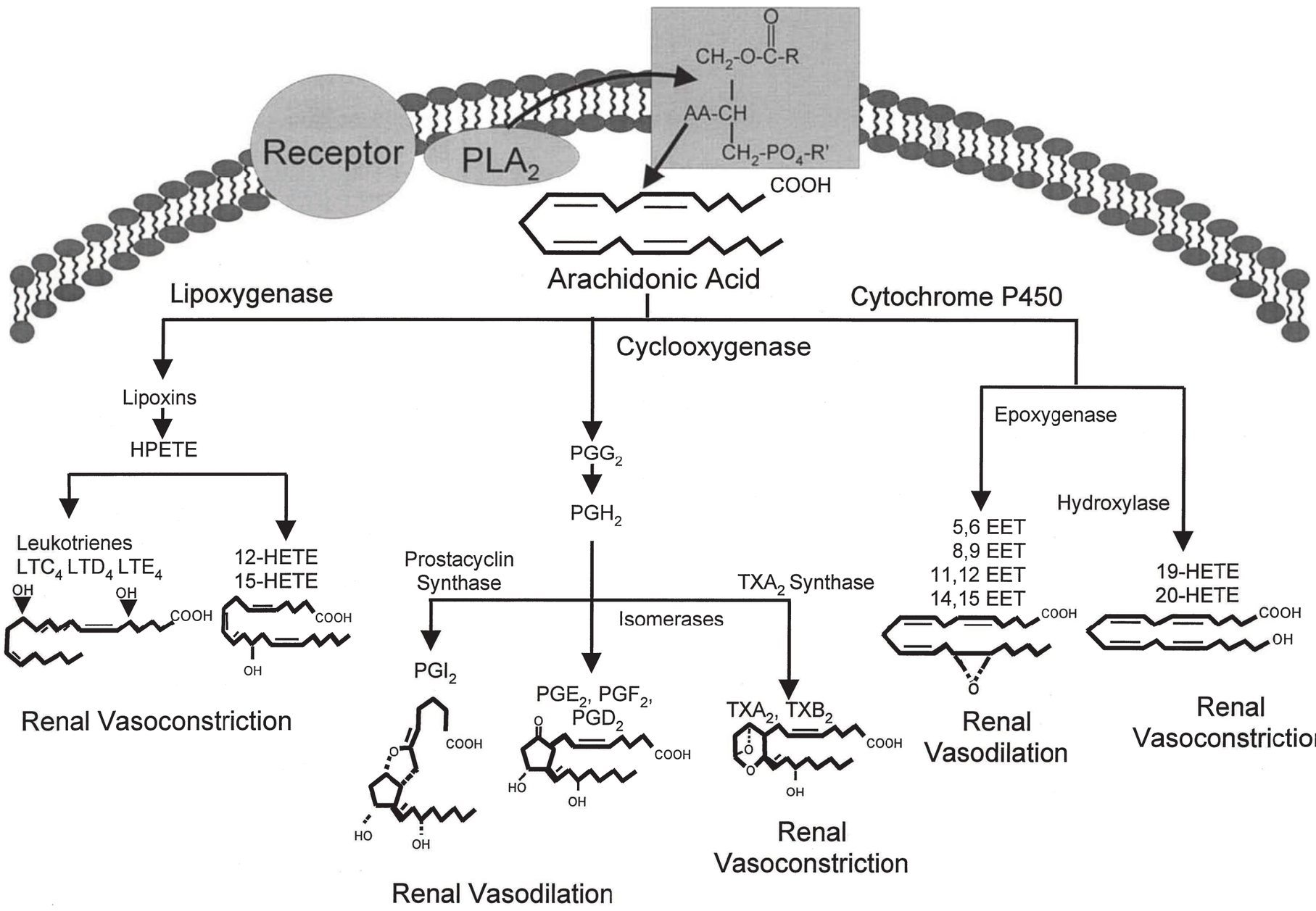

FIGURE 3.22 Three major pathways of eicosanoid synthesis from arachidonic acid involving cyclooxygenase, lipoxygenase, and cytochrome P-450 monooxygenase enzyme systems. Note that only the configuration of the cyclopentane ring is shown. *LT*, leukotriene; *PLA*$_2$, phospholipase A2; *PG*, prostaglandin; *TX*, thromboxane; *EET*, epoxy-eicosatrienoic acid; *HETE*, hydroxyeicosatetraenoic acid.

acid, an unsaturated 20-carbon fatty acid, from membrane phospholipids.[2]

A key regulatory, rate-limiting step for prostaglandin synthesis is the conversion of arachidonic acid to prostaglandin (PG) G_2/H_2 by COX. There are two major COX enzymes. COX-1, a constitutive enzyme, is found in renal arteries and arterioles, glomeruli, cortical and medullary collecting ducts, and medullary interstitial cells. COX-2 is a regulated constitutive enzyme that is important in development, and in adult animals is commonly activated by growth factors, inflammatory states, glucocorticoids, and low salt diet. Renal COX-2 mRNA is localized primarily to epithelial cells of the cortical thick ascending limb that includes the macula densa region and medullary interstitial cells, with greater amounts in the papilla than the cortex. COX-2 expression is noticeably absent from arterioles, glomeruli, and cortical or medullary collecting ducts, sites of COX-1 expression; COX-2 is also expressed in endothelial cells of medullary vasa recta. COX-2 expression in thick ascending limb cells and macula densa cells varies with salt diet, increasing during chronic sodium restriction and decreasing during sodium loading. COX-2 expression in medullary interstitial cells is induced by water deprivation and high interstitial osmolality, high levels of Ang II and AVP, as well as growth factors and cytokines through MAP kinase pathways. Leukotrienes are synthesized by another major pathway involving the enzyme lipoxygenase. Vasoactive metabolites are also formed via the cytochrome P-450 monooxygenase pathway. Medullary tubular and interstitial cells have a larger synthetic capacity than the vasculature in the cortex. Endothelial cells produce PGE_2 and PGI_2, whereas thromboxane A_2 seems to derive from vascular smooth muscle cells and mesangial cells.[118,180–184]

Prostaglandins

General stimuli for renal prostaglandin synthesis are renal vasoconstriction and states of volume depletion and hypoperfusion. Anesthesia, surgery, and associated stress may exacerbate prostaglandin production. The diuretics ethacrynic acid and furosemide also stimulate renal release of prostaglandins. Many vasoactive receptor agonists stimulate phospholipases that promote the release of arachidonic acid from membrane phospholipids. The stimulation of PGE_2 and PGI_2 production by Ang II is well characterized. In turn, the vasodilatory PGE_2 and PGI_2 usually buffer the vasoconstriction elicited by Ang II and stimulate renin release from juxtaglomerular cells. Other stimulants include ET_A receptor agonists. Vasodilators such as acetylcholine and bradykinin stimulate the production of PGE_2 or PGI_2 as well as NO. In addition, acetylcholine may stimulate the production of thromboxane A_2. The vasoactive peptides Ang II, ET-1, vasopressin, acetylcholine, and bradykinin increase the

availability of the substrate, arachidonic acid, secondary to membrane receptor–mediated Ca^{2+} influx and the activation of phospholipase A_2. Phospholipase A_2 is activated by increased activity of the Ca^{2+}-calmodulin complex, increased production of DAG, or phospholipase C-mediated phosphorylation of lipocortin, a membrane-bound enzyme that normally inhibits phospholipase A_2. Thus, there is a common pathway by which many vasoconstrictor agents can increase the production of COX-derived prostaglandins, primarily vasodilatory PGE_2 and PGI_2, which in turn can counteract vasoconstriction. Vasodilatory prostaglandins produced by the endothelium of glomerular arterioles and mesangial cells exert net effects to stimulate adenylate cyclase and the formation of cAMP and the activation of protein kinase A. α-Adrenergic neurotransmission can be inhibited prejunctionally and postjunctionally by prostaglandins.[71,185,186]

Four forms of PGE_2 receptors (termed EP_1 through EP_4) have been identified and cloned. The EP_4 receptor, coupled via $G\alpha_s$-proteins to generate cAMP and activate protein kinase A, predominates along the preglomerular vasculature and mediates the principal vasodilator actions of PGE_2. Similar actions are exerted by the single IP receptor for PGI_2 (prostacyclin). Low concentrations of the prostaglandins PGE_2 and PGI_2 normally formed by the arterial vasculature exert part of their physiologic effects by attenuating the actions of vasoconstrictors, with larger amounts acting as vasodilators that increase RBF. The buffering action of PGE_2 and PGI_2 in the preglomerular vasculature is primarily mediated by the ability of cAMP and protein kinase A activation to inhibit IP_3-induced release of Ca^{2+} from internal stores. A low density of a vasoconstrictor receptor (EP_1 or EP_3) may counteract some of the net dilation. The EP_1 receptor increases Ca^{2+} mobilization. The EP_3 receptor inhibits the production of cAMP via a pertussis-toxin-sensitive $G\alpha_i$ protein. EP_2 receptors, which act through a $G\alpha_s$ protein to increase cAMP in tubules, appear to be absent from the renal vasculature under normal conditions. Arachidonic acid dilates the isolated interlobular artery and afferent arteriole; a smaller response occurs in efferent arterioles. Intrarenal infusions of arachidonic acid, PGE_2 or PGI_2 increase RBF and reduce renal resistance without affecting GFR. $PGF_2\alpha$ has little or no effect on the renal circulation. Local administration of small amounts of PGE_2 or its stable analog cause renal vasodilation and attenuate the constrictor effects of Ang II, thromboxane A_2, and norepinephrine. PGE_2 and PGI_2 dilate both afferent and efferent arterioles such that glomerular capillary pressure is constant when the stimulatory effects of the prostaglandins on Ang II formation are blocked. However, the application of PGE_2 from the interstitial side causes vasoconstriction of juxtamedullary nephrons, a response due to subsequent metabolism to a vasoconstrictor agent or the activation of EP_1 or EP_3 receptors.[60,185,187,188]

Endogenous prostaglandins regulate RBF and GFR by direct effects on vascular smooth muscle and indirectly by modification of the action of other hormones or neural stimuli. The vasodilator prostaglandins serve an important protective function and homeostatically balance the hemodynamic effects of vasoconstrictor substances. Studies of inhibition of COX activity indicate that the major function of COX-derived prostaglandins is to attenuate the influence of vasoconstrictor substances during the activation of the renin–angiotensin system, the sympathetic nervous systems, or both. These counteracting effects provide a balance between the vascular effects of Ang II and those of prostaglandins during variations in plasma volume and sodium intake. As is shown in Figure 3.23, blockade of the compensatory dilator action of prostaglandins promotes vasoconstriction when the renin–angiotensin and sympathetic nervous systems are

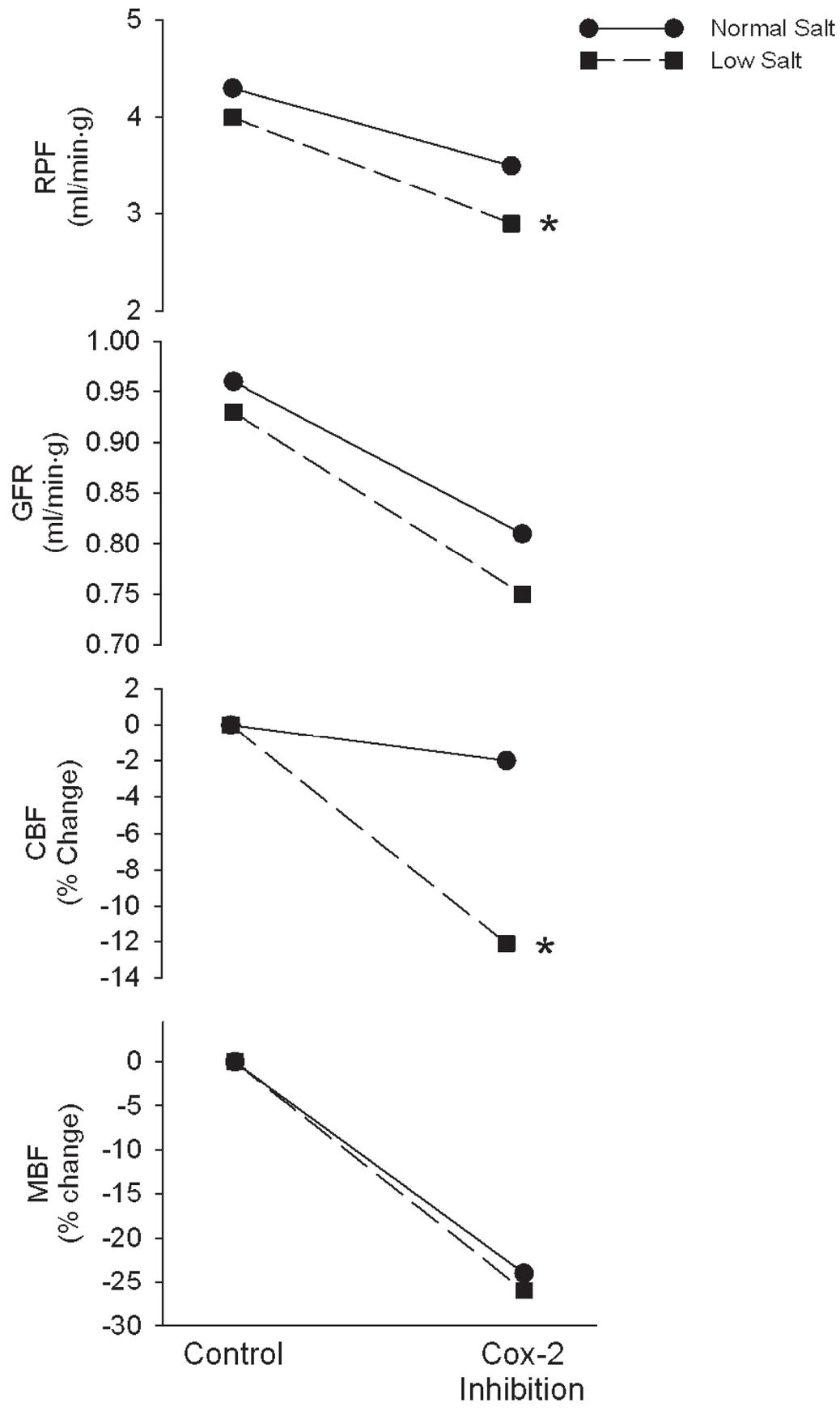

FIGURE 3.23 Renal plasma flow (*RPF*), glomerular filtration rate (*GFR*), cortical blood flow (*CBF*), and medullary blood flow (*MBF*) responses to the inhibition of cyclooxygenase (Cox-2, nimesulide) in anesthetized rats maintained on a normal salt diet (●) or on a low sodium diet (■). * $P < .05$. (Data from Green T, Rodriguez J, Navar LG. Augmented cyclooxygenase-2 effects on renal function during varying states of angiotensin II. *Am J Physiol Renal Physiol.* 2010;299:F954–F962.)

stimulated, such as during sodium depletion. Prostaglandins also antagonize the tendency for high concentrations of vasopressin to produce renal vasoconstriction. In contrast, in a healthy, unstressed individual with a normal plasma volume, the inhibition of prostaglandin synthesis has little or no effect on RBF and GFR (Fig. 3.23). Basic autoregulatory efficiency of whole-kidney RBF and GFR remains high even during the blockade of renal prostaglandin synthesis via the COX pathway. The inhibition of COX-2 produces renal vasoconstriction that may be greater in the medulla than in the cortex. During afferent arteriolar constriction induced by TGF, COX-2 generates vasodilatory metabolites in response to increased nNOS activity that attenuate the strength of feedback-mediated vasoconstriction.[2,116,182]

Renin release is increased by arachidonic acid, endoperoxides, PGE_2, and PGI_2. PGE_2 and PGI_2 act on their respective EP_4 and IP receptors on juxtaglomerular granular cells to stimulate renin release by stimulating adenylate cyclase activity and producing cAMP. COX inhibitors reduce renin release under basal as well as during stimulated conditions. α-Adrenergic neurotransmission can be inhibited prejunctionally and postjunctionally by prostaglandins.[13,116,121,133]

The COX-2 metabolite PGE_2 plays a key role in linking NaCl control of macula densa signaling to juxtaglomerular cells to regulate renin release and to afferent arterioles to regulate TGF. COX-2 activity in the macula densa increases in response to a salt deficient diet and to a loop diuretic, such as furosemide, with increases in PGE_2 production and renin release. nNOS in the same cells may serve as an upstream intermediate responding to salt transport. High levels of nNOS and COX-2 parallel each other, and the inhibition of nNOS causes a fall in COX-2 expression and an uncoupling to transport. On the other hand, high renin states suppress COX-2 expression in the cortical thick ascending limb and macula densa cells via Ang II and AT_1 receptors; ACE inhibition has the opposite effect. The recruitment of renin-containing cells retrograde along the afferent arteriole during chronic salt restriction or ACE inhibition appears to be COX-2 dependent as renin is restricted to juxtaglomerular cells during these stimuli in COX-2–deficient mice. Plasma renin activity is reduced in COX-2–null mice and also in nNOS-deficient mice. The inhibition of COX-2 produces renal vasoconstriction that may be greater in the medulla than the cortex.[117,182,189]

In disease states, endogenous vasodilator prostaglandins serve a protective role in maintaining renal function by means of their effects on vascular resistance, GFR, and renin release. The administration of nonsteroidal anti-inflammatory drugs to patients with clinical disorders such as advanced hepatic cirrhosis, severe congestive heart failure, and sodium depletion often produces deleterious effects with reductions in renal perfusion and GFR. In other pathophysiologic conditions, there may be enhanced production of the vasoconstrictor thromboxane A_2, which may contribute to the deterioration of renal function. Some of the vasoconstriction produced by Ang II may be mediated by thromboxane A_2 in pathophysiologic settings. Administration of a stable thromboxane-receptor agonist reduces RBF and GFR by causing afferent and efferent arteriolar constriction. K_f appears to be unaffected, although it is noteworthy that thromboxane A_2 causes the contraction of isolated glomeruli and cultured mesangial cells. As mentioned in the section on TGF, although very little thromboxane A_2 is produced under normal conditions, thromboxane plays a modulatory role in the preglomerular vasoconstriction associated with the activation of the TGF mechanism and the vasoconstrictor response to chronic Ang II infusion. The thromboxane A_2-PGH_2 receptor is coupled by the $G\alpha_q$ protein to the activation of phospholipase C, increased $[Ca^{2+}]_i$, and possible inhibition of adenylate cyclase. The renal vasoconstriction produced by thromboxane A_2 is primarily mediated by Ca^{2+} influx. Chronic activation of PKC appears to reduce the number of thromboxane A_2 TP receptors. In Ang II–dependent hypertension, the administration of COX-2 inhibitors decreases RBF and GFR due to increased RVR, reflecting a renal vasodilator action of intrarenal COX-2 metabolites; however, systemic vascular resistance is decreased, reflecting a vasoconstrictor role of COX-2 metabolites on the systemic circulation.[116,184,190]

Leukotrienes

Lipoxygenase enzymes convert arachidonic acid to leukotrienes (LTs), HETEs, and lipoxins (see Fig. 3.22). The major lipoxygenase products are monohydroxyeicosatetraenoic acids—12-HETE and 15-HETE—generated by glomeruli, mesangial cells, renal cortical tubules, and vascular tissues. LTs (LTB_4, LTC_4, LTD_4, and LTE_4) are hydroperoxy fatty acid products of the intermediate 5-hydroperoxyeicosatetraenoic acid (HPETE). Stereoisomers of 12-HETE are produced by different enzymatic pathways. The 12(S)-HETE isomer is predominantly produced in the cortex; however, the 12(R)-HETE is a primary biologically active metabolite of the cytochrome P-450 pathway. The renal vasculature constricts in response to 12(S)-HETE and 15-HETE due to the depolarization of smooth muscle cells, increased Ca^{2+} entry, and the activation of PKC. Vascular generation of 12-HETE is increased in pathologic conditions associated with oxidative stress and inflammation.[2,180,183,191]

Receptors for LTC_4 and LTD_4 have been identified on preglomerular arteries, glomeruli and cultured mesangial cells, and efferent arteriole. The infusion of LTC_4 or LTD_4 causes renal vasoconstriction, reduces GFR and filtration fraction, and activates the renin–angiotensin system. Leukotriene C_4 receptors are linked to ion channels, but specific mechanisms are not known. Some of the LT actions may be mediated by endothelial cells. LTD_4 is known to release NO in addition to activating a pertussis-toxin–sensitive G protein. Lipoxin A_4 increases renal plasma flow and GFR, with a small reduction in K_f. Interestingly, the effects are COX dependent and can be completely reversed by the inhibition of this enzyme. On the other hand, the ability of

lipoxin B_4 to decrease RBF and GFR is independent of COX activity.[180,183]

Cytochrome P-450 Metabolites

Vasoactive metabolites of the cytochrome P-450 pathway are produced by vascular smooth muscle cells, endothelial cells, renal tubular cells, and glomeruli. Arachidonic acid is oxygenated via NADPH-dependent microsomal monooxygenases. As is shown in Figure 3.22, epoxygenase enzymes are responsible for the production of epoxides or epoxyeicosatrienoic acids (EETs) (e.g., 11,12-EET and 14,15-EET). A second cytochrome P-450 pathway involves the ω-hydroxylase formation of 19- and 20-HETEs. The major metabolites of this pathway are EETs in the cortex and HETE in the medulla. Salt diet, Ang II and other hormones, and various pathophysiologic settings alter the renal cytochrome P-450 metabolism.[118,183,192–195]

Cytochrome P-450 ω-hydroxylase metabolites, in particular 20-HETE, participate in regulation of cortical and whole-kidney blood flow. Although rodent studies report effects of 20-HETE, studies in dogs and rabbits have failed to show an effect of cytochrome P-450 inhibition on autoregulation and pressure natriuresis. In isolated rat vessels, transmural pressure stimulates ω-hydroxylase activity to produce 20-HETE, which is thought to participate in myogenic vasoconstriction. Cytochrome P450-4A–derived eicosanoids may also participate in the renal hemodynamic effects of Ang II and endothelin. Products of the cytochrome P-450 pathway potentiate control of preglomerular vasomotor tone by the juxtaglomerular apparatus. Inhibition of P-450 metabolites blunts TGF activity, whereas the luminal perfusion of 20-HETE restores TGF responses. 20-HETE causes vasoconstriction by the inhibition of tonically active K^+ channels, thereby causing depolarization and activation of voltage-gated Ca^{2+} channels and increases intracellular Ca^{2+}.[118,181,183]

Epoxygenase metabolites elicit variable vascular responses. 11,12-EET is an endothelial-derived factor distinct from NO that produces vasodilation in interlobular arteries and afferent arterioles mediated by membrane hyperpolarization following the activation of cAMP/protein kinase A and of K^+ channels, actions independent of the vascular endothelium or COX activity. 5,6-EET or 8,9-EET induces vasoconstriction with a decrease in GFR, mediated in part by thromboxane TP receptor activation. However, COX inhibition changes the renal response to these EETs to vasodilation and an increase in GFR. Inhibition of epoxygenase activity enhances afferent arteriolar autoregulation. Not clear is whether epoxygenase/EET actions blunt the pressure-induced myogenic response, or TGF, or both. EETs, in particular 11,12-EET, have now been identified as being an endothelial-derived hyperpolarizing factor and activate potassium channels, thus increasing the membrane potential, leading to renal vasodilation and attenuated responses to vasoconstrictor influences.[183,196,197]

Isoprostanes such as 8-epi-$PGF_{2\alpha}$ are vasoconstrictor metabolites related to prostaglandins. They are stable products of nonenzymatic lipid peroxidation of arachidonic acid, formed in and released from cell membranes and excreted in urine. Isoprostane production is stimulated by peroxynitrate, a reactive oxygen species resulting from NO scavenging by superoxide anion.[114,198,199]

Kallikrein–Kinin System

Plasma and glandular/tissue kallikreins are distinct serine protease enzymes acting on kininogens (inactive α_2-glycoproteins) to form the biologically active nonapeptide bradykinin and also the decapeptide lysyl-bradykinin (kallidin). It is unlikely that circulating kinins affect the renal microcirculation because they are rapidly inactivated enzymatically by endothelial-bound kininase II (ACE) and neutral endopeptidase. Within the kidney, tissue kallikrein and its substrate, kininogen, are located predominantly in the distal convoluted and cortical collecting tubules. The synthesis and the release of kallikrein into the tubular fluid and interstitium are stimulated by prostaglandins, mineralocorticoids, Ang II, increased renal perfusion pressure, and several diuretic drugs. The renal vasculature and tubules contain the constitutive B_2 receptor and the inducible B_1 receptor. Bradykinin B_2 receptors on vascular endothelial cells cause renal vasodilation as a result of stimulation of NO, EET, and prostanoid production. The B_2 receptor density in glomeruli is reduced by a low sodium diet and water deprivation. B_2 receptors on tubular epithelial cells inhibit sodium reabsorption. The bradykinin B_1 receptor is not normally expressed and is silent under physiologic conditions. Its expression is highly inducible by inflammatory mediators and tissue damage, chronic ACE (kininase II) inhibition, or genetic deletion of B_2 receptors.[200–203]

The infusion of bradykinin elicits renal vasodilation characterized by a larger increase in RBF than GFR, and a natriuresis and diuresis. Exogenous kinins also produce an independent stimulation of prostaglandin formation (PGE_2 and PGI_2) and renin release. Kinin-induced vasodilation, however, may be similar in the presence and absence of COX inhibition of prostaglandin synthesis owing to major actions of NO and EETs. As a result of vasodilatorlike actions, exogenous bradykinin reduces the vasoconstrictor responses to Ang II and norepinephrine. The observed decline in glomerular K_f contrasts with the well-known effect of kinins to increase capillary permeability in other tissues. The mechanisms responsible for the reduction in K_f are not known. Additional effects include the enhanced conversion of inactive to active renin and the presynaptic inhibition of adrenergic neurotransmitter release. Isolated vessels and cultured mesangial cells devoid of endothelium exhibit B_2-receptor–dependent constrictor responses to bradykinin. Signal transduction appears to involve a pertussis-toxin–insensitive G protein, a PKC pathway, increased $[Ca^{2+}]_i$, and arachidonic acid metabolites.[1,2]

B_2 receptors reside on endothelial and vascular smooth muscle cells. Vasodilator effects of bradykinin are observed in isolated preparations of afferent and efferent arterioles. In the isolated perfused afferent arteriole, low concentrations of bradykinin ($<10^{-10}$ M) vasodilate by acting on endothelial B_2 receptors to produce NO and prostaglandins. Epoxygenase-dependent EETs also contribute. Higher concentrations ($>10^{-9}$ M) vasoconstrict by acting on B_2 receptors to produce COX-derived thromboxane. In contrast, the vasodilator effect of bradykinin in efferent arterioles (perfused in retrograde direction) via B_2 receptors is mediated by cytochrome P450 metabolites (probably EETs), but not by NO or COX products. Perfusion of bradykinin through glomeruli releases prostaglandins that dilate the efferent arteriole. Bradykinin relaxes pericytes surrounding the outer medullary descending vasa recta. Endogenous bradykinin dilates afferent and efferent arterioles in vivo to a greater extent in the deep versus superficial cortical glomeruli with primary mediation by NO. EETs also participate in vasodilation of afferent arterioles of juxtamedullary nephrons. Isolated vessels and cultured mesangial cells devoid of endothelium exhibit B_2-receptor–dependent constrictor responses to bradykinin. Signal transduction appears to involve a pertussis-toxin–insensitive G protein, a PKC pathway, increased $[Ca^{2+}]_i$, and arachidonic acid metabolites. Endogenous bradykinin, potentiated during ACE inhibition in animals fed a low sodium diet, dilates the renal vasculature with predominant actions in the medulla. During extracellular fluid volume expansion, bradykinin promotes sodium excretion and reduces regional autoregulatory efficiency to increase medullary blood flow, effects largely mediated by B_2 receptor stimulation of NO.[15,204–209]

Early studies evaluated endogenous kinin activity using an infusion of bradykinin-binding antibodies, the suppression of renal kallikrein activity with the serine protease inhibitor aprotinin, and the pharmacologic inhibition of kininase II. The results suggest that locally formed kinins attenuate renal vasoconstriction. Further understanding has been gained by employing more specific receptor antagonists. Use of a specific B_2 receptor antagonist reveals that basal kinin levels do not contribute appreciably to the regulation of renal function during normal conditions. However, when their levels are elevated as during sodium restriction and volume depletion, kinins act as vasodilators in a manner similar to the prostanoids, which buffer the renal vasoconstriction associated with elevated local levels of Ang II, norepinephrine, and vasopressin. A combined blockade of both degrading enzymes (ACE or kininase II and neutral endopeptidase) leads to increases in RBF and GFR in association with an increased urinary excretion of kinins. Kinins may participate in the autoregulation of GFR and the TGF mechanism, although overall steady-state RBF autoregulation is not affected by kinin receptor blockade. Kinins exert larger vasodilatory effects in the medulla than in the cortex.[208]

Animal studies on the role of the renal kallikrein–kinin system using kininogen-deficient rats and also B_2 receptor knockout mice indicate that this system primarily functions to promote a natriuresis that impacts on the pressure–natriuresis relation when there is excess sodium intake or high plasma aldosterone concentration. Genetic dysfunction of the renal kallikrein–kinin system leads to altered functional maturation of the kidney and the development of salt-sensitive hypertension. Rats with a genetic reduction in urinary kallikrein excretion have an altered pressure–natriuresis relationship, with this defect being corrected by an infusion of purified tissue kallikrein. Knockout mice lacking the B_2-receptor gene have elevated arterial pressure under basal conditions, reduced RBF, increased renin mRNA, and enhanced blood pressure sensitivity to salt.[210]

Purinergic Actions on Renal Microcirculation

Adenosine and ATP

Adenosine nucleosides and nucleotides have received considerable attention as regulators of renal hemodynamics and renin release. It is proposed that GFR and filtered sodium load are coupled to tubular transport capacity and O_2 consumption via the hydrolysis of ATP and the resultant adenosine production. Adenosine and other adenine nucleotides are secreted into the interstitial fluid in the extracellular compartment. ATP is released from cells through membrane channels and coreleased with transmitters from nerve terminals. In this fashion, purine-based substances act as paracrine agents to influence renal microcirculation.[1,2,121]

Purinergic Receptors

Renal purinoceptors display different sensitivities for ATP, ADP, AMP, and adenosine. P_1 purinoceptors respond primarily to adenosine and sometimes to AMP, but are relatively insensitive to ADP and ATP. Extracellular ATP predominantly activates P_2 purinoceptors. There are at least two subtypes of adenosine-responsive P_1 receptors in the renal vasculature. P_1-vasoconstrictor A_1 receptors are coupled to a pertussis-toxin–sensitive $G\alpha_i$ protein that inhibits adenylate cyclase and cAMP production. The stimulation of A_1 receptors decreases GFR and RBF. A local application of an A_1 receptor agonist constricts both preglomerular and postglomerular microvessels. A_2 receptors cause vasodilation of afferent and efferent arterioles, stimulating adenylate cyclase through a $G\alpha_s$ protein, and also increase EETs and NO, which contribute to the vasodilation. There are two subtypes (A_2a and A_2b), with A_2b being prevalent in afferent arterioles.

P_2 receptors, present on endothelial and vascular smooth muscle cells, have a greater affinity for ATP and ADP than for adenosine or AMP. Interstitial fluid ATP concentrations are sufficiently high to play a role in regulating the vascular resistance changes responsible for autoregulation and TGF. The receptor subtype P_{2X} increases $[Ca^{2+}]_i$ by increasing Ca^{2+} influx through voltage-gated and receptor-operated

Ca^{2+}channels. The low frequency stimulation of renal sympathetic nerves causes renal vasoconstriction that is mediated, in part, by a nonadrenergic component involving ATP corelease with the neurotransmitter acting on P_{2X} receptors. P_{2Y} receptor activation increases $[Ca^{2+}]_i$ via the PLC-IP_3 cascade. Endothelial cells have a high concentration of P_{2Y} receptors, and their activation by ATP leads to vasorelaxation mediated by NO, or PGI_2, or both. However, ATP causes a rapid, marked constriction of the afferent arteriole when NO synthesis is inhibited. The P_{2U} purinoceptor is termed a nucleotide or 5′-nucleotide receptor because it responds to all nucleotides with a similar potency of ATP and uridine triphosphate. P_{2U} receptor activation increases $[Ca^{2+}]_i$ by a G-protein stimulating the PLC pathway.[1,2,211,212]

Adenosine

The intrarenal administration of adenosine produces a biphasic renal response. Resistance vessels respond initially with transient vasoconstriction, mediated by P_1-A_1 receptors, followed by gradual vasodilation, mediated in part by P_1-A_2 receptors. In isolated preparations, adenosine A_1 receptor stimulation constricts both afferent and efferent arterioles. Endogenous adenosine serves as an important regulator of renal hemodynamics. The blockade of A_1 receptors decreases afferent arteriolar resistance and K_f in anesthetized animals.

Evidence about the importance of adenosine in intrinsic autoregulatory mechanisms is mixed. One set of results suggests that adenosine is not essential for autoregulation. Interstitial fluid concentrations of adenosine are unchanged over the autoregulatory range of arterial pressure, and the administration of adenosine-receptor antagonists does not impair autoregulatory capability. Other results, however, implicate a role of adenosine in the regulation of afferent arteriolar tone by TGF. Salt transport by the ascending limb of the Henle loop or macula densa cells requires metabolic energy and the use of ATP that is linked to control of preglomerular vascular resistance through macula densa signaling. As transport increases, more adenosine is liberated within cells and is available to diffuse to the afferent arteriole, where it elicits vasoconstriction to reduce RBF and GFR. Luminal perfusion of A_1 adenosine-receptor agonists cause TGF-induced vasoconstrictor changes in glomerular capillary pressure. Attenuated TGF-mediated responses of the afferent arteriole are observed when an adenosine antagonist is added to either the luminal fluid or peritubular capillary blood. The effects are insensitive to the salt transport inhibitor furosemide, indicating P_1-A_1 adenosine-receptor–mediated effects by acting on either the macula densa or vascular smooth muscle cells. Moreover, an adenosine-receptor blockade reduces TGF control of preglomerular resistance, and mice without functional A_1 receptors lack TGF activity. However, unopposed A_2 receptor activation may result in substantial vasodilation. Dynamic RBF studies on gene-targeted mice are consistent with A_1 receptors mediating pressure-induced TGF responses participating in RBF autoregulation but not the myogenic response of the preglomerular vasculature. In addition, mice lacking the enzyme NTPDase/CD39, which reduces the formation of adenosine, have impaired TGF activity.[1,104,213–217]

Although adenosine and Ang II can act independently of each other on glomerular arterioles, they also act synergistically. Adenosine enhances afferent arteriolar reactivity to Ang II by increasing Ca^{2+} sensitivity. On the other hand, Ang II amplifies afferent arteriolar constriction to adenosine by a different mechanism, one due to increased $[Ca^{2+}]_i$. In this manner, adenosine can amplify vasoconstriction during high renin states and increased endogenous Ang II. Adenosine causes larger long-term decreases in GFR and the filtration fraction in sodium-depleted animals with increased renin-angiotensin activity than in animals consuming a normal salt diet.[218,219]

Adenosine participates in the regulation of renin secretion. Adenosine infusion inhibits renin release in vivo. The mechanism involves adenosine receptors on juxtaglomerular cells, with A_1 and A_2 receptors having opposite effects. Renin release is inhibited by A_1 purinergic receptors coupled to a $G\alpha_i$ protein and stimulated by A_2 receptors. A tonic inhibitory effect of adenosine is observed in isolated afferent arterioles with and without macula densa cells attached.[220–222]

ATP

P_2 receptors are also involved in mediating autoregulatory adjustments in RVR involving TGF responses. Extracellular ATP, at high levels that saturate P_2 purinoceptors, causes renal vasoconstriction and impairs the autoregulatory ability of the vasculature to dilate in response to decreases in arterial pressure. The effect on autoregulation appears to be specific to ATP because similar studies show that RBF autoregulation is not impaired when the vasoconstrictor is norepinephrine or Ang II.

The renal arterial infusion of ATP increases RBF. The renal vasodilation is mediated by activation of endothelial receptors to release NO. The ATP effect is converted to vasoconstriction during NO synthesis inhibition. P_2 receptors are present on vascular endothelium, with P_{2Y} receptors being responsible for NO-dependent vasodilation. P_{2X} receptors on vascular smooth muscle cells produce vasoconstriction. In considering a paracrine role for ATP, it seems likely that endogenous ATP reaches vascular smooth muscle cells from the interstitium. The vasoconstriction is mediated by Ca^{2+} mobilization and entry through L-type Ca^{2+} channels.[1,2]

ATP acts on P_2 purinoceptors to selectively constrict afferent arterioles in a sustained manner, whereas ATP does not elicit any response in efferent arterioles. This is consistent with autoradiographic and immunohistochemical evidence of abundant P_{2x} receptors limited to the preglomerular vasculature. Pressure-induced TGF–mediated afferent arteriolar constriction is prevented by P_2 receptor saturation or desensitization by high arterial concentrations of ATP. Tubuloglomerular responses also are markedly

blunted during peritubular capillary perfusion with saturating doses of ATP or slowly metabolizable analogs. $P_{2(x)1}$ receptor blockade prevents TGF-initiated responses of afferent arterioles.

Renal interstitial fluid concentrations of ATP measured by microdialysis are closely associated with autoregulatory and TGF-mediated changes in RVR. A direct relationship is observed when TGF is stimulated by increased distal fluid delivery due to a carbonic anhydrase inhibition of proximal tubular reabsorption and when TGF is inhibited by the transport inhibitor furosemide. Mice lacking functional P_{2x} receptors have partially blunted whole kidney RBF autoregulation and a loss of TGF. Macula densa cells secrete ATP through a basolateral maxi-chloride channel in response to increases in luminal NaCl concentration. Although macula densa cells have abundant mitochondria, they have low levels of Na^+-K^+-ATPase, making them a good candidate for a source of extracellular ATP. The activation of macula densa signals trigger the rapid propagation of Ca^{2+} waves and associated afferent arteriolar constriction, which are prevented by blocking P_2 receptor activation or increasing ATP hydrolysis. Collectively, the data support an important role for the ATP-dependent activation of P_2 receptors in the mediation of autoregulation and TGF.[1,2,12,107,211,223,224]

Sympathetic Nervous System and Catecholamines

The renal vasculature is richly innervated with postganglionic adrenergic fibers originating from sympathetic celiac and aorticorenal plexuses that receive inputs from the sixth thoracic through the second lumbar spinal nerves. All arterial segments of the renal vasculature and the large veins are extensively innervated with neuroeffector junctions containing norepinephrine as the primary neurotransmitter. A heavy concentration appears in subadventitial layers of arcuate arteries, with notable innervation of smooth muscle cells of both the afferent and efferent arterioles as well as the juxtaglomerular granular cells and the outer medullary descending vasa recta. The incoming efferent innervation is predominantly adrenergic, although some nerve endings are reported to contain dopamine and neuropeptide Y (NPY) as well as ATP. Alpha$_1$-adrenoceptors, primarily α_{1A} and α_{1D}, increase $[Ca^{2+}]_i$ and Ca^{2+} sensitivity, and mediate nerve stimulation-induced vasoconstriction of glomerular arterioles in the renal cortex and medullary pericytes, an action opposed in part by concurrent activation of α_2-adrenoceptors. Beta-adrenoceptors stimulate renin release from juxtaglomerular granular cells via cAMP/PKA signaling.[132,225]

Renal nerves are divided into two types. Type I almost exclusively innervate afferent arterioles, whereas type II innervate both afferent and efferent arterioles. Type II nerves contain NPY, whereas type I terminals do not. The electrical stimulation of the greater splanchnic or renal efferent nerves produces frequency-dependent renal vasoconstriction that is abolished by α_1-adrenergic receptor antagonists and attenuated by NPY Y_1 receptor blockade. Very low levels of nerve stimulation affect renin release and tubular sodium reabsorption without causing major changes in renal hemodynamics. Intermediate nerve activity elicits renal vasoconstrictor responses in the medullary as well as the cortical circulation, with more pronounced reductions in cortical blood flow. The increased vascular resistance in the cortex is due to the constriction of preglomerular and efferent arteriolar segments. As is shown in Figure 3.24, low levels of stimulation cause an equivalent constriction of afferent and efferent arterioles such that glomerular pressure is unchanged. Similar results are obtained in isolated afferent and efferent arterioles when α_1-adrenoceptors are stimulated. Higher frequencies of nerve stimulation produce predominant constriction of preglomerular vessels and thus reduce glomerular capillary pressure and GFR. Intense stimulation at 10 Hz produces glomerular ischemia.[226]

The renal medullary circulation is less sensitive than the cortical vasculature to renal nerve stimulation, particularly at low stimulus intensities. This is largely due to more effective blunting of vasoconstriction by NO and eicosanoids. The electrical stimulation of renal nerves causes parallel reductions in RBF and cortical blood flow, whereas the decrease in medullary perfusion is approximately 50% less. Low frequency nerve stimulation ($<$2 Hz) increases NO production, which buffers the vasoconstriction in all regions of the kidney. More severe renal vasoconstriction produced by high frequency nerve stimulation ($>$4 Hz) leads to the activation of AT_1 receptors that magnifies nerve-induced constriction in both the renal cortex and the medulla. NO derived from nNOS is critical to protecting the medullary regions.[227,228]

Infusions of norepinephrine, epinephrine, or α_1-adrenergic agonists produce similar dose-related vasoconstrictor effects on the renal microcirculation. Studies on individual vessels indicate that norepinephrine constricts the interlobular artery and the afferent and efferent arterioles. Total RVR responses to norepinephrine are substantially attenuated by Ca^{2+} channel blockers and by the inhibition of IP_3-mediated release of stored Ca^{2+}. Ca^{2+} responses of the afferent arteriole to α-adrenoceptor stimulation are mediated by the mobilization from internal stores in combination with Ca^{2+} entry through voltage-gated L-type channels and channels insensitive to dihydropyridine Ca^{2+} channel blockers. The latter includes voltage-independent receptor-operated Ca^{2+} channels. Ca^{2+} sensitivity is also increased. Recent evidence implicates superoxide anion generation and downstream signaling involving cyclic ADP ribose, ryanodine receptors on the sarcoplasmic reticulum, and Ca^{2+}-induced Ca^{2+} release. Efferent arteriolar responses to norepinephrine appear to be independent of L-type channels.[20,58,143]

The renal nerves are not essential for efficient steady-state autoregulation of RBF and GFR or for the operation of either the myogenic or the TGF mechanism. These conclusions are

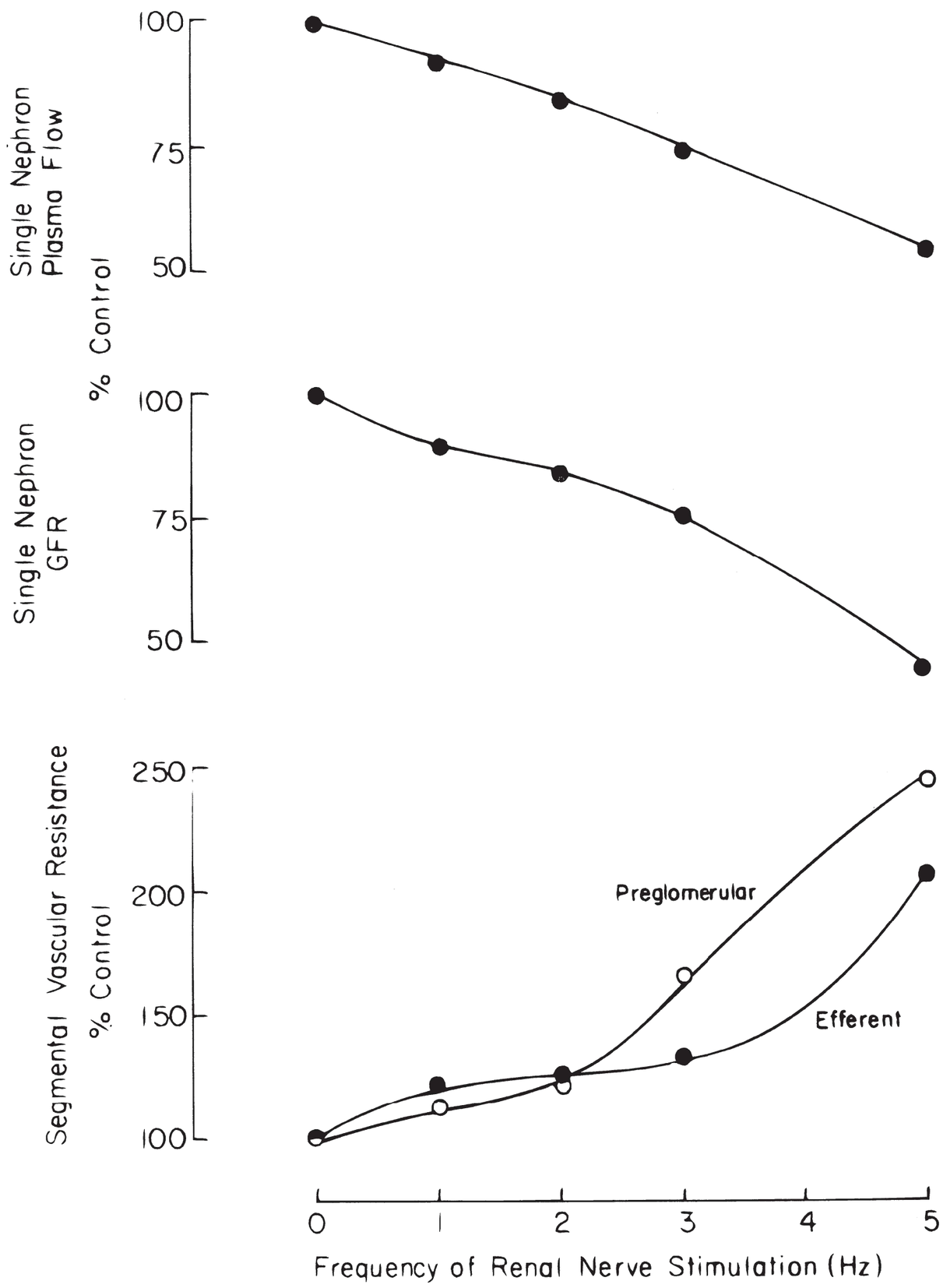

FIGURE 3.24 The effect of electrical stimulation of renal efferent nerves at 1 to 5 Hz on a single nephron glomerular filtration rate (*GFR*), plasma flow, and the resistance of preglomerular vessels and efferent arterioles.

reinforced by a frequency analysis of RBF dynamics indicating that baroreflex-mediated changes in renal sympathetic nerve activity and acute denervation have little impact on intrinsic autoregulatory mechanisms stabilizing renal perfusion in normotensive animals. Under unstressed conditions, while basal efferent sympathetic tone is low, neither acute nor chronic renal denervation affects RBF or GFR. Physiologic changes in renal efferent nerve activity during sleeping, grooming, and movement cause inverse changes in RBF in conscious animals. In anesthetized animals, moderate hypoxia and reflexively induced increases in renal sympathetic nerve activity reduce RBF more than GFR with increases in glomerular capillary pressure as a result of greater constriction of efferent over afferent arterioles. More severe hypoxia reduces RBF and GFR equally as afferent and efferent arteriolar resistances were increased to the same extent. Moderate increases of about 50% in renal nerve activity that are induced reflexively by high- or low-pressure receptors fail to alter renal hemodynamics, although this level of stimulation is capable of affecting tubular sodium reabsorption and renal release of renin and prostaglandins. Hypercapnic acidosis associated with an increase in PCO_2 from 25 to 70 mm Hg activates the renal efferent nerves but the neurally mediated renal vasoconstriction is partially counteracted by enhanced synthesis of vasodilatory prostaglandins. Neurally induced vasoconstriction is more readily demonstrable during more stressful states, such as during dehydration, blood-volume contraction, hemorrhage, and congestive heart failure. The pronounced activation of renal efferent nerve activity (+500%) produced by auditory or emotional stimuli causes intense renal vasoconstriction.[1,132,229]

In addition to a direct effect on vascular smooth muscle, the renal efferent nerves exert secondary hemodynamic effects as a consequence of an intrarenal stimulation of the constrictors Ang II and ET-1 and the formation of dilator prostaglandins and NO. Renal nerve stimulation elicits an increase in renin release from juxtaglomerular cells primarily by the activation of β_1-adrenoceptors signaling through the cAMP/PKA pathway. Prostaglandin synthesis is enhanced by the activation of phospholipase A_2 and the augmented availability of arachidonic acid. Ang II formation accentuates norepinephrine release and renal vasoconstriction

produced by increased renal nerve activity. α_1-Adrenoceptor activation enhances Ang II–mediated afferent arteriolar vasoconstriction by increasing calcium sensitivity. The vasoconstriction caused by moderate renal nerve stimulation is reduced in the presence of the Ang II/AT_1 receptor blockade, suggesting that part of the effect is mediated by increased intrarenal Ang II formation due to the neural stimulation of renin. On the other hand, vasodilatory prostaglandins buffer a significant fraction of the vasoconstriction elicited by nerve stimulation as evidenced by much larger changes in GFR, RBF, and vascular resistance during COX inhibition. Moreover, endothelium-dependent NO release, possibly via the activation of β_3-adrenergic receptors, functions as an inhibitory modulator of vasoconstrictor responses to the sympathetic transmitters norepinephrine and NPY. The effect of renal nerve stimulation on glomerular K_f is controversial. Some investigators report a reduction; others find that K_f is normally unaffected by nerve stimulation but that a reduction of K_f is evident during the inhibition of prostaglandin synthesis.[2]

Afferent renal nerves serve important functions in the neurohumoral control of arterial pressure, vasopressin release, and renal excretion of sodium and water. Afferent nerve endings contain neuropeptides such as calcitonin gene-related peptide, substance P, and vasoactive intestinal peptide. Afferent input from intrarenal sensory receptors in the pelvic wall participates in renorenal reflexes that modulate efferent nerve activity to the contralateral kidney. Baroreceptors or mechanoreceptors are activated by high venous or interstitial pressure. High renal pelvic pressure reduces efferent renal nerve traffic to the opposite kidney, which results in compensatory increases in RBF, GFR, and sodium excretion. Similar functional changes in the opposite kidney are elicited by ischemia-sensitive chemoreceptors responding to increased urinary potassium or very high sodium concentration. Outgoing afferent nerve activity is influenced also by incoming efferent nerve activity such that increased efferent input usually increases afferent nerve activity. The norepinephrine release from efferent nerves activates adrenoceptors on sensory nerves, with α_1-adrenoceptor activation increasing afferent nerve activity and α_2 receptor stimulation reducing afferent firing.[230,231]

Although cholinesterase-containing fibers and β-adrenergic sites have been identified histologically in the renal cortex, there is little functional evidence for neurogenic renal vasodilation mediated by acetylcholine or β-adrenoceptors. The neural release of acetylcholine, however, may exert a small indirect effect at presynaptic sites to inhibit norepinephrine release. Infusions of exogenous acetylcholine increase RBF and reduce RVR, whereas GFR is unaffected. The decline in total resistance is due to parallel reductions in afferent and efferent arteriolar resistance. Acetylcholine relaxes isolated preparations of the interlobular artery and the afferent and efferent arterioles. As pointed out earlier, however, the full vasodilatory effect of acetylcholine requires an intact endothelium and is primarily mediated by a combination of NO as well as prostanoids and EETs.[132]

Dopamine, a sympathomimetic amine precursor of norepinephrine, is a neurotransmitter capable of regulating renal hemodynamics, renin secretion, and sodium excretion. Histofluorescent evidence suggests dopaminergic innervation of the cortical vessels, primarily the glomerular vascular poles. However, dopamine synthesized from L-dopa via L-dopa decarboxylase in proximal tubular cells is clearly the major source of urinary dopamine and its metabolites, with the greatest production during high salt diet and lowest synthesis during sodium restriction. There are two major receptor families: D_1-like (D_1 and D_5 receptors) and D_2-like (D_2, D_3, D_4 receptors). D_1 receptors are postsynaptic receptors located on vascular smooth muscle and tubular cells, but are absent from glomeruli. They cause vasodilation by cAMP/PKA signaling. D_2 receptors are either presynaptic or postsynaptic and are located in glomeruli as well as vessels and tubules in both the cortex and medulla. The presynaptic D_2 receptor indirectly dilates vessels by the inhibition of norepinephrine release. Dopamine is commonly used to evaluate the renal functional reserve—the ability to dilate the renal vasculature—in various pathologic conditions.[232–236]

Although receptor blockers abolish renal effects produced by exogenous dopamine, such antagonists do not consistently affect basal renal hemodynamics, questioning the role of physiologic levels of dopamine. Further, the prejunctional stimulation of dopamine receptors during moderate levels of renal efferent nerve activity has little influence on RVR. Small amounts of exogenous dopamine or a D_1 receptor agonist dilate the renal vasculature. Responses include an increase in RBF and a decrease in filtration fraction; GFR and glomerular capillary pressure are unaffected. Most of the dopamine-induced renal vasodilation is mediated by D_1 receptors, which are coupled to cAMP/PKA signaling. Dopamine and D_1 receptor agonists relax afferent and efferent arterioles and interlobular and arcuate arteries. D_1 receptor stimulation attenuates the constriction of both afferent and efferent arterioles produced by either Ang II or ET-1. The D_1-induced dilation of the preglomerular vasculature does not impair autoregulatory adjustments in vascular resistance to changes in perfusion pressure. D_1-receptor stimulation attenuates tubuloglomerular feedback responsiveness. The vasodilation elicited by dopamine is more pronounced after denervation or pharmacologic blockade of α-adrenoceptors, suggesting that presynaptic D_2-dopamine receptors augment norepinephrine release from nerve terminals. D_2-receptor stimulation is associated with increases in GFR and the attenuation of Ang II–induced contraction of glomerular mesangial cells. D_1 receptors also stimulate a renin release from juxtaglomerular cells. D_1 agonists inhibit fluid and electrolyte transport indirectly via hemodynamic mechanisms and directly by the occupation of D_1 receptors in the proximal tubule, the thick ascending limb of Henle, and the collecting duct. D_1 receptor null mice have elevated proximal tubular salt transport and hypertension.[2,132]

NPY is another neurotransmitter. Nerve endings containing immunoreactive neuropeptides are localized along the interlobular and arcuate arteries extending to glomerular arterioles, innervating both afferent and efferent arterioles. NPY is a cotransmitter of the renal sympathetic nerves that is coreleased with and may potentiate the vascular pressor effects of norepinephrine and ATP. NPY is less potent than norepinephrine. The kidney expresses NPY receptors that can also be activated by peptide YY (PYY), a circulating hormone released from gastrointestinal cells. NPY and PYY produce renal vasoconstriction via the Y_1 receptor. Administered NPY constricts both the afferent and efferent arterioles and inhibits renin release. Nerves innervating the efferent arteriole contain more immunoreactive NPY than those to the afferent arteriole. NPY acts prejunctionally to inhibit norepinephrine release via Y_2 receptors. Despite marked reductions in RBF, systemic NPY infusion elicits a diuresis and natriuresis that is mediated in part by bradykinin. NPY antagonists increase basal RBF but do not alter basal urinary excretion.[226,228,237]

Other Vasoactive Agents

Atrial Natriuretic Peptide

ANP is a 28-amino acid peptide family involved in the physiologic regulation of renal function and sodium excretion. A high molecular weight precursor of ANP is constitutively synthesized in cardiocytes of the atria. ANP is released into the circulation as a function of atrial volume or distention in association with changes in sodium and water balance. In addition to ANP, related peptides include ventricular brain natriuretic peptide (BNP) and renal urodilatin. Studies implicate renal conversion of ANP to urodilatin, which is closely related to sodium excretion under a variety of conditions.[238,239]

ANP receptors are concentrated in glomerular capillaries and the collecting duct; they are also present along the cortical arterioles and the medullary arterioles and the vasa recta; cell types include the collecting duct, the vascular smooth muscle, and the endothelial and mesangial cells. There are three subtypes of natriuretic peptide receptors (NPR), termed NPR-A, NPR-B, and NPR-C. The A- and B-type receptors include a cytoplasmic catalytic domain and the active particulate guanylate cyclase, exerting physiologic effects by increasing cell cGMP. ANP may buffer the action of vasoconstrictors such as Ang II and norepinephrine via an interaction of cGMP with intracellular Ca^{2+}, perhaps secondary to the inhibition of Ca^{2+} mobilization and the stimulation of Ca^{2+} efflux. The renal vasculature also has biologically silent, NPR-C "clearance" receptors, that remove ANP from the circulation with no role in signal transduction or guanylate cyclase activity. [240–242]

ANP is a rapid-acting, potent natriuretic and diuretic peptide that is also capable of lowering arterial pressure by direct vasodilatory effects on the systemic vasculature and reducing cardiac output. Physiologic concentrations of ANP inhibit tubular reabsorption without altering RBF, GFR, or the filtered sodium load. Medullary blood flow is increased during the administration of ANP, but the effect seems to be secondary to the ANP-induced natriuresis. Several tubular sites have been evaluated, including a Na-ATPase of the proximal tubule and an amiloride-sensitive sodium channel in the collecting duct and an angiotensin-sensitive exchanger in the proximal tubule. ANP has a direct inhibitory action on renin release in cultured juxtaglomerular cells that is mediated by cGMP.[243,244]

The renal vascular effects of ANP are mediated by NPR-A and NPR-B receptors. Activation of NPR-A receptors dilates preglomerular resistance vessels, including arcuate and interlobular arteries, afferent arterioles, and efferent arterioles. The NPR-B receptor contributes to the dilation of the preglomerular vasculature. The reported effect of ANP on K_f is variable. Although glomerular capillary pressure is increased, GFR is usually unchanged, presumably because of a decrease in K_f. ANP infusions increase glomerular permeability to macromolecules. The preglomerular vasodilation produced by ANP does not impair autoregulation of either RBF or GFR. ANP exerts both indirect and direct effects on the TGF mechanism. ANP markedly inhibits TGF control of glomerular hemodynamics when feedback activity is evaluated by the perfusion of the Henle loop with artificial fluid. Studies on mice with underexpression and overexpression of the NPR-A receptor highlight the important role ANP has in mediating the renal vascular and tubular changes resulting from blood volume expansion. Overexpression of NPR-A receptors is associated with enhanced renal vasodilatory and natriuretic responses, whereas the responses are markedly attenuated in mice lacking NPR-A receptors. Synthetic ANP (M-ANP), being tested for use in hypertension and congestive heart failure, are potent agents for lowering blood pressure and systemic vascular resistance, while increasing RBF, GFR, and sodium excretion. Transgenic mice with an overexpression of natriuretic peptide receptor A respond to blood volume expansion with marked natriuresis, along with increases in RBF and GFR, whereas mice deficient in the NPR-A fail to show the increases in RBF and GFR or sodium excretion. These data demonstrate the importance of ANP in regulating renal function in response to volume expansion.[78,107,244–247]

Vasopressin

In addition to its antidiuretic properties, AVP is a vasoconstrictor, with predominant actions in the renal medulla. The plasma AVP concentration is directly related to plasma osmolality and inversely with blood volume and pressure. AVP produces effects on renal function by binding to V_{1a} or V_2 membrane receptors. The well-known osmoregulatory function of low plasma AVP concentrations is mediated through a V_2 tubular membrane receptor that signals through cAMP/PKA to alter water permeability and sodium reabsorption. Vasopressin also stimulates the production of medullary NO via the activation of V_2 receptors, which serves a protective

function to prevent excessive vasoconstriction. AVP affects renal hemodynamics by acting on V_1a receptors localized to cortical arteries/arterioles (interlobar, arcuate and interlobular arteries, afferent and efferent arterioles, glomeruli) and outer medullary vasa recta. V_{1a} mRNA and protein in preglomerular resistance arteries/arterioles are upregulated in response to reduced plasma AVP concentration associated with water loading and downregulated as plasma AVP is increased during water deprivation. Desensitization of V_1 receptors and reduced receptor density are related to PKC-mediated phosphorylation. AVP activates vascular V_{1a} receptors to exert pressor effects that contribute to the maintenance of arterial pressure during conditions of chronic water deprivation, graded hemorrhage, and possibly in various forms of hypertension.[107,248,249]

The vascular effects of AVP are due to the activation of V_{1a} receptors on vascular smooth muscle cells, which is coupled to Gα_q protein that leads to increased IP_3 production and PKC activation. In afferent arterioles, $[Ca^{2+}]_i$ is increased by a combination of Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels and voltage-insensitive store-operated cation channels. The contraction of efferent arterioles is more dependent on Ca^{2+} release from sarcoplasmic reticular stores with little Ca^{2+} entry through L-type channels. In addition to increasing $[Ca^{2+}]_i$, AVP increases Ca^{2+} sensitivity via PKC and Rho-kinase signaling. The K_f lowering effect of AVP may be mediated by a direct action on mesangial cells. It was found, however, that K_f could be normalized by a combined blockade of the vascular action of both AVP and Ang II, whereas the selective blockade of either peptide was ineffective. The V_{1A} receptor on endothelial cells mediates AVP-induced NO release. Also, AVP interacts with the renal arachidonic acid and the renin–angiotensin systems. AVP stimulates renal release of PGE_2 in vivo. Prostaglandin production is increased by AVP-receptor interaction with either V_1 or V_2 receptors on medullary interstitial cells or glomerular mesangial cells. Vascular smooth muscle V_{1A} receptor activation stimulates phospholipase A_2 and prostaglandin production. Mice lacking the V_{1A} receptor are hypotensive and have decreased blood volume and sympathetic activity. They also have decreased activity of the renin–angiotensin system and lower aldosterone levels. The V_{1A} receptor is expressed in macula densa cells and colocalizes with COX-2 and nNOS. The reduced RBF and GFR are due, in part, to reduced blood pressure.[1,55,107,142,248,250–252]

The administration of exogenous AVP produces renal vasoconstriction that is buffered more by NO than COX-dependent prostaglandins, effects mediated by V_1 but not V_2 receptors. Vasoconstriction in the renal medulla is more prominent than in the renal cortex. AVP-induced cortical vasoconstriction is effectively buffered by the cytochrome P450 epoxide production of vasodilator EETs such that cortical blood flow is basically unchanged by AVP. In contrast, the medullary vasoconstriction elicited by AVP is unaffected by the inhibition of cytochrome P450 epoxide activity and EET production.[107,253]

In normal, unstressed conscious animals, the antagonism of V_1 receptors has no effect on basal RBF or arterial pressure, indicating minimal pressor activity of AVP when its plasma concentration is low. Physiologic increases in plasma AVP concentration during 48-hour water restriction and maximum urine osmolality reduce blood flow to the inner medulla via V_1 receptors while maintaining a constancy of blood flow to the outer medulla. V_2 receptor antagonism produces a diuresis. Increases in plasma AVP concentrations up to 8 pg per millimeter reduce medullary perfusion selectively and greatly attenuate the arterial pressure–blood flow and pressure–natriuresis relations without affecting total RBF or renal cortical blood flow. AVP-induced vasoconstriction in the renal medulla is normally modulated by AVP-stimulated local release of NO, perhaps mediated by V_2 receptors, reflecting a compensatory response that buffers the magnitude of the vasoconstriction and stabilizes medullary perfusion.[15]

Adrenomedullin

Adrenomedullin, α- and β-calcitonin gene-related peptide (CGRP), calcitonin, and amylin are homologous polypeptides with overlapping biologic actions such as vasodilation, inhibition of bone resorption, and antiproliferative activity. Adrenomedullin is a potent renal vasodilating and natriuretic peptide (52 amino acids with a disulfide ring), which is made in the kidney as well as the adrenal gland. mRNA for adrenomedullin and its receptor are colocalized to renal vessels, glomeruli, and inner medullary collecting ducts. The proximal tubule is abundant in adrenomedullin mRNA, whereas the greatest amounts of receptor mRNA are in the papilla. Changes in the salt diet do not appear to change the expression of the peptide or its receptor in either the cortex or the medulla. This hormone/paracrine agent increases RBF and sodium excretion without affecting GFR. Natriuretic potency increases during the inhibition of neutral endopeptidase, an enzyme that cleaves endogenous peptides with a disulfide ring such as adrenomedullin and ANP.

Adrenomedullin produces vasodilation by cAMP/PKA signaling and by stimulating endothelial NO production, leading to the activation of ATP-sensitive K^+ channels and Ca^{2+}-dependent K channels and the hyperpolarization of vascular smooth muscle cells. Adrenomedullin increases renin release via increasing cAMP in juxtaglomerular granular cells. CGRP exerts NO-dependent as well as cAMP/PKA vasodilation. The administration of adrenomedullin or CGRP increases RBF and to a lesser extent GFR; both are natriuretic and diuretic. However, the renal vasodilator effects of adrenomedullin are not dependent on CGRP. Adrenomedullin and CGRP dilate afferent arterioles and blunt Ang II- and norepinephrine-induced renal vasoconstriction. Increased adrenomedullin levels in diabetic rats are associated with

the early hyperfiltration and may contribute to the afferent arteriolar vasodilation.[107,254–257]

Reactive Oxygen Species

Small amounts of ROS constantly produced by aerobic metabolism have important roles in signal transduction in the vasculature under physiologic conditions. In pathophysiologic states, ROS initiate and amplify deleterious events such as lipid oxidation and tissue/DNA damage associated with glomerular inflammation and proteinuria, and vascular hypertrophy in addition to vasoconstriction. ROS are products of partial reduction of oxygen, generated by enzymatic and nonenzymatic reactions. Common oxidative enzymes include nicotinamide adenine dinucleotide(NADH)/reduced NADPH oxidases (NOX), COX, cytochrome P450, and xanthine and glucose oxidases. The reduction of molecular oxygen ($O_2 + e^-$) produces superoxide anion ($\bullet O_2^-$), which is normally balanced by its degradation. Superoxide dismutases (SOD) catalyze the conversion of $\bullet O_2^-$ to hydrogen peroxide (H_2O_2) that has oxidizing potential. H_2O_2 is subsequently neutralized by glutathione peroxidases and catalase. An alternative pathway for $\bullet O_2^-$ degradation is to rapidly react with NO to form peroxynitrite ($ONOO^-$), which clearly limits the half-life, the diffusion distance, and the biologic activity of NO as well as $\bullet O_2^-$. Vitamins A, E, and C and bilirubin are scavengers of ROS.[199,258–261]

As intracellular signals, ROS may activate or inactivate redox-sensitive protein kinases and phosphatases to modulate GPCR phosphorylation and transcription factors. Reactive nitrogen species such as S-nitrosothiols can modulate GPCR signaling and internalization through S-nitrosylation of β-arrestin and GRK. Normally there is a fine balance between activities of oxidative and antioxidant enzymes, optimizing NO activity and minimizing superoxide anion generation. $\bullet O_2^-$ functions as the counterpart to NO and its antiproliferative and vasodilatory actions. In the kidney, ROS are formed in arteries and arterioles, glomeruli, and juxtaglomerular cells endowed with oxidases such as NOX, NOS, and COX. NOX1, NOX2, and NOX4 are expressed in the kidney. Predominant isoforms in the renal vasculature are NOX1 and NOX2 that primarily produce $\bullet O_2^-$; epithelial NOX4 mainly generates H_2O_2. $\bullet O_2^-$ is degraded by superoxide dismutases, of which the cytosolic or intracellular isoform accounts for ~70% of the enzyme in the kidney, with lesser amounts in extracellular and mitochondrial compartments. In the juxtaglomerular apparatus, endothelial cells produce NO via eNOS and macula densa cells via nNOS. Stimulants of $\bullet O_2^-$ production include vasoconstrictor agents (e.g., Ang II, ET-1, norepinephrine), growth factors, and stretch. Chronic high Ang II and a high salt diet are potent stimulants, increasing the expression of NOX subunits and reducing superoxide dismutase isoforms. Overall renal actions of $\bullet O_2^-$ are vasoconstriction and the enhancement of tubular sodium reabsorption. NOX-derived $\bullet O_2^-$, largely independent of H_2O_2, contributes to GPCR signaling and participates in renal vasoconstriction elicited by Ang II, catecholamines, and ET-1 activation of ET_A and ET_B receptors.[199,262]

$\bullet O_2^-$ exerts tonic renal vasoconstriction in normotensive rats, an action that becomes more pronounced when NO production is inhibited, indicating an interaction between $\bullet O_2^-$ and NO that have opposing actions on vascular resistance. This is evident because the administration of superoxide dismutase and NOX inhibition increases RBF. Moreover, NOX2-deficient mice have an increased basal RBF with normal GFR and arterial pressure, with less renal vasoconstriction in response to NOS inhibition than observed in wild-type mice that are able to produce the vasoconstrictor via NOX2. NOX-derived $\bullet O_2^-$, largely independent of H_2O_2, contributes to GPCR signaling and participates in renal vasoconstriction elicited by Ang II, catecholamines, and ET-1 activation of ET_A and ET_B receptors. Ang II–induced renal vasoconstriction and reduced GFR are magnified during NOS inhibition and weakened during administration of superoxide dismutase to scavenge $\bullet O_2^-$. Increased endogenous $\bullet O_2^-$ activity produced by acute pharmacologic inhibition of superoxide dismutase (diethyldithiocarbamate) reduces total renal, as well as both cortical and medullary blood flow. The vasoconstriction is greater when the buffering afforded by NO is removed by NOS inhibition. This is also the case for renal vasoconstriction produced by norepinephrine, phenylephrine, and ET-1. Other studies show that increased $\bullet O_2^-$ production stimulated by the acute infusion of Ang II produces more pronounced renal vasoconstriction when NADPH oxidase is intact than when NADPH oxidase is rendered nonfunctional in transgenic animals with NOX2 mutated. Ang II elicits less pronounced renal vasoconstriction in the absence of NOX2. Extracellular superoxide dismutase inactivation of the $\bullet O_2^-$ plays an important role in buffering acute Ang II–induced constriction of the afferent arteriole and increased RVR produced by chronic Ang II infusion.[198,263–266]

The mechanism by which $\bullet O_2^-$ causes or modulates renal vasoconstriction in normal kidneys is still unresolved. Ang II stimulates NADPH oxidase activity and $\bullet O_2^-$ production in the renal cortex and medulla. Ang II and ET-1 receptor activation rapidly stimulates NOX2-mediated O_2- release from afferent arterioles that increases cytosolic Ca^{2+} concentration in smooth muscle cells by increasing ADP ribosyl cyclase activity. The metabolite cyclic ADP ribose sensitizes ryanodine receptors on the sarcoplasmic reticulum of smooth muscle to release Ca^{2+}. $\bullet O_2^-$ may also scavenge NO and reduce bioavailability of this vasodilator, a major factor contributing to endothelial dysfunction in disease states. Accordingly, $\bullet O_2^-$ activity is enhanced during NOS inhibition. It should be appreciated that $\bullet O_2^-$-induced acute renal vasoconstriction is observed during NOS inhibition, highlighting a principal direct action on vascular smooth muscle independent of NO.[20,113,160,198,263,264,266–269]

Increased intrarenal $\bullet O_2^-$ produced by infusions of hypoxanthine and xanthine oxidase increases sodium excretion,

while GFR is reduced more than RBF and arterial pressure is unchanged. In these rats, $\bullet O_2^-$ did not affect the efficiency of pressure-induced steady-state RBF autoregulation. However, other evidence convincingly indicates that $\bullet O_2^-$ regulates both myogenic and TGF mechanisms responsible for renal autoregulation. The pressure-induced myogenic constrictor response of isolated afferent arterioles is mediated or enhanced by $\bullet O_2^-$ independent of H_2O_2 and of eNOS production of NO. In addition, $\bullet O_2^-$ produced by NOX2 in macula densa cells plays a role in TGF-induced afferent arteriolar vasoconstriction via direct action on afferent arterioles. This direct action complements quenching NO availability. The effects of $\bullet O_2^-$ are attenuated by increased levels of superoxide dismutase. Thus, ROS are important signaling molecules that participate in intrinsic autoregulatory responses of the preglomerular vasculature to changes in renal perfusion pressure. Local production of NO and ROS modulates reactivity of descending vasa recta pericytes that control medullary perfusion.[114,171,270–274]

eNOS and NADPH oxidase are both expressed in tubular epithelial cells within the renal medulla, particularly the thick ascending limb of the Henle loop, with the production of NO and $\bullet O_2^-$ participating in the regulation of medullary blood flow and influencing the set point of the pressure–natriuresis relation. Sodium retention and hypertension result when the balance of production of these free radicals favors $\bullet O_2^-$ in conditions such as in activation of the renin–angiotensin system, NOS inhibition, and diabetes. For example, during chronic NOS inhibition, renal vascular actions of $\bullet O_2^-$ are largely unopposed. Intrarenal infusion of the superoxide dismutase tempol in hypertensive L-nitro-arginine-methyl-ester (L-NAME) treated rats increases total RBF and blood flow to both the renal cortex and the medulla, and GFR, while urinary excretion of 8-isoprostane is reduced.[275–277]

Peroxynitrite ($ONOO^-$) is formed endogenously by NO reacting with $\bullet O_2^-$, exerting NO-like biologic activity as well as nitrating proteins during oxidative stress. The administration of low $OONO^-$ concentrations causes renal vasodilation as RBF and GFR increase in parallel in a NO-dependent manner, reverting to the constriction during NOS inhibition. High $ONOO^-$ concentrations produce renal vasoconstriction with larger reductions in GFR than in RBF. The reductions in RBF and GFR are magnified during NOS inhibition.[278]

$ONOO^-$ can oxidize arachidonic acid to form the vasoconstrictor 8-iso-$PGF_2\alpha$ (F2-isoprostane), which activates a thromboxane TP receptor to elicit vasoconstriction. Isoprostanes may increase ET-1 release from endothelial cells. Chronic exposure to high levels of Ang II stimulates isoprostane production by the kidney. Oxidative stress and exaggerated isoprostane levels acting on TP receptors enhances the strength of TGF in some models of hypertension. Such signaling may explain the afferent arteriolar vasoconstriction associated with oxidative stress in Ang II–induced hypertension, which appears to be mediated by an endothelial-derived and COX-derived vasoconstrictor that acts on TP receptors on vascular smooth muscle cells.[279]

Endogenous H_2O_2 appears to have little effect on preglomerular resistance vessels in the renal cortex because the combination of catalase with a superoxide dismutase mimetic has no additional effect as compared to superoxide dismutase quenching O_2^- alone. As mentioned in the following, increases in H_2O_2 above basal levels of 100 to 500 nmol per liter cause vasoconstriction in the renal medulla. H_2O_2, at apparent pharmacologic concentrations in micrometer per millimeter range in vitro, is reported to have biphasic effects on nonrenal resistance arterioles, dilating at low concentrations, perhaps due to NO, and constricting at high concentrations (>50 μmol per liter), possibly due to an isoprostane. H_2O_2 inhibits Ang II increases in $[Ca^{2+}]_i$ in afferent arterioles when the concentration is ≥ 1 μm but not less. Electrophysiologic studies of endothelium of freshly isolated porcine renal arteries indicates that a large conductance (300 pS) Ca^{2+}-activated K^+ channel (BK_{Ca}) is inhibited dose dependently by ROS in nanomole per liter range and H_2O_2 in the micromole per liter range. If tonically active, closure of BK_{Ca} channels causes depolarization and leads to vasoconstriction. Consistent with this view, H_2O_2 was found to reduce bradykinin-induced dilation of isolated renal arteries.[272,280,281]

MECHANISMS REGULATING MEDULLARY MICROCIRCULATION

Blood flow to the renal medulla is only about 20% of the total RBF; however, it is of major importance in regulating sodium homeostasis and in the maintenance of the medullary hypertonic environment. Expressed per gram of tissue, medullary blood flow is lower than cortical blood flow. The unique architecture of the renal medullary microcirculation preserves the axial osmotic gradient generated by the countercurrent exchange of water and solutes between the descending and ascending vasa recta while allowing for the removal of the water and solutes reabsorbed from the descending limb of the Henle loop and the medullary collecting ducts. Blood flow to the renal medulla is supplied from efferent arterioles of juxtamedullary nephrons, which give rise to the vascular bundles located in the outer medulla. The descending vasa recta (DVR) gradually transform until the smooth muscle layer, present in the outer medulla, is replaced by the discontinuous rings of pericytes. Thus, the vascular smooth muscle cells of the juxtamedullary nephron arterioles and of the outer medullary DVR are primarily responsible for the differential regulation of the medullary circulation. The outer zone of the inner medulla is partitioned into two distinct compartments with intercluster regions, one consisting of the ascending and the descending vasa recta and another consisting of the ascending vasa recta (AVR) and collecting ducts. Efferent arterioles and the DVR

are the main medullary structures with sympathetic innervation, which terminates when pericytes replace the smooth muscle cells. Extravascular renomedullary interstitial cells also exhibit contractile properties, but their role in regulating medullary blood flow is unclear. It has been more difficult to study the medullary circulation due to its inaccessibility and the fact that it is positioned in series with the glomerular circulation of juxtamedullary nephrons. Although there is considerable discrepancy regarding the absolute values of medullary blood flow obtained using various techniques, most of them provide a reasonable index of relative changes in blood flow.[1,15,18,89,282,283]

Hematocrit

The hematocrit of renal medullary blood is lower than that of systemic blood or blood derived from the renal cortex. Red blood cell (RBC) transit time is shorter than for plasma, and tissue hematocrit varies inversely with the medullary axis. Studies using videomicroscopic techniques and complementary direct measurements of hematocrit with micropuncture have demonstrated low microvessel hematocrit in the renal medulla. Shrinkage of RBCs in the hypertonic medulla also shifts water from the interior of RBCs to plasma and also contribute to the lower medullary microvessel hematocrit.[2,15]

In contrast to the peritubular capillary plexus that arises from efferent arterioles in the cortex to reabsorb massive volumes of tubular fluid, the vasa recta serve different needs specific to the medulla. Through their countercurrent arrangement, the DVR and the AVR trap NaCl and urea deposited to the interstitium by collecting ducts and the loops of Henle and maintain corticomedullary osmotic gradients. However, metabolic substrates, including O_2, that enter the DVR diffuse to the AVR to be shunted back to the cortex, leading to lower O_2 levels in the medulla than in the cortex. Paracrine agents regulate the renal medulla perfusion in a complex manner involving tubular-to-vascular and vascular-to-tubular paracrine signaling cross-talk. Protection of the medulla from hypoxia by these agents involves the local generation of paracrine agents by tubular and vascular structures and trapping by countercurrent exchange to yield axial concentration gradients that exert variable effects on the medullary vessels.[90]

Autoregulation

As already discussed, overall RBF is autoregulated with very high efficiency over a wide range of systemic perfusion pressure. Although cortical blood flow is well autoregulated within the physiologic range, the extent to which medullary blood flow is autoregulated is more controversial, especially in the rat. Some studies suggest that medullary blood flow autoregulation is not as efficient as in the cortex and that changes in medullary blood flow contribute to pressure natriuresis and the regulation of salt and water excretion as perfusion pressure changes. As mentioned previously, however, the renal medulla is largely perfused by the efferent arterioles from juxtamedullary nephrons, which have very high autoregulatory efficiency. Although a small population of shunt vessels bypass glomeruli, and may escape the autoregulatory adjustments, the extent of periglomerular shunting is very small and unlikely to contribute significantly to overall medullary blood flow responses. Both cortical and medullary blood flows are efficiently autoregulated in the dog. This holds for both outer and inner medullary perfusion. The same is true for cortical blood flow in the rat. Inner and outer medullary blood flows are also autoregulated efficiently in hydropenic rats, but not during marked volume expansion. Thus, it appears that loss of medullary blood flow autoregulation occurs primarily during marked volume expansion in rats subjected to increases in perfusion pressure. Recruitment of flow through the previously unperfused vasa recta may also contribute to that process, in particular because individual medullary vessels also exhibit autoregulatory responses. Volume-expanded sodium-replete dogs and rabbits exhibit efficient intact medullary blood flow autoregulation. The in vitro juxtamedullary nephron preparation that evaluates blood flow through nephrons that give rise to the vasa recta clearly exhibits normal autoregulatory behavior of the preglomerular vasculature. Thus, the extent of medullary blood flow autoregulation efficiency as well as the role of medullary perfusion in the generation of pressure natriuresis varies with species and degree of volume expansion. Nevertheless, paracrine factors act within the medulla to exert local control of medullary blood flow. The pressurization of in vitro perfused DVR increases endothelial $[Ca^{2+}]_i$ and the generation of NO. The release of NO by the vasa recta could increase local blood flow as well as inhibit salt reabsorption by adjacent tubules. A role for NO to provide a diffusible signal between the vasculature and nephrons seems likely.[1,2,15,18,107,153,277,284–286]

Vasopressin

Increases in medullary blood flow reduce the efficiency of passive countercurrent exchange, leading to "solute washout" and reductions in the corticomedullary gradients for NaCl and urea. Vasopressin exerts an important role in reducing medullary blood flow which contributes to increased urinary concentrating ability during antidiuresis. Homozygous Brattleboro rats that lack vasopressin have central diabetes insipidus and elevated medullary plasma flow. Vasopressin exerts its actions via subtype specific V_1 (vasoconstrictor) and V_2 (antidiuretic) receptors and reduces vasa recta blood flow through the activation of both V_1 or V_2, indicating roles for both vasoactive and reabsorptive mechanisms. A selective V_1 receptor agonist reduces inner medullary more than outer medullary blood flow. Similarly, elevated circulating vasopressin in water-deprived rats reduces inner medullary blood flow with weaker effects in the cortex and the outer medulla; these effects are blocked

by a V_1 antagonist, supporting V_1-mediated vascular effects of vasopressin to modulate inner medullary blood flow and to promote antidiuresis by maintaining the elevated osmolality in the medullary interstitium. Vasopressin reduces medullary perfusion by constricting juxtamedullary afferent and efferent arterioles. Afferent arteriolar vasopressin–mediated constriction is dependent upon voltage-gated Ca^{2+} entry via voltage-gated L-type and store-operated channels, whereas efferent constriction may be primarily related to Ca^{2+} mobilization from stores. Vasopressin also constricts outer medullary DVR. A vasopressin V_1 agonist reduces medullary blood flow without constricting either afferent or efferent arterioles, suggesting a greater sensitivity of the vasa recta to vasopressin.[15,252,287,288]

In addition to V_1 mediated constrictor effects of vasopressin, vascular V_2 receptors may cause vasodilation via the stimulation of NO release. V_2 agonists dilate preconstricted afferent arterioles and the outer medullary DVR in vitro. The V_2 agonist, dDAVP, stimulates medullary NO release and increases medullary blood flow. AVP-induced V_2 receptor activation in collecting ducts stimulates the phosphoinositide pathway and causes the mobilization of Ca^{2+} to increase NO production in the medulla and to protect the outer medulla from excessive vasoconstriction and ischemia.[1,15,248,286]

Angiotensin

In addition to its actions on the afferent and efferent arterioles, Ang II tonically constricts the medullary microcirculation via effects on the vasa recta. A NOS blockade constricts DVR and intensifies the constriction by Ang II. Similar to NO, PGE_2 and adenosine blunt Ang II constriction of DVR.[15,275,289]

AT_2 receptor activation elicits vasodilation via the generation of NO and the synthesis of vasodilatory cytochrome P450 epoxygenase-derived EETs. AT_2 activation vasodilates DVR where it inhibits reactive oxygen species formation and facilitates endothelium-dependent $[Ca^{2+}]_i$ signaling that leads to the release of vasodilators. There are also mechanisms leading to the Ang II–induced enhancement of medullary perfusion via AT_1 receptors due to the generation of compensatory vasodilators, particularly NO and vasodilator prostaglandins. Interstitial Ang II concentrations in the renal medulla are higher than in the cortex and Ang II receptor density is also higher in this region.[15,129,228,290–292]

Nitric Oxide

NO is particularly important in defending the renal medulla against hypoxia and ischemia. Chronic and acute systemic or intrarenal NOS inhibition reduces medullary blood flow more than cortical blood flow and elicits hypertension. Per volume of tissue, NO production in the renal medulla exceeds that in the cortex. NO production is closely coupled to L-arginine availability and cellular uptake via an amino acid transporter. Accordingly, dietary L-arginine supplementation increases renal medullary interstitial NO and medullary blood flow and reduces blood pressure in hypertensive Dahl rats and spontaneously hypertensive rats (SHR). DVR is dilated by NO donors and endothelium-dependent vasodilators. Similarly, NOS inhibition increases DVR vasomotor tone, producing as much as a 35% decrease in blood flow and blunts the dilation of preconstricted DVR by the endothelium-dependent vasodilators, acetylcholine and bradykinin. NO has a particularly important role to protect against the excessive reduction of medullary perfusion associated with hypoxia and oxidative stress, and NO levels in the medullary interstitium rise in response to Ang II, norepinephrine, and vasopressin. Conversely, medullary inhibition of NOS and NO production increases vascular sensitivity so that infusion of otherwise ineffective doses of vasoconstrictors reduce perfusion and generate tissue hypoxia.[284,293]

NO has both vasodilatory and natriuretic effects and is synthesized by epithelial as well as endothelial cells, suggesting critical tubular–vascular interactions. NO generated by the medullary thick ascending limb influences DVR tone. Conversely, NO generated by the DVR inhibits sodium reabsorption by adjacent nephron segments.[15,284,294]

The bioavailability and actions of NO are modified to a great extent by opposing the generation of ROS. NO levels in the renal medulla are modulated through reactions with oxygen radicals. ROS generation plays a role in the agonist-induced constriction of renal microvessels in the cortex and the medulla and in Na reabsorption by the ascending limb of the loop of Henle. In the DVR, ROS are generated upon stimulation with Ang II and PKC agonists; superoxide anion generation by the medullary thick ascending limb may limit availability of NO delivery and the vasodilation of DVR. The SOD inhibitor, diethyldithiocarbamate, reduces medullary blood flow, indicating a vasoconstrictor action of superoxide anion. Infusion of the SOD mimetic, tempol, increases medullary blood flow and sodium excretion, an effect that is more pronounced when H_2O_2 is simultaneously eliminated with catalase.[228,270,275,294–296]

Arachidonic Acid Metabolites

Prostaglandins, generated from arachidonic acid by COX-1 and COX-2 enzymes, alter intrarenal hemodynamics and increase blood flow toward the juxtamedullary cortex. Medullary blood flow is protected from excessive vasoconstrictors by prostaglandins as well as NO. Nonselective COX blockade decreases vasa recta blood flow by up to 50% and potentiates medullary hypoxia with a relative sparing of cortical perfusion. PGE_2 is generated in large quantities in the renal medulla and blunts Ang II–induced constriction of isolated perfused DVR. Both COX-1 and COX-2 isoforms contribute to renal prostaglandin synthesis and are predominantly expressed in the renal medulla; COX-2 is subject to greater regulation. Renomedullary interstitial cells express receptors and release paracrine substances including PGE_2, EETs, and medullipin, and express both COX-1 and COX-2. Medullary COX-2 expression is

stimulated by tonicity. Genetic deficiency of COX-2, or its chronic inhibition, reduces medullary blood flow and enhances vasoconstrictor responses to Ang II. COX-2–derived prostanoids serve to maintain medullary blood flow under most conditions.[15,180,290,297,298]

Products of arachidonic acid are also generated by cytochrome P450 isoforms to yield EETs, the HETEs, and their products, dihydroxyeicosatetraenoic acids (DHETs). 20-HETE reduces medullary blood flow and the inhibition of 20-HETE with HET0016 enhances medullary blood flow. In contrast, EETs have been linked to endothelial-derived hyperpolarizing factors and cause vasodilation directly and also oppose vasoconstrictor actions of vasopressin.[118,253,299]

Kallikrein–Kinin System

Kallikreins release kinins from kininogens and are expressed by the outer and inner medullary collecting ducts. Kinins exert their actions by activating B_1 and B_2 receptors. B_2 receptors are expressed throughout the outer and inner medulla, and modulate blood flow and sodium reabsorption in the renal medulla. The infusion of a kinin antagonist causes a 20% reduction of papillary blood flow. The enhancement of kinin activity through the infusion of bradykinin or the inhibition of kininases with enalaprilat or phosphoramidon increases both medullary blood flow and sodium excretion. Blocking bradykinin receptors decreases medullary blood flow, an effect that is blocked by NOS inhibition. ACE inhibition in the presence of an AT_1 receptor blockade causes greater increases in MBF than cortical blood flow, and the effects are blocked by the B_2 receptor antagonist, icatibant. Low sodium diets augment the kinin-mediated component elicited by ACE inhibition. In volume-expanded anesthetized rats, a B_2 receptor blockade with icatibant reduces papillary blood flow. Bradykinin, acting through B_2 receptors, generates robust endothelial cytoplasmic calcium $[Ca^{2+}]_i$ responses in isolated DVR leading to marked NO production and EETs leading to vasodilation of Ang II preconstricted vessels.[15,208,300]

Adenosine

As discussed earlier, adenosine exerts its actions on the renal vasculature predominantly through A_1 and A_2 receptor subtypes. Adenosine A_1 receptor activation transiently reduces cortical and medullary blood flow, whereas A_2 receptor stimulation leads to medullary vasodilation and natriuresis. Both A_1 and A_2 receptors are expressed by DVR, and their respective stimulation induces constriction or dilation. Interstitial adenosine concentrations are near the affinity for the A_2 receptor so that changes should modulate vasodilatory and natriuretic effects. A_1-induced constriction is mediated by the pertussis-toxin–sensitive $G\alpha_i$ protein and phospholipase C activation. A_2-mediated dilation is mediated by the stimulation of the $G\alpha_s$ protein and the activation of K_{ATP} channels via enhanced levels of 11,12-EET. During hypoxia, the medullary thick ascending limb of Henle synthesizes adenosine, which serves a paracrine vasodilator function to preserve medullary perfusion and to augment medullary pO_2.[15,61,212,217,301,302]

Endothelin

Medullary ET receptors are present on medullary vascular bundles, medullary interstitial cells, and adjoining collecting duct cells. ET1 concentrations in the renal medulla are regulated by osmolality. ET1 binds to and stimulates both ET_A and ET_B receptors, thus inducing vasoconstriction. Isolated ET_B receptor stimulation, however, mediates vasodilation. Preglomerular smooth muscle cells show $[Ca^{2+}]_i$ responses to ET_A and ET_B agonists. The DVR from the outer medulla constrict in response to ET1, but medullary perfusion is not greatly affected due to the enhanced production of NO from ET_B-receptor stimulation, counteracting the vasoconstriction. ET_B-receptor stimulation of NO production plays a significant role in protecting the medullary circulation from excessive vasoconstriction.[15,150,156,162,303,304]

ET1 treatment selectively reduces cortical blood flow while transiently increasing medullary blood flow. Medullary vasodilation and natriuresis are prevented by blocking ET_B receptors or NO synthesis. Renal hypoxia stimulates ET1 production. The effects of endothelins on medullary blood flow vary with dietary salt intake. Chronic Ang II infusion, combined with salt loading, increases cortical and medullary immunoreactive ET. The administration of ET1 into ET_B-receptor deficient rats or wild-type rats in which the ET_B receptor is blocked fails to increase sodium excretion. Mice with the collecting duct-specific knockout of the ET1 gene have impaired sodium excretion in response to sodium loading and have salt-dependent hypertension.[156,304]

Reactive Oxygen Species

The production of ROS in the kidney is greatest in the outer medulla. eNOS and NADPH oxidase are both expressed in tubular epithelial cells within the renal medulla, particularly the thick ascending limb of the Henle loop, with the production of NO and $\bullet O_2^-$ participating in the regulation of medullary blood flow by acting on pericytes encircling the DVR. $\bullet O_2^-$ and H_2O_2 cause vasoconstriction, effects that are partially offset by the vasodilator NO. The inhibition of superoxide dismutase in the renal medulla increases local $\bullet O_2^-$ levels and reduces medullary perfusion. This is also the case for increased H_2O_2. Chronically increased oxidative stress such as in the activation of the renin–angiotensin system and diabetes, reduces medullary blood flow and sodium excretion, resetting pressure–natriuresis to produce hypertension.[270,275,277,305]

Collectively, the various vasoactive agents selectively regulate medullary blood flow through their actions on the pericytes that surround the vasa recta. Interestingly, the pericytes respond relatively weakly to vasoconstrictors such as Ang II, ET1, norepinephrine, and vasopressin, but

more robustly to the vasodilators including NO, CO, PGE_2, and adenosine. Furthermore, endogenous Ang II enhances, whereas NO reduces, the impact of increased renal sympathetic activity on medullary blood flow. The continuous interactions among these components regulate medullary blood flow and local oxygen supply.[15,18,208,306]

ADAPTATION OF RENAL HEMODYNAMICS TO ALTERED PHYSIOLOGIC CONDITIONS

Changes in Salt Intake

In healthy young adults, chronic changes in salt in the diet cause proportional changes in extracellular fluid volume and sodium excretion while having a subtle or minor influence on renal hemodynamics and arterial pressure. RBF and GFR are usually maintained within normal limits as a result of basically parallel adjustments in vasoconstrictor and dilator systems; this most often is also the case for arterial pressure. Some people, especially the elderly with compromised renal functions, develop salt-sensitive hypertension when consuming a high sodium diet.

As discussed earlier, the sodium diet and the extracellular fluid volume are major regulators of renin synthesis and release from juxtaglomerular granular cells. Renin secretion and Ang II production are stimulated by volume contraction due to macula densa signaling and increased sympathetic activity. A reduced delivery of salt to the macula densa stimulates COX-2 activity and PGE_2 production and, thereby, renin release. Macula densa COX-2 and microsomal PGE synthase mRNA are induced to increase PGE_2 production during a low sodium diet, whereas the EP_4 receptor expression is increased in glomeruli and in renin-secreting juxtaglomerular granular cells. Other important stimuli to renin-containing cells are sympathetic nerve activity and baroreceptor input. High salt intake inhibits renin release and Ang II production by these three major mechanisms of regulating renin. During sodium restriction, the elevated circulating and intrarenal Ang II levels constrict both afferent and efferent arterioles to either maintain or increase glomerular capillary pressure. K_f tends to be reduced. GFR is maintained in the normal range or is slightly reduced, whereas RBF declines to a greater extent, thus increasing the filtration fraction. Single nephron studies indicate that the TGF regulation of the afferent arteriolar tone is augmented during chronic salt restriction and high endogenous Ang II.[13,121,131,182,307]

The adaptive renal vasoconstrictor component is primarily the elevated Ang II acting on vascular AT_1 receptors because the Ang II receptor blockade increases RBF and GFR and reduces arterial pressure, with predominant effects in reducing afferent arteriolar resistance. Intrarenal ACE inhibition increases RBF and GFR in sodium-restricted dogs as a result of equal dilation of afferent and efferent arterioles in the presence of unchanged arterial pressure; K_f increases slightly. It is noteworthy that recent studies of direct renin inhibition with aliskiren in humans on a low salt diet reveal larger increases in RBF and GFR than in inhibition of the renin–angiotensin system using an ACE inhibitor or AT_1 receptor antagonist.[308]

A high salt diet increases the urinary excretion of salt to maintain a steady-state balance. There is a moderate increase in extracellular fluid volume, whereas the arterial pressure and the RBF and GFR usually remain within the normal range in individuals who are not salt sensitive. The stability of renal hemodynamics is achieved by a balance between vasoconstrictor and dilator systems. The vasoconstrictor effects of the renin–angiotensin system are reduced due to multiple mechanisms, including the suppression of renin release and Ang II production. Macula densa nNOS and COX-2 expression and activity are reduced during sodium loading. Reduced PGE_2 production by macula densa cells contributes to a reduced renin release along with reduced β-adrenergic stimulation by sympathetic nerves and increased baroreceptor inhibition in juxtaglomerular granular cells. Accordingly, inhibition of the renin–angiotensin system has little influence on renal hemodynamics and arterial pressure in animals fed a high salt diet. This also is the case for the AT_1-receptor antagonism and for ACE inhibition. Renal efferent sympathetic nerve activity is also suppressed, as is the case for opposing actions of the major vasodilator systems of NO and prostaglandins, which are reduced in parallel. TGF activity is reduced, whereas distal tubular flow is increased during volume expansion. The underlying mechanisms are not known, but low ambient levels of Ang II may contribute to reduced reactivity.[2]

Renal vascular reactivity to exogenous Ang II is enhanced during sodium loading and attenuated during a low salt diet, being related to AT_1-receptor density that is reciprocally associated with endogenous Ang II levels. Thus, Ang II–induced vasoconstriction is less pronounced when Ang II is chronically elevated because of the downregulation of vascular AT_1 receptors. This is not the case for norepinephrine as glomerular hemodynamic responses to renal nerve stimulation are similar whether animals are maintained on a low, normal, or high salt diet.[146] Intrarenal blood flow varies during changes in the sodium diet, with cortical and outer medullary but not inner medullary blood flow paralleling sodium intake. A high salt diet and reductions in the plasma Ang II concentration lead to increased cortical blood flow but not outer or inner medullary perfusion. Medullary blood flow is relatively well maintained during sodium restriction.[111,309]

The renal hemodynamic influence of vasodilator systems such as eNOS/NO, COX/PGE_2-PGI_2, and epoxygenase 11,12-EET are upregulated by a low salt diet, effectively counteracting the exaggerated vasoconstrictor influence of high Ang II and sympathetic nerve activity. In this setting, COX inhibition reduces RBF, renal cortical blood flow, and GFR, along with PGE_2 production as compared to no effect on renal hemodynamics in sodium-replete animals (Fig. 3.25). Whether the prostanoids are produced

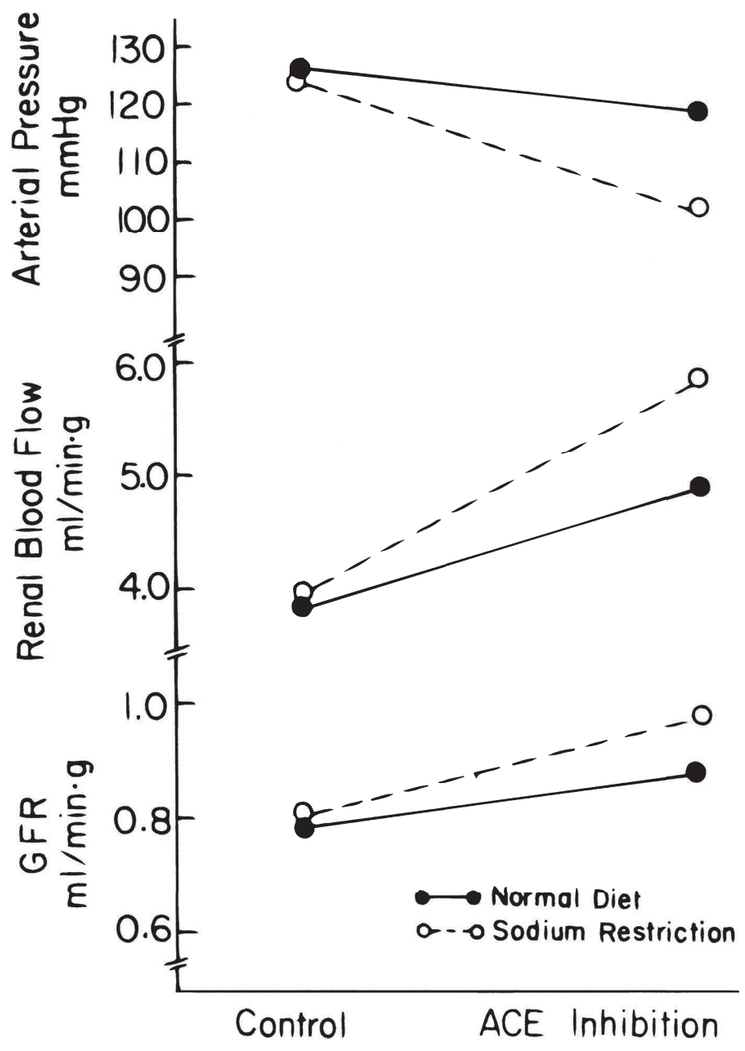

FIGURE 3.25 A comparison of renal hemodynamic and arterial pressure responses to angiotensin converting enzyme (ACE) inhibition in sodium-replete (*solid lines*) and sodium-depleted (*dashed lines*) anesthetized dogs. *GFR*, glomerular filtration rate.

by COX-1 or COX-2 is uncertain. One study finds that the renal hemodynamic changes are dependent on COX-2 activity, whereas another study reports that selective COX-2 inhibition is without effect on whole kidney hemodynamics during sodium depletion. COX-2 inhibition decreases medullary blood flow equally in animals on normal and low salt diets.[71,116,310,311]

COX activity and renal cortical production of vasodilatory prostanoids are low during volume expansion. COX inhibition in high salt animals has essentially no effect on whole kidney RBF or GFR. On the other hand, a high salt diet increases COX-2 expression in the renal medulla. Selective medullary COX-2 inhibition reduces medullary blood flow, which is associated with sodium retention and hypertension in rats consuming a high salt diet.[312–315]

Macula densa nNOS activity and NO productivity are inversely related to the sodium diet. Both endothelial eNOS and macula densa nNOS are upregulated during a low salt diet. NO generation in the kidney from L-arginine participates in adapting renal function to changes in salt intake. Dietary salt loading in animals enhances eNOS expression and activity and NO generation in the renal vasculature. Urinary nitrate + nitrite excretion, an index of NO production, increases during high sodium intake. NOS inhibition produces greater increases in arterial pressure (AP) and RVR and greater reductions in RBF in animals and humans on high versus low salt diets. NOS inhibition reduces medullary more than cortical perfusion during low and normal sodium diets. During sodium loading, NO control is similar for cortical and medullary blood flow. Macula densa nNOS is downregulated during a high salt diet. In contrast, protein expression of eNOS, iNOS, and nNOS is increased in the inner medulla. Afferent arteriolar vasodilatory responses to acetylcholine and sodium nitroprusside were unaffected by chronic high salt diets.[316–318]

Sodium restriction upregulates the kallikrein–kinin as well as the renin–Ang II system. The AT_1-receptor antagonism and ACE inhibition in dogs maintained on a low salt diet increase RBF and cortical blood flow. ACE inhibition causes larger increases in medullary flow than AT_1 receptor antagonism, an effect normalized by the blockade of bradykinin B_2 receptors. The vasodilator kallikrein–kinin system also participates in the renal adaptation to salt loading. Mice lacking vasodilator B_2 receptors respond to a high salt diet by becoming hypertensive with reduced RBF and increased RVR. In contrast, wild-type mice are normotensive with tendencies toward increased RBF and reduced RVR.[206]

Renal vascular reactivity to exogenous ET-1 is reduced by a low salt diet. ET_A receptor blockade has little effect on RBF during volume contraction. Sodium restriction upregulates pre-pro-ET-mRNA in the renal cortex, whereas ET_A and ET_B receptor density is unchanged. Low sodium intake and elevated Ang II acting on AT_1 receptors increase renal medullary ET-1 and ET_A and ET_B receptor mRNA, but not the sensitivity of renal medullary perfusion or sodium excretion to the ET_A or ET_B receptor blockade.[319,320]

Endogenous ET-1 levels are increased in the renal cortex and the medulla of animals fed a high salt diet, and renal vascular reactivity to exogenous ET-1 at the whole kidney level is enhanced. ET-1 reduces renal cortical blood flow and increases medullary perfusion in rats on a high salt diet as compared to cortical vasoconstriction, and has no effect on medullary blood flow in animals on a normal salt diet. Juxtamedullary-afferent arteriolar vasoconstriction produced by ET-1 and the ET_B receptor agonist are reduced. Arteriolar ET_B receptors, but not ET_A receptors, are upregulated presumably on endothelial cells during salt loading, and their vasodilation appears to buffer ET-1–induced vasoconstriction mediated by ET_A receptors. The ET_A-receptor blockade has no effect on whole kidney renal hemodynamics in rats placed on a high salt diet.[158,321]

Sodium loading increases renal ET-1 content and NO production that is in part mediated by ET_B receptors that are responsible for increasing medullary blood flow. A genetic deficiency of ET_B receptors leads to salt-induced hypertension. The more specific deletion of ET-1 or ET_B receptors in the collecting duct also causes salt-sensitive hypertension. In contrast, the specific mutation of ET_B receptors in vascular endothelial cells does not affect the AP sensitivity to salt.[156,322]

Normal kidneys, however, exhibit highly efficient whole kidney steady-state autoregulation of RBF whether animals are fed either a low or high salt diet and thus is independent of endogenous Ang II. Sodium depleted dogs have a normal RBF and GFR, with efficient autoregulation of RBF,

GFR, and single nephron GFR to changes in renal perfusion pressure. RBF is also effectively autoregulated during a low salt diet whether or not ACE is inhibited or AT_1 receptors are antagonized.[2] A high salt diet has little impact on dynamic RBF autoregulation, with either no change or an increased responsiveness of the myogenic mechanism in Sprague-Dawley or Long-Evans rats, respectively. Normal myogenic responses of afferent arterioles to increased luminal pressure is observed in mice fed a high salt diet for 3 months. A slight attenuation of pressure-induced myogenic responses is noted during high salt versus low salt diets for isolated interlobular arteries and afferent arterioles of Dahl salt-resistant rats. In hypertensive states, efficient autoregulatory responses to an increased arterial pressure play an important role in protecting sensitive glomerular capillaries and other renal structures from severe barotrauma. In some individuals, high salt intake may increase RBF and impair autoregulatory mechanisms such that the increased arterial pressure is transmitted to glomeruli, with the eventual development of proteinuria and glomerular sclerosis, along with interstitial inflammation.[93,323–325]

Renal cortical cytochrome P450-2 epoxygenase protein levels in renal microvessels and urinary metabolite EET excretion are increased during the consumption of a high salt diet. Epoxygenase products such as 11,12-EET exert antihypertensive effects as a result of vasodilator and natriuretic actions. Epoxygenase inhibition leads to salt-sensitive hypertension. In contrast, cytochrome P450-4A ω-hydroxylase protein levels are reduced in the renal cortex and in the renal vasculature. Nevertheless, a high salt diet increases overall renal excretion of 20-HETE in an adaptive response that contributes to increased sodium excretion. The pharmacologic inhibition of 20-HETE formation reduces sodium excretion and leads to salt-sensitive hypertension in rats.[183,318,326]

Adenosine produces more pronounced A_1 receptor–mediated renal vasoconstriction in high renin/Ang II states such as during sodium restriction. In contrast, adenosine produces dilation mediated by A_2 receptors in animals on a high salt diet, with increases observed in both the renal cortex and the outer and inner medulla. The reduction in RVR is due in part to enhanced cytochrome P450 activity and EET production during high salt intake. The pharmacologic stimulation of A_1 receptors decreases RBF and perfusion to the cortex and both the outer and inner medulla. In contrast, an A_1 receptor agonist causes reductions in RBF and cortical and outer medullary blood flow, but not inner medullar perfusion in low salt animals.[218,327,328]

Conditions that shift the balance to favor increased vasoconstrictor actions of superoxide and other reactive oxygen species over the vasodilator buffering effects of NO promote salt-sensitive hypertension that is characterized by renal vasoconstriction and enhanced salt retention. A hallmark of endothelial dysfunction is reduced NO production that can result from a reduced expression of eNOS and nNOS and impaired eNOS activation and NO production in the kidney. Salt-sensitive hypertension can be induced by pharmacologic NOS inhibition or genetic deletion of eNOS; the pressor response is reversed by the superoxide dismutase mimetic tempol. Enhanced renal vasoconstriction is associated with stronger than normal actions of Ang II and amplification by oxidative stress and NADPH oxidase–derived ROS as well as isoprostane. A chronic high salt diet leads to oxidative stress and inflammation in the kidney and vascular tissues and, eventually, hypertension, which is independent of Ang II activation of AT_1 receptors. Salt loading increases renal cortical NADPH oxidase subunit expression (NOX2 and $p47^{phox}$) and activity, increases superoxide generation, and increases urinary H_2O_2, 8-isoprostane, malondialdehyde, and thromboxane B_2 excretion, and decreases plasma NO end products. This is accompanied by the reduced expression of antioxidant superoxide dismutase isoforms. Such changes are limited more to the renal cortex than the renal medulla. It is noteworthy that the functional changes associated with oxidative stress and high salt intake are counteracted by oral L-arginine supplementation and improved NO production.[262,329–335]

Sodium restriction increases plasma renin activity and AT_1-receptor dependent oxidative stress in the kidneys as urinary excretion of 8-isoprostane is increased. Chronic activation of the renin–angiotensin system induces oxidative stress in the kidney. The administration of Ang II acting on AT_1 receptors enhances renal cortical NOX1, $p22^{phox}$ expression, superoxide production, and urinary excretion of 8-isoPGF2α (isoprostane). mRNA is decreased for NOX4 and extracellular superoxide dismutase. Although weaker, AT_2 receptors tend to blunt the actions of AT_1 receptors. The administration of the superoxide dismutase tempol increases RBF and arterial pressure, whereas GFR and sodium excretion are unchanged, suggesting predominant actions of superoxide on the renal vasculature.[262,336,337]

Changes in Protein Intake

The Western-style diet is characterized by highly processed and refined foods with a high content of sugars, salt, and fat, and high protein from meat that is chronically associated with dyslipidemia, oxidative stress, and inflammation. It is a major contributor to metabolic disturbances and the development of obesity-related diseases, including type 2 diabetes, hypertension, and cardiovascular and renal disease.

It has long been recognized that variations in the protein diet and plasma amino acid concentrations can have significant effects on the renal circulation. The consumption of protein in excess of 1 g/kg/day is usually associated with renal vasodilation in animals and humans. In dogs, the consumption of a high protein meal leads to increases in RBF and GFR, which are maximal at 3 to 6 hours and then progressively return to normal by 24 hours. The effect of protein feeding on renal function in humans is less marked than that in dogs. A short-term intravenous infusion of casein produces renal vasodilation that is sustained for up to 8 hours even though the blood amino acid concentrations

rapidly return to preexisting levels after the infusion is stopped. Various combinations of amino acids usually produce renal vasodilation and increase GFR; the changes are rapid in onset and reversible. It has been noted that only amino acids that are metabolized dilate the renal vasculature, whereas nonmetabolized amino acids do not affect RBF.[338–341]

The postprandial response to a protein-rich meal or a response to metabolizable amino acid infusion involves renal hyperemia and hyperfiltration because both RBF and GFR increase in both humans and animals. The ability of the kidneys to vasodilate and increase RBF and GFR in response to an acute protein or amino acid load is used clinically as a diagnostic index of vascular adaptability of "renal functional reserve." The greater the response, the more adaptable and thus the healthier the reserve; a weak or absent response is associated with severe nephron loss and aging. The mechanisms responsible for the renal vasodilation are multiple, involving blood-borne vasoactive agents, including pancreatic glucagon and the intrarenal release of COX-derived vasodilator prostanoids, NO, as well as reduced renin–Ang II and reduced TGF activity. Plasma renin activity and Ang II levels are unchanged and ACE inhibition does not impact on the protein-induced renal vasodilation.[154]

In healthy humans and laboratory animals, a high protein diet or the infusion of amino acids exert proportional effects on RBF, GFR, and urinary urea nitrogen excretion. Renal vascular resistance is reciprocally related to protein intake, with larger changes in RBF than in GFR. Arterial pressure remains stable and is independent of protein intake. Some reports, however, find that vegetarians have a reduced GFR relative to omnivores. The high protein diet leads to renal vasodilation and increased RBF and GFR in the face of normal arterial pressure and normal levels of plasma renin activity. Increased RBF and GFR are mediated by COX-dependent prostanoids and are largely prevented by COX inhibition. Glomerular COX and PLA_2 activities are increased, and the glomerular (but not renal papillary) production of PGE_2, $PGF_2\alpha$, and TxA_2 are increased under basal conditions and in response to Ang II. The urinary excretion of PGE_2 and PGF_2 metabolites are increased while plasma renin activity is elevated. Renal vasodilation is little affected by Ang II, as protein-induced changes in renal hemodynamics are unaffected by ACE inhibition. Nevertheless, vascular reactivity to Ang II is reduced during high protein feeding, whereas that of norepinephrine is normal. One mechanism contributing to renal vasodilation and increased GFR on a high protein diet is suppressed TGF control of preglomerular vascular resistance. Nevertheless, glomerular hyperfiltration is observed in adenosine A_1 receptor–deficient mice lacking TGF.[2,111,342,343]

Athletes and exercisers often use high-protein diets to enhance strength and muscle hypertrophy and recovery from intense exercise or injury. High protein diets combined with carbohydrate or fat restriction are also advocated for weight loss. However, it should be appreciated that chronic consumption of large amounts of protein may exacerbate or cause kidney damage. A long-term high protein intake often leads to proteinuria and increased GFR with renal hypertrophy that is accompanied with larger glomeruli and more glomerulosclerosis and tubulointerstitial fibrosis. The glomerular hypertrophy is due in part to increased vascular endothelial growth factor production.[344,345]

During a low protein diet, arterial pressure is normal, whereas plasma renin, Ang II, and aldosterone levels are reduced. Nevertheless, renal renin content is increased and exerts tonic actions on RVR. RBF and GFR are markedly reduced by protein restriction. The reduction in RBF is due to equal increases in both afferent and efferent arteriolar resistance. GFR is reduced due to reductions in plasma flow and the ultrafiltration coefficient. ACE inhibition increases RBF and GFR while reducing RVR, which is consistent with a prominent vasoconstrictor role of intrarenal Ang II. Glomerular AT_1 receptor density in the renal cortex and the medulla is increased during low protein feeding. Dietary protein restriction lowers plasma renin activity as a result of reduced PGE_2 production.[346]

In general, high protein intake aggravates renal injury, whereas restriction of dietary protein (~1 g protein per kilogram of body weight per day) while avoiding malnutrition ameliorates the progressive development of glomerular disease in various models. Based on animal studies, a low protein diet tends to be renoprotective in that it delays damage and proteinuria associated with hypertension, diabetes, obesity, or reduced renal mass and chronic renal disease, thus reducing GFR, glomerular growth, and interstitial infiltrate. A restricted protein diet reduces uremia, the progression of proteinuria, and the decline in GFR in adult patients with moderate-to-severe chronic renal disease. Dietary protein restriction reduces the glomerular permselective defect responsible for proteinuria in human renal disease. Implicated mechanisms include a hemodynamic basis, with reduced blood flow and glomerular capillary pressure, and nonhemodynamic factors related to reduced glomerular growth and less oxidative stress and inflammation. A low protein diet may reduce RBF by increasing TxA_2 production. Local Ang II levels may also participate. A protein restrictive diet lowers plasma renin activity by attenuating renin release, mediated in part by reduced PGE_2 stimulation, and increases AT_1 receptor density in the renal cortex and the medulla.[347–351]

CONCLUSION

As is evident from the previous discussion, there are many exciting issues concerning the area of renal hemodynamics and the multiple control mechanisms that are under active investigation. Modern technologic developments have allowed a more detailed and a more direct evaluation of the characteristics of specific segments of the renal microvasculature and of the various membrane and cellular mechanisms mediating differential responses. The direct assessment of

responses of individual arterioles has allowed for the clarification of long-standing controversies. In addition, developments related to interactions between endothelial cells and vascular smooth muscle cells are now receiving greater attention from investigators studying the renal circulation, as has been the case for interactions between distal tubular macula densa cells and vascular cells of the afferent arteriole and glomerulus. This has led to exciting new concepts with far-reaching implications. Additionally, many of these integrative mechanisms are now being addressed in terms of dynamic as well as steady-state characteristics. This combination of new developments has provided the impetus for renewed interest in the area of renal hemodynamics and the interactions with other intrarenal systems. These new investigations should result in a much better appreciation of the exact mechanisms that regulate renal microvascular contractility and reactivity and how disruptions of these mechanisms can lead to or predispose the kidneys to dysfunction and increased injury.

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CHAPTER

4 Regulation of Water Balance: Urine Concentration and Dilution

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Water is by far the largest component of the body and accounts for approximately 50% to 65% of body weight. Maintenance of body fluid homeostasis, including fluid volume and solute concentration, is essential for cell function and whole-organism survival. The osmolality of body fluid, a concentration of all of the solute in water, is kept within a remarkably narrow range (280 to 295 mOsm per kilogram of water), in spite of large fluctuations of solute and water intakes and losses. Although this constancy is made by a variety of regulatory mechanisms in the body, the most critical regulatory capacities are provided by the kidney's urine concentration and dilution mechanisms.

Body water homeostasis is maintained by the balance between the input and output of water. Each side has regulated and unregulated components. The regulated component of water input is oral intake of fluids in response to a perceived sensation of thirst. The unregulated components of water input are oral intake of liquids and water in foods, and metabolic water of oxidation. Oral intake of water usually varies enormously in excess of homeostatic need because of social, cultural, or psychological influences. Solute-free water excretion by the kidney is the only route of regulated water output. The unregulated component of water excretion occurs via various kinds of insensible water losses including sweat, evaporative loss, gastrointestinal loss, and the obligate amount of water that is required to excrete the solutes in the urine. Sweat volume is determined by the requirements of temperature regulation. Evaporative loss is determined by body temperature and surface area, ventilation, and environmental temperature and humidity. Gastrointestinal water loss is affected by disturbance of its function. Both the input and output of water have very substantial unregulated components, and these can vary tremendously as a result of factors that are unrelated to the maintenance of body water homeostasis. Therefore, the regulated components, which are urine excretion and water intake caused by thirst, must compensate for whatever perturbations result from the unregulated water gains and losses. The daily urine excretion range is as low as 0.5 L to as high as 25 L depending on the requirements for water balance. When the kidney's capacity to conserve water is maximized to the limit due to dehydration, a sensation of thirst is activated, causing oral water intake to be increased.

Because the solute concentrations in water in the body must be kept nearly constant, water loss must be regulated by a mechanism that decouples water and the solutes. The kidney can excrete the appropriate amount of water without marked perturbations in solute excretion. When water intake is too large and dilutes blood plasma, urine diluted more than plasma is excreted to concentrate the plasma. When water intake is too small to concentrate plasma, urine concentrated more than plasma is excreted to dilute the plasma. In both cases, the solute excretion varies little. Renal solute-free water excretion is mainly regulated by the antidiuretic hormone vasopressin. Vasopressin is secreted from the posterior pituitary gland into systemic circulation in response to increases in the tonicity, which is an effective osmotic pressure in the plasma, to decreases in the effective circulating volume or pressure, or to several other stimuli. In response to changing levels of vasopressin in the plasma, the kidney is capable of wide variations in free water excretion. The molecular entity of a major effector of vasopressin in the kidney is aquaporin-2 (AQP2), which is a member of the aquaporin (AQP) water channel family. Molecular identification of the AQP family has revolutionized the understanding of water transport in the body, including urine concentration and dilution.[1,2] AQP2 is abundant in the collecting duct, which is the chief site of regulation of water reabsorption.[3] Acute stimulation of vasopressin promotes AQP2 translocation from an intracellular reservoir to the luminal cell surface, and its chronic stimulation increases the cellular abundance of AQP2, both of which elevate the water permeability of the collecting duct cells, resulting in the promotion of water reabsorption from the urinary tubule. Its impairments result in various water balance disorders including nephrogenic diabetes insipidus (NDI). In addition to AQP2, six other AQP isoforms are expressed in the kidney and play key roles in water transport activities specific for their localization in the nephron segments without

affecting solute transport. In addition to AQPs, the localizations of urea transporters and ion transporters are highly specific through the renal tubule segments. Together with a three-dimensional configuration of the nephron, the specific transport activities of water, urea, and ion all through the renal tubule segments enable the mechanism of urine concentration and dilution.

ANATOMIC CONSIDERATIONS FOR URINE CONCENTRATION AND DILUTION MECHANISMS

General Features of the Nephron Structure

The kidney consists of two populations of nephrons: short-looped nephrons and long-looped nephrons (Fig. 4.1). Both types of loops have a hairpin or a U-shape configuration, and are defined by the length of their Henle loop. Short-looped nephrons originate from superficial glomeruli and have a Henle loop, which turns near the inner-outer medullary border and consists of a proximal straight tubule, a thin descending limb, and a thick ascending limb. Long-looped nephrons originate from the juxtamedullary glomeruli, extend into the inner medulla, bend at various levels of the inner medulla, and contain a thin ascending limb in addition to segments found in short-looped nephrons. Thin ascending limbs are found only in the inner medulla and their transition to thick ascending limbs defines the inner-outer medullary border. Thus, only thick ascending limbs are found in the outer medulla.

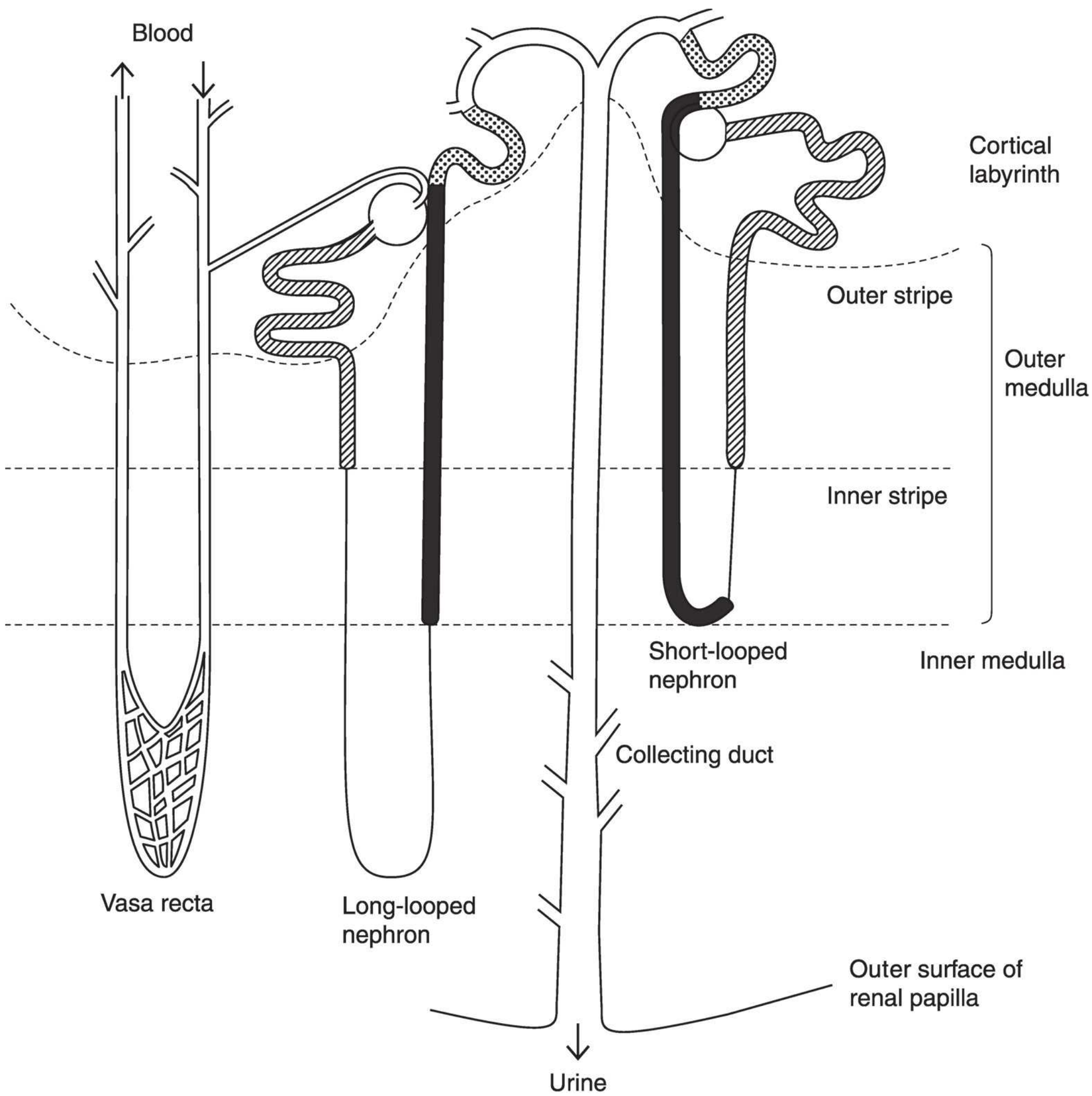


FIGURE 4.1 The kidney structure. The configurations of both a long-looped and a short-looped nephron are shown. The vasa recta is shown in the left. The major portions of the nephron are glomeruli (*circles*), proximal tubules (*hatched*), the thin limbs of the Henle loop (*single lines*), thick ascending limbs of the Henle loop (*solid*), distal convoluted tubules (*stippled*), and the collecting duct system (*open*). (Modified from Knepper MA, Stephenson JL. Urinary concentrating and diluting processes. In: Andreoli TE, Fanestil DD, Hoffman JF, Schultz SG, eds. *Physiology of Membrane Disorders*, 2nd ed. New York: Plenum, 1986:713, with permission.)

The Descending Part of the Henle Loop

The descending part of the Henle loop consists of the S2 proximal straight tubule in the medullary ray, the S3 proximal

straight tubule in the outer stripe of the outer medulla, and the thin descending limbs in the inner stripe of the outer and inner medulla. AQP1 is abundant in both the apical membrane and the basolateral plasma membrane in the S2 and S3 proximal tubules and the descending thin limbs.[4–6] AQP1 is constitutively active and is not regulated by vasopressin. AQP1 is present in sufficient abundance to account for the observed water permeability of these tubule segments.[7] The osmotic water permeability of the thin descending limb is extremely high due to the presence of the AQP1 water channel.[6] However, there is an axial heterogeneity in the expression levels of AQP1 in the thin descending limb; AQP1 is present in entirety through the thin descending limb of the short-looped nephron, whereas it is located in the upper half of the thin descending limb of long-looped nephron.[8–10] A comparison of water permeability and the abundance of AQP1 in these tubule segments indicates that AQP1 is the main route for water movement across the tubules.[8] The high water permeability of these segments is critical for the countercurrent multiplication system that is described in the section Countercurrent Multiplication in the Outer Medulla and Other Mechanisms in the Inner Medulla, which follows. As expected, a severely impaired urine concentrating ability is observed in AQP1 knockout mice.[11]

In addition to AQP1, AQP7 is expressed in the apical membrane of the S3 proximal tubule. AQP7 transports glycerol as well as water, thus AQP7 is an aquaglyceroporin.[12] Studies in AQP7 knockout mice suggest that water absorption mediated by AQP7 is small compared to that of by AQP1 and minimally contributes to urine concentration; whereas glycerol absorption by AQP7 may be important in glycerol metabolism in the body.[13]

A urea transporter UT-A2 is present in the thin limb of the long-looped nephron in the inner medulla and the thin descending limb of the short-looped nephron in the outer medulla. Thus, these segments have a high urea permeability[14] and play an important role for urea-recycling pathways that serve to maintain high urea concentrations within the inner medullary interstitium, which is critical for the urinary concentration that is described in the section Urea Accumulation in the Inner Medulla, which follows.

The Ascending Part of the Henle Loop

The ascending part of the Henle loop consists of the thin ascending limb in the inner medulla, the medullary thick ascending limb in the inner stripe of the outer medulla, and the cortical thick ascending limb in the medullary rays. The thin ascending limb is located exclusively in the inner medulla, is present only in the long-looped nephron, and becomes a thick ascending limb at the inner-outer medullary border. The thin ascending limb has extremely low water permeability and AQPs are not detected. By contrast, the ClC-K1 chloride channel is expressed in the apical and basolateral membrane of the entire length of the thin ascending limb.[9,15,16] Studies with immunohistochemical labeling and computer-assisted reconstruction in rats' inner medullas show that the thin ascending limb marked by ClC-K1 expression occurs about 160 μm before the loop bend.[9]

The thick ascending limb has essentially no water permeability and does not express AQPs. On the other hand, Na-K-2Cl cotransporter (NKCC2) is present in the apical membrane,[17] and Na-K-ATPase, a Na pump, is present in the basolateral membrane in the thick ascending limb. NaCl is actively absorbed by these transporters, resulting in the dilution of the luminal fluid in this segment, which works as the single effect in the countercurrent multiplication (described in the following sections). Urea permeability in the cortical thick ascending limb is higher than in the medullary thick ascending limb.[18] This may also contribute to the dilution of the luminal fluid as it flows up to the cortex by passive urea absorption.

The Distal Convoluted Tubule and Connecting Tubule

After exiting the Henle loop, the luminal fluid enters the distal convoluted tubule that expresses the thiazide-sensitive Na-Cl cotransporter NCC.[17,19] NCC is important for NaCl reabsorption that reduces fluid delivery to the collecting duct, leading to increases in urinary concentrating ability. Water permeability in the distal convoluted tubule is low and the expression of AQP2 or vasopressin V2 receptor is not observed.

Several distal tubules merge to form a connecting tubule arcade. The connecting tubule cells express both AQP2 and vasopressin V2 receptor, suggesting that, like the collecting ducts, the connecting tubules are sites of regulated water absorption. Moreover, vasopressin-regulated water absorption in the connecting tubule can result in a considerable amount of free-water absorption during antidiuresis because the large aggregate epithelial surface area of the connecting arcades is estimated to be roughly equivalent to that of the cortical collecting ducts.[20]

An AQP2 knockin mice model of nephrogenic diabetes insipidus, a rare disease manifested by an inability to respond to vasopressin, shows a severe urinary concentrating defect that results in neonatal death.[21] Meanwhile, AQP2 conditional selective knockout in the collecting duct but not in the connecting tubule rescues mice from the lethal phenotype that is observed in mice lacking AQP2 globally.[22] Although all types of mice show a severe urinary concentrating defect, these findings confirm an important role of the connecting duct for urinary concentration.

The Collecting Duct

The collecting duct is the final structure in the nephron. The luminal fluid from the connecting tubules then enters the collecting tubules in the superficial cortex. The collecting duct spans all the regions of the kidney. From the superficial cortex, the collecting duct descends through the cortex and the outer medulla. In the inner medulla, these collecting ducts

continually merge, finally forming the ducts of Bellini that open into the renal pelvis at the papillary tip. The renal pelvis is continuous with the ureter. The collecting ducts are arrayed parallel to the Henle loop. The collecting ducts have several morphologically discrete tubule segments: the cortical collecting duct, the outer stripe portion of the outer medullary collecting duct, the inner stripe portion of the outer medullary collecting duct, the initial part of the inner medullary collecting duct (IMCD), and the terminal part of the IMCD.

The collecting duct, under the control of vasopressin and other factors, is the nephron segment responsible for the final control of water excretion. The collecting duct expresses the vasopressin V2 receptor and AQP2, which are the primary targets for vasopressin regulation. In the absence of vasopressin, AQP2 resides in the vesicles below the apical cell surface and the entire collecting duct is very water impermeable. When the body is dehydrated, plasma osmolality is increased, which is sensed by the hypothalamic osmoreceptors and results in vasopressin secretion by the posterior pituitary. Vasopressin binds to V2 receptors in the basolateral membrane of the principal cells of the collecting duct, stimulates adenylate cyclase to produce cAMP, activates protein kinase A, phosphorylates AQP2, and inserts this water channel into the apical cell surface, which results in a significant increase in the collecting duct water permeability and water reabsorption.[23–25] This process is described in detail in the section Aquaporin-2, which follows. Not only are AQP2 water channels present, but also are AQP3 and AQP4 water channels in the collecting duct principal cells. There is axial heterogeneity in the abundance of AQP3 and AQP4; AQP3 is abundant in the connecting tubule, the cortical collecting duct, and the outer medullary collecting duct; whereas AQP4 is most abundant in the inner medullary collecting duct.[26] AQP3 and AQP4 are located in the basolateral membrane of these cells and represent a potential exit pathway from the cell to the interstitium for water entering via AQP2.

Water reabsorption mainly occurs in the cortex and the outer medulla. The inner medulla has the highest osmolality and is important for the reabsorption of the remaining water when maximal water reabsorption is required. Rather, water reabsorption by the terminal part of IMCD is greater during diuresis than during antidiuresis[27] because of its large transepithelial osmolality difference and relatively high basal water permeability compared to other portions of the collecting duct.

The connecting tubule and the cortical collecting duct express the amiloride-sensitive sodium channel ENaC.[28,29] Sodium is actively absorbed via ENaC in the apical membrane of the principal cells and exits the cells via Na-K-ATPase in the basolateral membrane. Sodium reabsorption via ENaC is increased by vasopressin or aldosterone and plays an important role for reducing fluid delivery to the medullary collecting duct, thus leading to increases in urinary concentrating ability.[30] On the other hand, the sodium permeability is low in the medullary collecting duct.

The urea permeability is extremely high only in the terminal IMCD and low in other portions of the collecting duct.[31] UT-A1 and UT-A3 urea transporters are present in the terminal IMCD cells.[32] Urea reabsorption in this segment is important for the preservation of high urea concentrations in the inner medullary interstitium, which are essential for its urine concentrating ability and are also important for minimizing the urinary loss of urea that is a major part of the urea recycling pathway. This process is described in detail in the section Urea Accumulation in the Inner Medulla, which follows.

The Vasculature

The blood vessels named the vasa recta carry blood into and out of the renal medulla. Like the Henle loop, there are the descending and ascending parts in the vasa recta, both of which are arranged in parallel and in mutual proximity. Blood from efferent arterioles of juxtamedullary nephrons enters into the descending vasa recta in the medulla, passes through the capillary plexus located at various depths within the medulla, then merges to form the ascending vasa recta. Different from the Henle loop, the descending and ascending vasa recta are separated by the capillary plexus (see Fig. 4.1).

The AQP1 water channel and the UT-B urea transporter are present in the descending vasa recta and enhance the countercurrent exchange of water and urea between the descending and ascending vasa recta in the medulla.[6,33] This countercurrent exchange reduces the effective blood flow, contributing to the conservation of osmolality gradients in the medullary interstitium.

The Medullary Interstitium

The interstitium of the outer medulla and the outer portion of the inner medulla is a narrow space that is important for limiting solute diffusion upward along the medullary axis.[34] To the contrary, the interstitium of the inner half of the inner medulla is much larger. In this region, the interstitial cells are interspersed in a gelatinous matrix of highly polymerized hyaluronic acid,[35] which is largely devoid of capillary plexuses or lymphatics. This structure slows the diffusion of solute and water in the inner medulla.

The Pelvis

The collecting duct finally opens at the papillary surface and urine enters the pelvic space. In the kidney with only one papilla, such as in hamsters and rats, the renal pelvis is an intrarenal urinary space surrounding the papilla. In the kidney with many papillae, such as in humans, each papilla is surrounded by a funnel-shaped calyx. In the human kidney, it is the compartment between the calyces and the ureter that is called the pelvis.[36] This compartment is not present in kidneys with one papilla, where the pelvis is a direct extension of the ureter. The renal pelvic (calyceal) wall contains smooth muscle layers. Contractions of these muscles induce regular peristaltic contractions of the wall that strongly

compress the medullary tissue, resulting in intermittent flow in the collecting ducts and the Henle loop.[37]

URINE CONCENTRATION MECHANISMS

General Features of Urine Concentration

In an adult human, glomerular filtrate is about 180 L per day, most of which is reabsorbed by the high water permeable proximal tubule and descending limb of the Henle loop. However, regulation of water excretion mainly occurs after the luminal fluid reaches the distal tubule. The osmolalities of luminal fluid along the rat nephron are shown in Figure 4.2. The luminal fluid in the proximal tubule is isosmotic to plasma, regardless of antidiuresis or diuresis. This fluid then enters the thin descending limb of Henle and is concentrated as it flows down to the bend of the Henle loop because of water reabsorption. As the fluid flows up the ascending limb of the Henle loop, the luminal fluid becomes diluted because this segment is impermeable to water, and the thick ascending limb actively absorbs NaCl. This diluted fluid finally enters the collecting duct system. In diuresis (low AVP), the fluid remains hypotonic. On the other hand, in antidiuresis (high AVP), the fluid is concentrated to a level far greater than plasma by water reabsorption as it flows down the collecting duct. This is due to a vasopressin-induced significant increase in water permeability of this segment and the existence of an axial osmolality gradient in the medulla. This osmolality gradient with the highest degree of hyperosmolality at the papillary tip is maintained by several mechanisms, including countercurrent multiplication, countercurrent exchange, and urea accumulation in the inner medulla, as described subsequently.

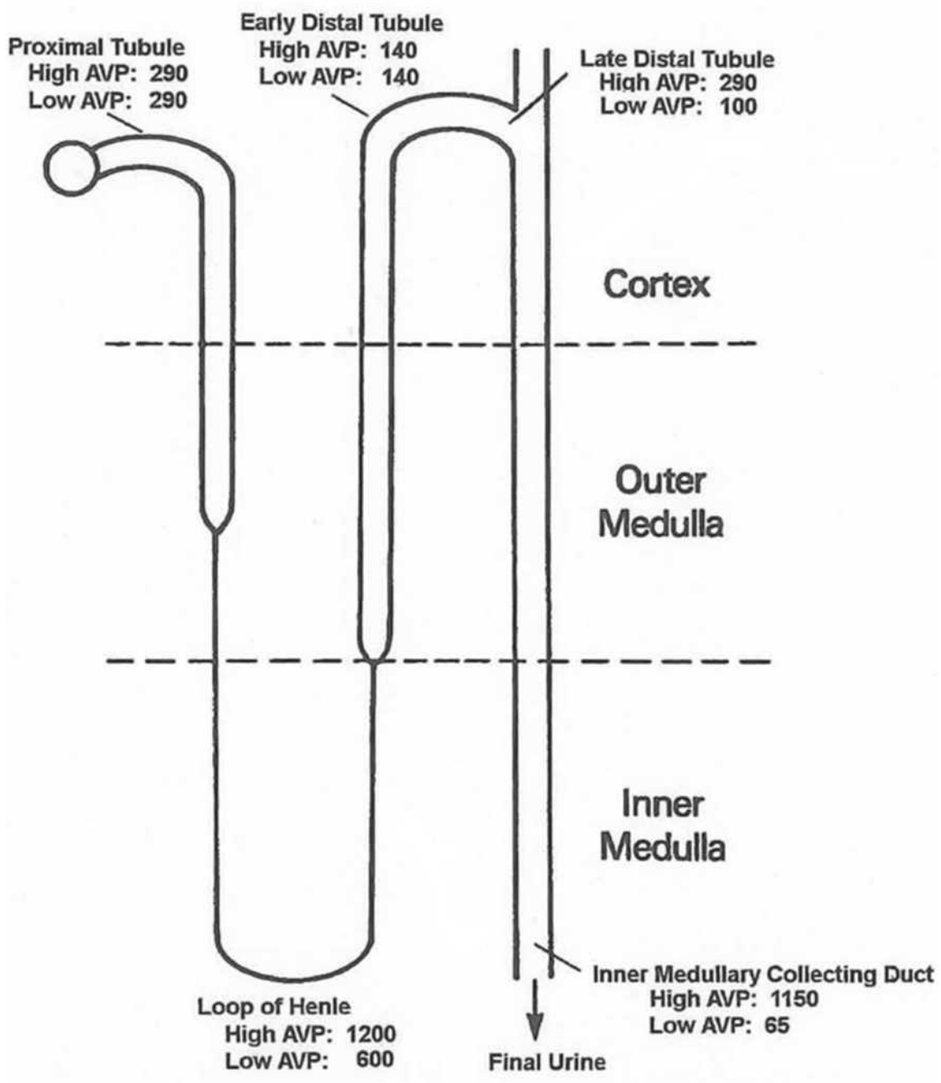


FIGURE 4.2 The typical tubular fluid osmolalities (in milliosmoles per kilogram of water) found along the nephron segments of the rat kidney. Fluid osmolality in the proximal tubule is isotonic, and it increases to 600 to about 1,200 mOsm per kilogram of water at the bend of the loop. Fluid emerging from the thick ascending limb is hypotonic, and final urine osmolalities are determined by urine concentration in the collecting duct depending on circulating vasopressin levels (65 to about 1,150 mOsm per kilogram of water). *AVP*, arginine vasopressin. (Based on micropuncture studies by Wirz H. Der osmotische Druck in den corticalin Tubuli der Ratten niere. *Helv Physiol Pharmacol Acta*. 1956;14:353; Gottschalk CW, Mylle M. Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis. *Am J Physiol*. 1959;196:927; Jamison RL, Buerkert J, Lacy F. A micropuncture study of collecting tubule function in rats with hereditary diabetes insipidus. *J Clin Invest*. 1971;50:2444, with permission.)

Countercurrent Multiplication in the Outer Medulla and Other Mechanisms in the Inner Medulla

Countercurrent multiplication is an essential process for generating a medullary osmotic gradient along the corticomedullary axis and occurs in the Henle loop.[38] The counterflow arrangement of this loop makes the osmolality difference between the ascending and descending limbs to be multiplied, resulting in an enormous increase in osmolality toward the bend of this loop. Figure 4.3 illustrates the basic components of this mechanism in the short Henle loop. In the thick ascending limb of Henle, which corresponds to the right limb in Figure 4.3, NaCl is actively reabsorbed at any level of this ascending limb. Because this segment is impermeable to water, the luminal fluid is diluted and NaCl concentrations of the surrounding interstitium become higher than the luminal fluid. On the other hand, the descending limb of the Henle loop, which corresponds to the left channel in Figure 4.3, is highly permeable to water due to the presence of an AQP1 water channel, but is impermeable to NaCl. Water is passively reabsorbed from the descending limb lumen to the interstitium, which has a high osmolality due to NaCl accumulation. The luminal fluid in the descending limb is concentrated by water reabsorption and is continually concentrated downward to the bend of the loop, and then enters to the ascending limb. High NaCl concentrations of this fluid further promote NaCl reabsorption in the ascending limb. This fluid is then diluted by this NaCl absorption while flowing toward the top of the loop. Because the transverse concentration difference between the two limbs is always maintained by active NaCl absorption by the ascending limb (this is called the single effect) and water permeability of the descending limb at each level of the longitudinal axis is high, the bend of the loop achieves a progressively higher osmolality. This results in an enormous increase in osmolality toward the bend of the Henle loop.

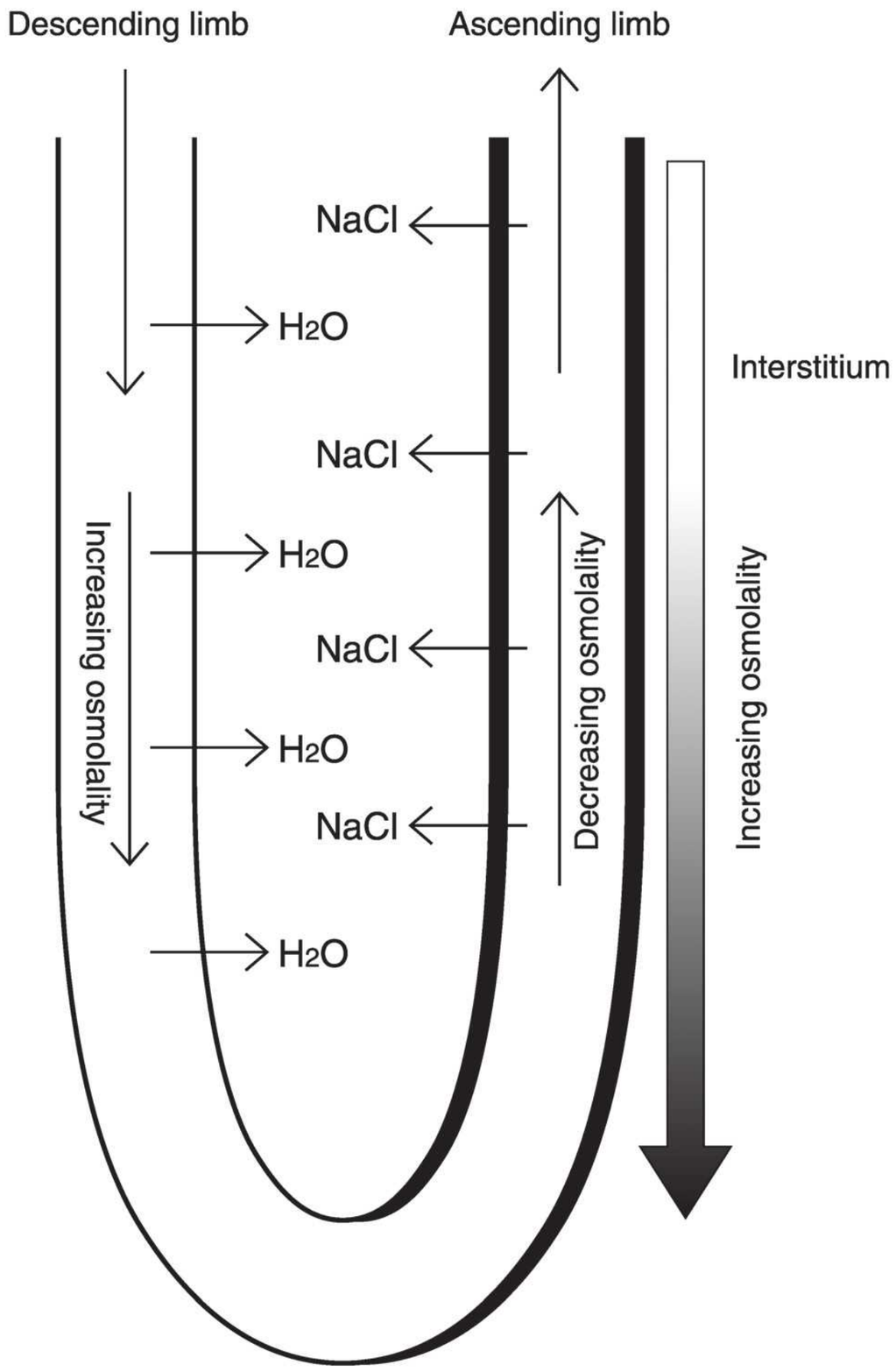


FIGURE 4.3 A countercurrent multiplication in the short Henle loop. In the thick ascending Henle limb, NaCl is actively reabsorbed. Because this segment is impermeable to water (indicated by *bold line*), the luminal fluid is diluted and the interstitium around this segment has high NaCl concentrations. On the other hand, the descending limb of the Henle loop is highly permeable to water, and water reabsorption occurs from the descending limb lumen to the interstitium with high NaCl concentrations. This results in an enormous increase in osmolality toward the bend of the Henle loop.

A countercurrent mechanism is accepted for generating the axial osmolality gradient in the outer medulla, where active NaCl reabsorption occurs in the thick ascending limb. However, in the inner medulla, there is little activity of NaCl transport despite the fact that osmolality continues to increase toward the tip of the papilla. The thin ascending limb of the Henle loop in the inner medulla cannot actively reabsorb NaCl. The high inner medullary interstitial osmolality provides a critical driving force for osmotic water flow across the collecting ducts, where the water permeability is regulated by vasopressin and AQP2 water channel, as described later. For explaining this concentrating effect in the inner medulla, a passive mechanism is widely accepted.[39,40] In this model, urea efflux from the terminal IMCD, which is mentioned previously, results in high urea concentrations in the inner medullary interstitium. This causes osmotic withdrawal of water from the thin descending limb and increases NaCl concentrations of the luminal fluid. This highly concentrated NaCl then exits passively from the thin ascending limb (works as single effect), and the luminal fluid is progressively diluted as it flows up. This passive transport process may produce an axial NaCl concentration gradient in the inner medulla. This model requires that the thin descending limb is highly permeable to water and but not to NaCl or urea, whereas the thin ascending limb is permeable to NaCl but not to water or urea. A microperfusion study of the thin ascending limb shows that the permeability of chloride and sodium are higher than urea, and that luminal dilution takes place when tubule segments are perfused in a condition simulating an in vivo situation.[41] Consistent with these physiologic studies, the ClC-K1 chloride channel is exclusively expressed in the thin ascending limb. ClC-K1 knockout mice show a large urinary concentrating defect with impaired urea as well as NaCl accumulation in the inner medulla.[42,43] This finding confirms the importance of a rapid chloride exit in the thin ascending limb and supports the passive mechanism.

Simulations based on many mathematical models incorporating physiologic parameters have been examined to show the validity of the passive model, but they usually failed to show satisfactory results. However, this does not necessary neglect the passive model because solute permeability and water permeability are sometimes different in species, in nephrons (long looped versus short looped), and within the segment (axial heterogeneity). Moreover, based on extensive studies of the rat inner medulla by immunohistochemical labeling and computer-assisted reconstruction, Pannabecker et al.[10] show that three-dimensional tubular and vascular relationships are more complex than thought before and a more comprehensive understanding of three-dimensional functional architecture is critically important for modeling urine concentration mechanisms of the inner medulla.

Countercurrent Exchange

Although the hypertonic medulla is essential for the ability to concentrate urine, blood flow may decrease the high solute concentrations in the medulla. The osmolality of blood entering to the medulla from the general circulation is far lower than the medullary interstitium and may decrease the osmolality of the interstitium as they come to equilibrium. Meanwhile, blood going out the medulla to the general circulation can carry out the solutes from the interstitium. To minimize this dissipation of the high solute concentrations in the medulla, there is a process called countercurrent exchange. Blood supply to the medulla is done by the vasa recta with the descending and ascending limbs arranged in a counterflow configuration. The vasa recta are permeable to water, sodium, and urea. Therefore, in the descending vasa recta, blood loses water and gains solutes as it flows down because

of the increasing osmolality in the medullary interstitium. After entering the ascending vasa recta, blood gains water and loses solutes as it flows up because of the decreasing osmolality of the surrounding interstitium. This exchange of water and solute between the descending and ascending vasa recta is called countercurrent exchange. This mechanism minimizes the solute washout from the inner medulla to the systemic circulation. Thus, this exchange at each level in the medulla preserves the axial solute concentration gradients in the medullary interstitium. If the blood flow is decreased, for instance in the conditions of volume depletion in the body, the efficiency of countercurrent exchange is further increased by getting enough time for achieving osmotic equilibration, leading to an increase in urinary concentrating ability.

The AQP1 water channel and the UT-B urea transporter are located in the descending vasa recta, which helps the rapid countercurrent exchange of water and urea.[6,33] Indeed, an impaired urine concentration ability is observed in AQP1 and UT-B knockout mice.[11,44]

Urea Accumulation in the Inner Medulla

The major solute responsible for the inner medullary osmolality gradient is urea and NaCl; although, in the outer medulla, it is NaCl.[45] High osmolality in the inner medulla is maintained by urea accumulation in this region in addition to NaCl. There are several mechanisms to maintain the high urea accumulation in the medulla that are called urea recycling (Fig. 4.4).[46,47]

Among the collecting duct, only the terminal IMCD has a high urea permeability due to the presence of UT-A1 and UT-A3 urea transporters in this segment.[31,32] During antidiuresis, water is absorbed in the collecting duct segments in the cortex and the outer medulla, which are impermeable to urea. Thus, the urea concentrations of the luminal fluid are progressively increased as it flows down through the connecting tubule, the cortical collecting duct, and the outer medullary collecting duct. When the luminal fluid reaches the terminal IMCD, which is highly permeable to urea, urea is rapidly absorbed from the lumen to the surrounding interstitium. During antidiuresis, the urea permeability of the inner collecting duct is increased by vasopressin, and this accounts for further rapid reabsorption of urea.[48,49]

Once reabsorbed, urea is not rapidly washed out to the general circulation because of countercurrent exchange and the low effective blood flow of the vasa recta, resulting in the high urea accumulation in the inner medullary interstitium. In addition, during antidiuresis, urea reabsorption by the terminal IMCD has another advantage for decreasing the luminal osmolality and preventing the osmotic diuresis when luminal fluid is very concentrated by enhanced water reabsorption in the upper portions of the collecting duct.

After being reabsorbed by the terminal IMCD, some urea in the inner medullary interstitium enters into the thin limbs of the long Henle loop where urea permeability is high due to the presence of UT-A2.[50] Urea entered in the thin limbs is then carried upward to the cortex through the thick ascending limb, the distal convoluted tubule, the connecting tubule, and the cortical collecting duct, and again enters into the medulla through the outer and inner medullary collecting ducts. Because these segments before the terminal IMCD have low urea permeability, urea can be returned to this region with little loss. Urea is again reabsorbed by the terminal IMCD and recycled to the inner medullary interstitium (Fig. 4.4).

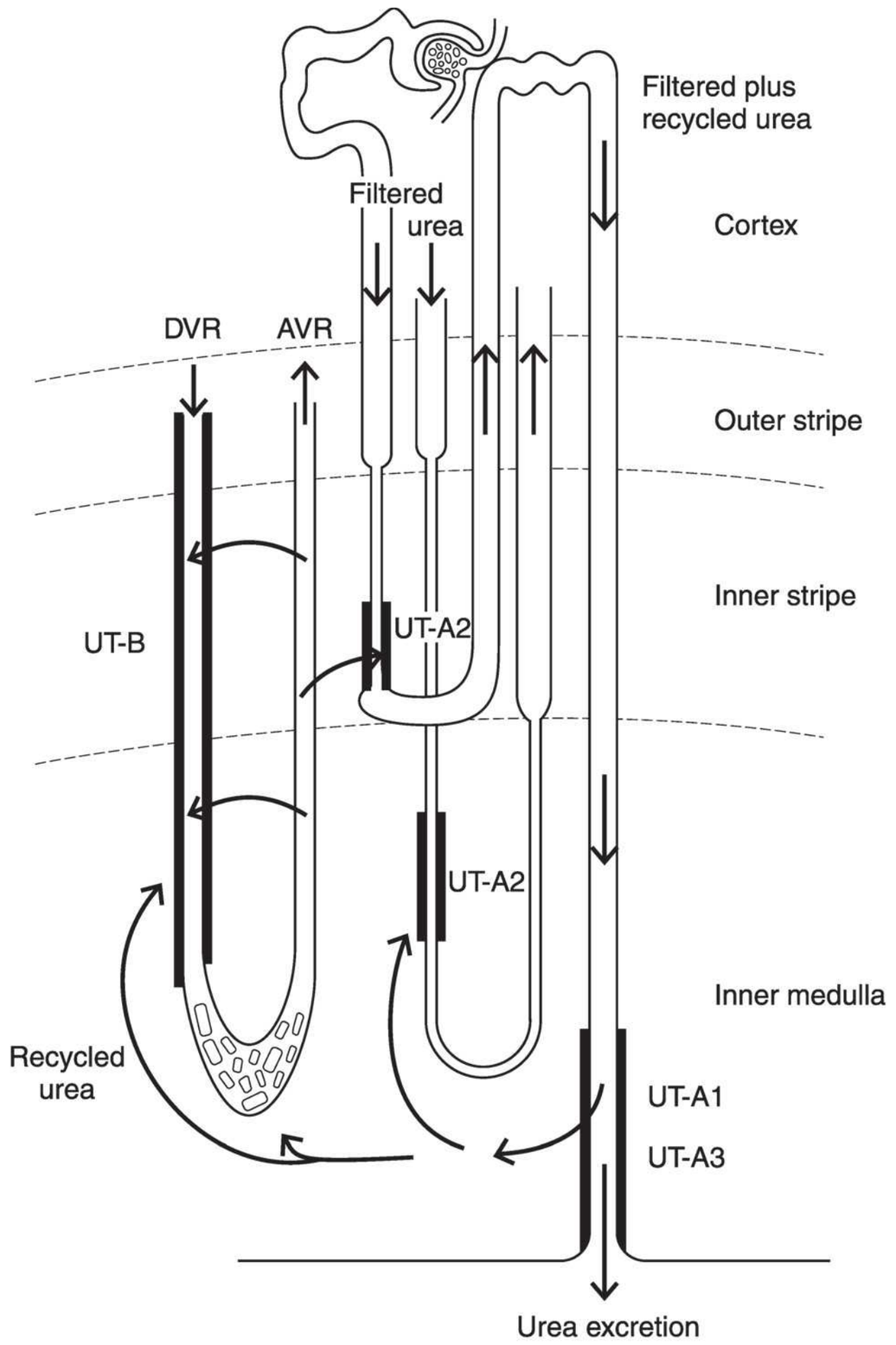


FIGURE 4.4 The urea recycling pathways within the kidney. The terminal portion of the inner medullary collecting duct (IMCD) efficiently reabsorbs urea through urea transporters UT-A1 and UT-A3, and accumulates urea in the inner medullary interstitium. Some of the urea is recycled to the IMCD by being reintroduced to the thin descending limb where UT-A2 is located. A high urea concentration in the medulla is maintained by countercurrent exchange (recycling) of urea between the descending vasa recta (*DVR*) and the ascending vasa recta (*AVR*), which is helped by UT-B in DVR. Some of the urea in the AVR is reintroduced to the thin descending limb and fed again to the IMCD. (Redrawn from Yang B and Bankir L. Urea and concentrating ability: new insights from studies in mice. *Am J Physiol Renal Physiol.* 2005;288:F881, with permission.)

In addition to the recycling pathway via the long loop in the inner medulla, there is another recycling pathway using the short loop in the outer medulla. Some parts of the urea reabsorbed by the terminal IMCD exit the inner medulla via the vasa recta. The vasa recta and the short Henle loop are arranged in parallel and in mutual proximity, and the descending limb of the short loop expresses the UT-A2 urea transporter and has a high urea permeability. Therefore, urea carried by the vasa recta is able to enter the short loop, then is convected through the distal convoluted tubule, the connecting tubule, and the collecting duct. When the luminal fluid finally reaches the terminal IMCD, urea is again reabsorbed to the surrounding inner medullary interstitium (Fig. 4.4).

The reabsorption sites among the collecting duct system are different between water and urea. Water is reabsorbed mainly in the cortex and the outer medulla, where blood flow is so high that water can be rapidly supplied to the systemic circulation. Furthermore, this reabsorption does not dilute the inner medulla. On the other hand, urea is reabsorbed in the inner medulla. Owing to low blood flow in this region and urea recycling pathways, urea can be trapped in the inner medulla, which is required for the passive mechanism, as described previously.

Regulated Water Reabsorption in the Collecting Duct

A countercurrent multiplication in the Henle loop generates a hypertonic medulla. A countercurrent exchange in the vasa recta minimizes the dissipation of this osmotic gradient. However, either of these processes has the ability to regulate water reabsorption in response to the body water balance. This function is performed by the collecting duct. The collecting duct is responsible for the final control of urine concentration, and its water permeability is regulated by vasopressin and other factors. In the absence of vasopressin, the collecting duct has an extremely low water permeability. Because the luminal fluid exiting the Henle loop is diluted, the fluid remains diluted after passing through the collecting duct, yielding a large volume of hypotonic urine. In the presence of vasopressin, the water permeability of the collecting duct is dramatically increased. Based on the hypertonic medullary interstitium generated by countercurrent multiplication and other processes, as described previously, an increase in water permeability of the collecting duct results in significant water reabsorption by the osmotic gradient between the lumen and the surrounding interstitium (Fig. 4.2). The molecular entity of this regulation of water permeability of the collecting duct is vasopressin V2 receptors and the AQP2 water channel expressed in the principal cells of the collecting duct.[26,51–55] V2 receptors and AQP2 are present in the connecting tubule and all segments of the collecting duct. When vasopressin binds to V2 receptors in the basolateral membrane of these cells, it stimulates adenylate cyclase to produce cAMP, activates protein kinase A, phosphorylates AQP2, and inserts this water channel into the apical cell surface, which results in a significant increase in the collecting duct water permeability and water reabsorption. In addition, vasopressin stimulation increases AQP2 protein abundance in the cell (Fig. 4.5). These processes are described in detail in the section Aquaporin-2, which follows. On the basolateral cell surface, AQP3 and AQP4 are located and represent a potential exit pathway from the cell to the interstitium for water entering via AQP2.

Water reabsorption mainly occurs in the connecting tubule, the cortical collecting duct, and the outer medullary collecting duct. In the cortex and the outer medulla, blood flow is sufficiently high so that absorbed water can be carried out to the general circulation without diluting the interstitium. On the other hand, the inner medulla has the highest osmolality and is important for reabsorption of the remaining water when maximal water reabsorption is required.

URINE DILUTION MECHANISMS

Approximately 50 mOsm per kilogram of water is the limit of the urinary diluting ability of humans. The mechanisms responsible for urinary dilution nearly overlap with that for urinary concentration. These two conditions between diuresis and antidiuresis are switched mainly by the collecting duct water permeability, which is regulated by vasopressin stimulation and the AQP2 water channel.

In the thick ascending limb, the luminal fluid is diluted by active NaCl absorption through NKCC2[56] and Na-H exchanger NHE3,[57] regardless of antidiuresis or diuresis. In addition, water impermeability of this segment preserves the luminal low osmolality by preventing water fluxes. The distal convoluted tubule also has the ability to actively absorb NaCl due to the presence of Na-Cl cotransporter NCC[19] and is impermeable to water, resulting in the dilution of the luminal fluid to an osmolality of about 100 mOsm per kilogram of water. In diuresis, this diluted fluid passes through the collecting duct because its water permeability is very low. Active Na reabsorption through Na channel ENaC in the collecting duct[29] can further dilute the luminal fluid. [58]

In addition to diuresis, there is another mechanism that further promotes urinary dilution. The terminal IMCD has a higher basal water permeability than other portions of the collecting duct and is surrounded by a high inner medullary interstitium. Because the osmolality of the luminal fluid reaching the terminal IMCD in diuresis is lower than that in antidiuresis, the transepithelial osmolality gradient in diuresis is larger than that in antidiuresis, resulting in a higher water reabsorption in this segment.[27] This reduces the inner medullary interstitial osmolality, leading to a further decrease in urinary concentrating ability.

VASOPRESSIN

The primary determinant of solute-free water excretion is the regulation of urinary water excretion by circulating levels of vasopressin in plasma. This section describes the regulation of vasopressin secretion from the neurohypophysis.

Structure and Synthesis

Arginine vasopressin is the antidiuretic hormone of most mammals, although members of the pig family have lysine vasopressin, in which a lysine replaces the arginine in position 8 of arginine vasopressin. Vasopressin is produced by the hypothalamic neurohypophyseal tract. This tract is composed of magnocellular neurons that arise bilaterally in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and project medially to merge in the pituitary stalk and form the posterior pituitary gland in the sella tunica. Vasopressin is synthesized as part of a protein precursor of approximately 21,000 Da of molecular mass that incorporates a signal peptide at its amino terminus and vasopressin, neurophysin, and copeptin at its carboxyl terminus. In the endoplasmic reticulum, the signal peptide is removed. The prohormone then moves through the Golgi apparatus and into the neurosecretory granules that travel down the axon. In the neurosecretory granules, the prohormone is processed to yield amidated vasopressin, neurophysin, and copeptin.[59] Vasopressin and neurophysin form an insoluble complex in the nerve terminal and dissociate from each other after its release into the general circulation.

Regulation of Vasopressin Secretion by the Tonicity of Body Fluid

Under physiologic conditions, the most important determinant of vasopressin secretion is the tonicity of body fluid. Tonicity defines the forces that determine the net flux of water between two solutions separated by a membrane permeable to water but impermeable to certain solutes. On the other hand, osmolality refers to the forces generated by solutes that reduce the random movement of water molecules. The osmolality is a concentration of all of the solute in water, whereas the tonicity is an effective osmotic pressure and a concentration of all of the osmotically effective solutes. There are two types of solutes: osmotically effective and noneffective. Osmotically effective solutes, such as Na and Cl, are characterized by their inability to move across the cell membrane, resulting in a difference in their concentrations between the intracellular and extracellular compartments. On the other hand, osmotically noneffective solutes, such as urea and ethanol, are characterized by their ability to diffuse freely across the cell membrane and their concentrations between these two compartments are similar. Glucose is confined in the extracellular compartment and is osmotically effective in the absence of insulin; whereas in the presence of insulin, glucose is able to enter the cell by insulin-activated transporters, thereby becoming osmotically noneffective. Vasopressin secretion is finely regulated by the tonicity of body fluid.

The changes in the tonicity of body fluid are sensed by a group of cells called osmoreceptor neurons. These neurons are located in several brain areas including the organum vasculosum laminae terminalis (OVLT), the SON, and the PVN nuclei of the hypothalamus.[60] It is postulated that an increase in the tonicity of the extracellular compartment causes water withdrawal and shrinkage of the osmoreceptor neurons. Owing to the presence of mechanosensitive cation channels in these cells, cell shrinkage induces a positive charge influx and depolarizes the cell membrane, resulting in the triggering of neuronal action potentials. Such neurons send axonal projections to the SON, where they release the excitatory transmitter glutamate to synaptically excite the magnocellular neurons that release vasopressin in their distal axons.[60,61]

Recent studies show that the transient receptor potential vanilloid (TRPV) channels are likely to be the major component of the osmoreceptor. The N-terminal truncated TRPV1 channel is selectively expressed in osmosensory neurons in OVLT. The responses of these neurons to hypertonic stimuli, including reactive increases in cation channel conductance, membrane depolarization, and increased frequency of neuronal action potentials, were greatly inhibited in TRPV1 knockout mice. Furthermore, TRPV1 knockout mice showed a higher serum osmolality than wild-type mice, indicating a decreased sensitivity of osmosensation.[62] However, these results were not reproduced by another study.[63] In addition to TRPV1, TRPV2 and TRPV4 are present in osmoreceptor neurons and functional characteristics of these channels show responsiveness to the tonicity. Further studies, such as examining a possible hetero-oligomerization of these TRPV channels, are necessary for a molecular understanding of osomosensation.[60,61]

The functional properties of osmoregulatory mechanisms have a discrete threshold for vasopressin secretion, above which a linear relationship between plasma osmolality and vasopressin levels occurs.[64] When the plasma osmolality is below a threshold level, vasopressin secretion is suppressed to low or undetectable levels (0.5 pg per milliliter). Above this threshold, vasopressin secretion increases linearly in direct proportion to plasma osmolality. Both the threshold level and the slope of the regression line relating vasopressin secretion to plasma osmolality vary between persons due to unknown genetic factors and between the different conditions in the same individual. In general, the threshold is approximately 280 mOsm per kilogram of water, and above this threshold, a rise in plasma osmolality of 1% increases plasma vasopressin by approximately 0.4 to 1.0 pg per milliliter. The renal response to circulating vasopressin is also linear, with urinary concentration that is proportional to vasopressin levels from 0.5 to 5 pg per milliliter, above which urinary osmolality is maximal and cannot increase further. Therefore, changes of as little as 1% in plasma osmolality are sufficient to cause a significant increase in urinary concentration, and maximal antidiuresis is achieved by an increase in plasma osmolality of only about 10 to 15 mOsm per kilogram of water above the threshold. Furthermore, vasopressin secretion occurs within minutes in response to changes in plasma osmolality. After release into the systemic circulation, vasopressin distributes quickly because of its small size, and the equilibration between the vascular and extravascular compartments is almost complete within 10 minutes. The half-life of vasopressin in plasma is within

30 minutes. Therefore, the changes in plasma osmolality are rapidly transferred to changes in urinary concentration.

There are several factors that can alter the threshold and the sensitivity, which is the slope of the regression line relating vasopressin secretion to plasma osmolality. The most important factor is hemodynamic changes, including blood pressure and effective arterial blood volume, which are described in the next section. Aging enhances the sensitivity of vasopressin secretion.[65] In pregnancy, relaxin, an ovarian hormone produced by the corpora, reduces the threshold and increases vasopressin secretion, contributing partly to the increase in blood volume.[66]

Hemodynamic Regulation of Vasopressin Secretion

Vasopressin secretion is also affected by changes in blood volume and pressure.[67] A decrease in blood volume, such as with bleeding, sequestration or redistribution of blood, or fluid loss by sweating, diarrhea, or vomiting, can increase vasopressin secretion. The vasopressin release mechanism is much less sensitive to small changes in blood volume than to comparable changes in osmolality. Small reductions under 8% in blood volume usually have little effect on plasma vasopressin concentration. On the other hand, further acute reduction in blood volume significantly stimulates vasopressin secretion. Usually, 20% to 30% reductions in blood volume increase vasopressin secretion to the levels of 20 to 30 times normal. The stimulus-response relationship follows an exponential pattern. The vasopressin response to an acute reduction in blood pressure is similar to the response to blood volume. Reductions in blood pressure under 10% have little effect on vasopressin secretion, whereas blood pressure decreases of 20% to 30% result in significant increase in plasma vasopressin. The effects of reductions in blood volume and pressure are exerted through shifting the threshold and sensitivity of vasopressin secretion to osmotic stimuli.[68]

These hemodynamic effects on vasopressin secretion are mediated at least in part by neural pathways that originate in stretch-sensitive receptors called baroreceptors in the cardiac atria, the aorta, and the carotid sinus. From these receptors, afferent nerve fibers ascend in the vagus and glossopharyngeal nerves to the nuclei of the tractus solitarus (NTS) in the brainstem.[69] A variety of postsynaptic pathways from the NTS then project, both directly and indirectly, to the SON and the PVN nuclei of the hypothalamus.

Vasopressin secretion through the baroreceptor mechanism is promoted not only by a blood volume decrease but also by the reduction in effective arterial circulating blood volume. For example, upright posture, congestive heart failure, and cirrhosis stimulate vasopressin secretion.[70,71]

Other Influences for Vasopressin Secretion

The sensation of nausea, with or without vomiting, is a powerful stimulus to vasopressin secretion.[72] Nausea increases plasma vasopressin in excess of 200 to 400 pg per milliliter. The pathways responsible for this effect are located in the chemoreceptor zone in the postrema area of the brainstem. Water loading blunts, but does not abolish, the effect of nausea on vasopressin secretion, suggesting that the mechanism of emetic effect has a possible common pathway with that of an osmotic effect.

The renin-angiotensin system also influences the osmotic regulation of vasopressin secretion.[73] Neurons in the subfornical organ (SFO) contain angiotensin II and project axons onto hypothalamic magnocellular neurosecretory cells (MNCs) located in the SON and PVN nuclei. It is suggested that the release of angiotensin II may contribute to the potentiation of osmotically evoked action potential firing and vasopressin release.

Acute hypoglycemia induces a modest increase in plasma vasopressin,[65] although the pathways responsible for this effect are unknown.

The effects of pregnancy on the osmotic regulation of vasopressin secretion are complex. The systemic hemodynamic profile of pregnancy is characterized by a decrease in mean arterial pressure, a rise in cardiac output and plasma volume, and a decrease in body tonicity. This is due to a decrease in the osmotic threshold for vasopressin secretion because of arterial underfilling secondary to systemic arterial vasodilatation.[74,75] In addition, relaxin, an ovarian hormone produced by the corpora, reduces the threshold and increases vasopressin secretion, which contributes partly to the increase in blood volume.[66] On the other hand, the metabolic clearance of vasopressin is increased, owing to circulating cystine aminopeptidase (vasopressinase) produced by the placenta. This mechanism rarely causes transient diabetes insipidus in late pregnancy[76] because of the increased release of vasopressin.

MOLECULAR MECHANISMS OF VASOPRESSIN-REGULATED WATER PERMEABILITY OF THE COLLECTING DUCT

Vasopressin-regulated water permeability of the collecting duct is responsible for the final control of urine concentration. Upon vasopressin stimulation, the water permeability of the collecting duct is dramatically increased. Based on the hypertonic medullary interstitium, the increased water permeability results in significant water reabsorption and urine concentration. The vasopressin V2 receptor and AQP2 in the collecting duct are the primary targets for vasopressin. Vasopressin-regulated AQP2 translocation and abundance change the water permeability of the collecting duct principal cells. The essential roles of the vasopressin V2 receptor and AQP2 in urine concentrations are demonstrated by mouse models. Male mice that were introduced with a nonsense mutation into the AVPR2 gene, which codes the vasopressin V2 receptor and is located in the X chromosome, died within 7 days after birth due to hypernatremic

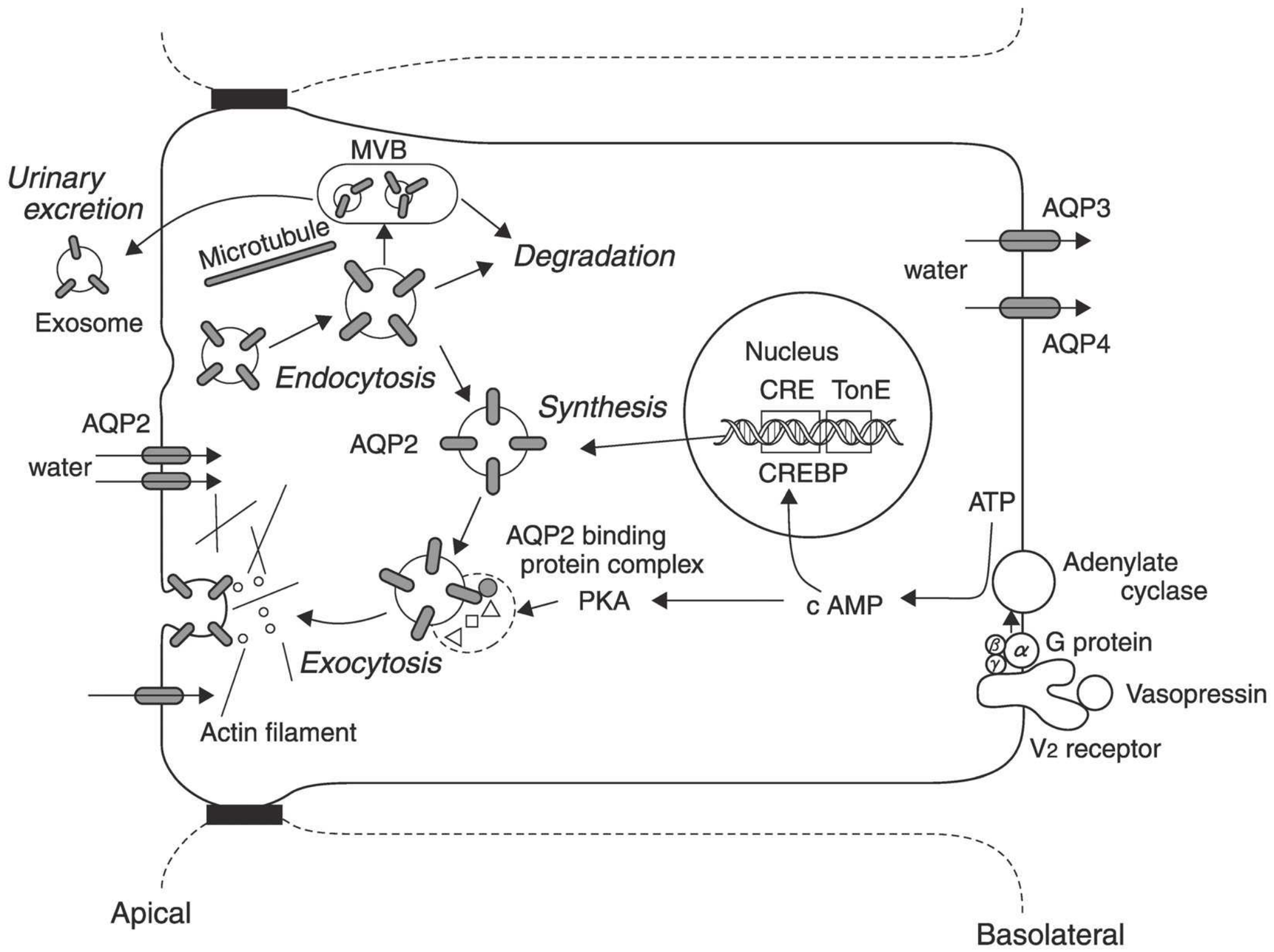


FIGURE 4.5 A schematic representation of the AQP2 protein dynamics within the cell. Vasopressin binds to the V2 receptor and this stimulates adenylate cyclase via G protein, resulting in the production of cAMP. cAMP stimulates the synthesis of the AQP2 protein through the interaction of the cAMP responsive element (*CRE*) of the AQP2 gene and the CRE binding protein (*CREBP*). Synthesized AQP2 are stored in subapical vesicles. Protein kinase A (*PKA*) directly phosphorylates AQP2, and this alters the binding dynamics of AQP2 and its binding proteins (AQP2 binding protein complex), which induces a reorganization of actin and stimulates the exocytosis of AQP2 containing vesicles to the apical membrane. Upon withdrawal of vasopressin stimuli, AQP2 is internalized by endocytosis and degraded. Some of the internalized AQP2 is transferred to the multivesicular body (*MVB*) and is excreted to the urine as an exosome. Thus, water transport—crossing the apical membrane through AQP2 and exiting the basolateral membrane through AQP3 and AQP4—is regulated by vasopressin.

dehydration.[77] Mice models with AQP2 protein deletion in the collecting duct also showed a severe defect in urinary concentration.[22,78] This section describes the molecular mechanisms for the regulation of water permeability of the collecting duct principal cells. Figure 4.5 illustrates several major mechanisms.

Vasopressin V2 Receptor

When circulating vasopressin reaches the kidney, vasopressin binds to the vasopressin V2 receptor expressed on the basolateral plasma membrane of the collecting duct principal cells and initiates the signal transduction in the cell. The V2 receptor is a 371 amino acid protein with 7 membrane-spanning domains and couples to heterotrimeric G-proteins.[79,80] The binding of the V2 receptor to vasopressin promotes the disassembly of the bound heterotrimeric G-protein (Gs) into Gα and Gβγ subunits. GDP-GTP exchange occurs in the Gα subunit and this activated Gsα then stimulates adenylate cyclase, resulting in an increase in intracellular cAMP levels (Fig. 4.5). Increased cAMP activates protein kinase A, which phosphorylates AQP2, and this phosphorylation event is required to increase the water permeability and water reabsorption of renal principal cells, which is described in the next section.

Aquaporin-2

AQP2 is a 271 amino acid protein with 6 membrane-spanning domains and 2 conserved, membrane-embedded asparagine–proline–alanine (NPA) motifs, and selectively permeates water.[3] AQP2 is predominantly expressed in the collecting duct principal cells (Fig. 4.6).[81,82] In response to vasopressin, AQP2 recycles between the luminal cell surface membrane (also called the apical membrane) and the intracellular subapical storage vesicles of the collecting duct principal cells. In the absence of vasopressin, AQP2 predominantly resides in the subapical vesicles. Because water molecules slowly diffuse through the lipid bilayer, the water permeability of the cell membrane without AQPs is low. Therefore, basal water permeability of the principal cells is also low. In the presence of vasopressin, AQP2-containing vesicles fuse with the apical membrane via exocytosis, thus inducing an exceedingly high

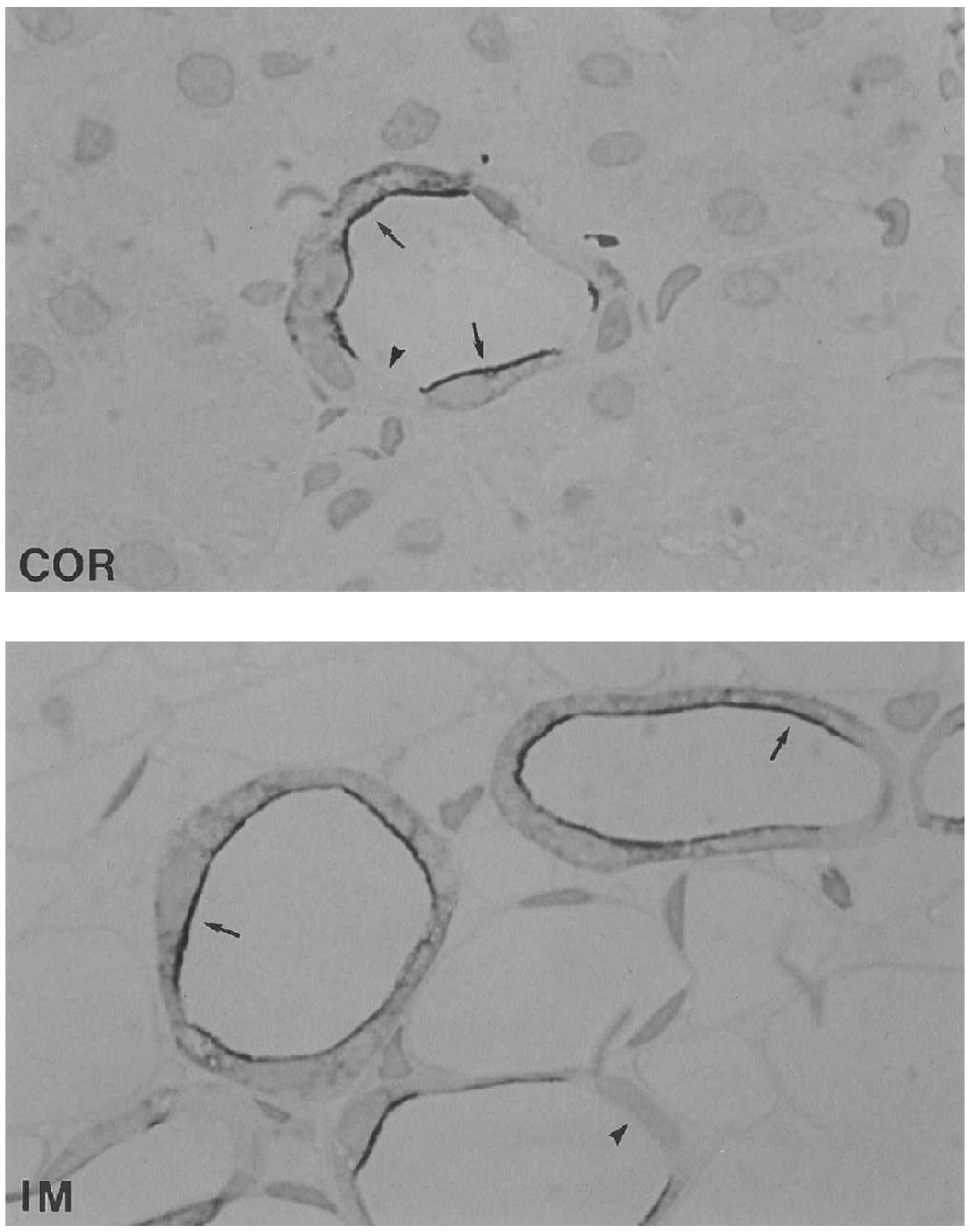


FIGURE 4.6 The immunoperoxidase labeling of AQP2 in the cortical (*COR*), the outer medullary (*OM*), and the inner medullary (*IM*) collecting duct. AQP2 is very abundant in the apical plasma membrane domains, as well as in the subapical domains (*arrows*), whereas intercalated cells are unlabeled (*arrowheads*). In the inner medullary collecting duct, AQP2 is also present in the basolateral part of the cell. (Magnification × 1,100.) (Reprinted from Nielsen S, Knepper MA, Kwon TH, Frøkiaer J. Urinary concentration and dilution. In: Schrier RW, ed. *Diseases of the Kidney & Urinary Tract*, 8th ed. Philadelphia: Lippincott Williams & Wilkins 2005:98, with permission.)

water permeability of this apical membrane (Fig. 4.7).[23–25] Vasopressin increases the water permeability by a factor of 10 to 100 in the cortical collecting duct,[49] 20 to 30 in the outer medullary collecting duct,[83] and 10 to 30 in the initial IMCD.[49] The terminal IMCD has a higher basal water permeability and vasopressin increases the water permeability by a factor of 10 in the terminal IMCD.[49]

The water permeability of the basolateral membrane of the principal cells is always high because of the presence of AQP3 and AQP4, and is not rate limiting for water transport. Thus, water that enters into the cell from the lumen via AQP2 can exit to the hypertonic medullary interstitium by the osmotic gradient. Upon the binding of vasopressin to its receptors, AQP2 is phosphorylated and this phosphorylation event is a requisite for AQP2 translocation to the apical membrane (Fig. 4.5).

Aquaporin-2 Phosphorylation and Trafficking

AQP2 forms homotetramers,[84] and at least three of four monomers in AQP2 tetramers must be phosphorylated for successful apical membrane localization.[85] Phosphorylation of serine 256 is required for the trafficking of AQP2 to the cell surface in cultured cells.[86,87] Protein kinase A and its substrates are present throughout the cell; therefore, localization of protein kinase A in specific sites is necessary for PKA to effectively phosphorylate its target. The phosphorylation process is assisted by protein kinase A anchoring proteins

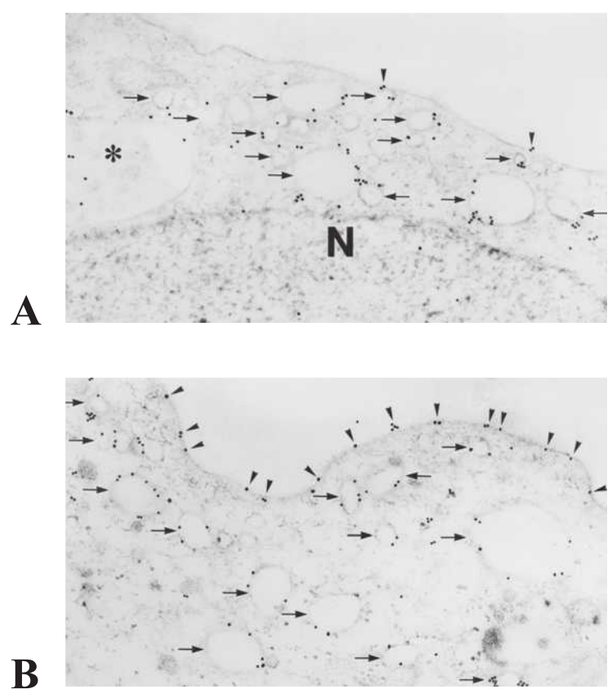


FIGURE 4.7 AQP2 immunogold labeling in the inner medullary collecting duct cells of control **(A)** and vasopressin-treated **(B)** Brattleboro rats. Immunogold labeling is obvious in the subapical cytoplasm in close association with cytoplasmic vesicles (*arrows*) or in the multivesicular body (*) but is sparse on the apical membrane (*arrowheads*). In contrast, heavy AQP2 labeling is observed on the apical membrane after vasopressin treatment (*arrowheads*). *N*, nucleus. (Magnification × 64,000.) (Reprinted from Yamamoto T, Sasaki S, Fushimi K, et al. Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. *Am J Physiol.* 1995;268:C1546, with permission.)

(AKAPs). The tethering of PKA to AKAPs is required for AQP2 shuttling to the cell surface.[88] A splice variant of AKAP-18, AKAP-18δ, is specifically involved in AQP2 shuttling[89] and the involvement of AKAP-220 has also been reported.[90]

AQP2 phosphorylation by kinases other than PKA might also be involved in the regulation of AQP2 trafficking. Serine 256 in AQP2 is also a substrate for Golgi casein kinase. AQP2 transition through the Golgi apparatus is associated with a PKA-independent increase in AQP2 phosphorylation at serine 256, suggesting that phosphorylation by Golgi casein kinase may be required for Golgi transition.[91] van Balkom et al.[92] showed that the activation of protein kinase C mediates AQP2 endocytosis, which is independent of the phosphorylation state of serine 256. In addition, a cyclic-GMP-dependent pathway is shown to be involved in AQP2 exocytosis,[93] and an inhibitor of cyclic GMP phosphodiesterase is able to induce AQP2 translocation to the cell surface.[94]

In addition to serine 256, there are three additional phosphorylation sites near the AQP2 C-terminus. These modifiable residues are serine 261, serine 264, and serine 269. Vasopressin induces the phosphorylation of AQP2 also at serine 264, and serine 264-phosphorylated AQP2 is translocated to the plasma membrane similarly to serine 256-phosphorylated AQP2.[95] On the other hand, vasopressin decreases the phosphorylation levels at serine 261, and the localization of serine-261-phosphorylated AQP2 is different from that of serine 256-phosphorylated AQP2, which suggests distinct roles for these residues in AQP2 trafficking.[96] Lu et al.[97] reported that the phosphorylation state of serine 261 does not affect AQP2 trafficking. Serine 269 is shown to be involved in the plasma membrane retention of AQP2.[98] Moeller et al.[99] showed that the phosphorylation of serine 264 and serine 269 depends on the prior phosphorylation of serine 256, and that the phosphorylation of serine 261 partially depends on the phosphorylation of serine 264 and serine 269. In contrast, serine 256 phosphorylation is not dependent on the state of any of the other phosphorylation sites, suggesting that serine 256 is the most important phosphorylation site of AQP2.

Role of Calcium in Aquaporin-2 Regulation

Intracellular Ca^{2+} mobilization is also involved in vasopressin-mediated AQP2 trafficking,[100] although its precise role remains unclear. In addition to increasing cAMP levels in the cytoplasm of the principal cells of the collecting duct, vasopressin binding to V2 receptor triggers a rapid increase of intracellular Ca^{2+}, which is followed by sustained temporal oscillations of the level of this ion. This process seems to be involved in AQP2 exocytosis. Balasubramanian et al.[100] suggest several plausible candidates as downstream effectors of this signaling cascade, such as calmodulin and MLCK. MLCK is a calmodulin-dependent kinase that regulates actin filament organization by phosphorylating the regulatory light chain of myosin II, and thus also activates myosin motor activity. Myosin II and its regulatory light chain are found in the AQP2-binding protein complex,[101] supporting their involvement in AQP2 trafficking. Nevertheless, Lorenz et al.[102] demonstrated that cyclic AMP alone is sufficient to induce AQP2 translocation without the need for an increase in cytosolic Ca^{2+} levels in the inner medullary collecting duct cells.

Involvement of extracellular Ca^{2+} in AQP2 regulation has also been indicated by several findings.[103–106] Urinary AQP2 excretion correlates with the severity of enuresis, a disease characterized by nocturnal polyuria and hypercalciuria.[103] Clinical amelioration demonstrated by a low calcium diet is accompanied by the regulation of urine output through the remodulation of AQP2 expression/trafficking.[104] Drug-induced hypercalcemia/hypercalciuria causes polyuria and reduces AQP2 expression in rats.[105] AQP2 translocation to the apical membrane prompted by forskolin-induced increases in cyclic AMP levels is inhibited by increased levels of extracellular Ca^{2+}.[106] This process is probably mediated by the endogenous calcium-sensing receptor and is associated with an increase in F-actin levels.

Other Influences for Aquaporin-2 Trafficking

Several other factors have recently been reported to affect AQP2 trafficking. Nejsum et al.[107] used Madin–Darby canine kidney epithelial cells transfected with AQP2 and showed that prostaglandin E2 and dopamine induce the internalization of AQP2, regardless of AQP2 dephosphorylation. de Seigneux et al.[108] reported that aldosterone induces a basolateral expression of AQP2, suggesting a role for aldosterone in water metabolism in conditions of increased sodium reabsorption in the collecting ducts.

Role of the Cytoskeleton in Aquaporin-2 Trafficking

The actin cytoskeleton is reported to function as a barrier for AQP2 exocytosis. Actin depolymerization is necessary for the cAMP-dependent translocation of AQP2. [109] In fact, the stimulation of prostaglandin E3 receptors has been shown to inhibit vasopressin-induced inactivation of Rho GTPase, vasopressin-induced F-actin depolymerization, and AQP2 translocation induced by vasopressin, cAMP, or forskolin.[110] Rho GTPase activation by bradykinin stabilizes cortical F-actin and inhibits AQP2 trafficking.[111]

GTPase-activating protein Spa-1 (SPA-1) binds to the C-terminus of AQP2, and this binding is required for AQP2 trafficking.[112] SPA-1 may inhibit Rap1 GTPase activating protein, which triggers F-actin disassembly and may maintain the basal mobility of AQP2.[55,113] SPA-1-deficient mice show impaired AQP2 trafficking and hydronephrosis.[112] In humans, mutations in the C-terminus of AQP2, which is the binding region of SPA-1, causes nephrogenic diabetes insipidus (NDI), a disease characterized by a massive loss of water through the kidney.[114,115] Furthermore, AQP2 binds to a multiprotein complex that includes the actin cytoskeleton, and a pattern of competing interactions between AQP2 and G-actin or tropomyosin directs AQP2 trafficking to the apical membrane (Fig. 4.5).[101,116,117]

F-actin assembly might have both inhibitory and facilitatory effects on an AQP2 transport to the cell surface.[118] Drug-induced actin depolymerization inhibits AQP2 translocation from the early endosomes that express early endosome antigen 1 to the subapical storage vesicles that express Rab-11.[119] Myosin II and its regulatory light chain are found in an AQP2-binding protein complex,[101] and vasopressin induces myosin light chain phosphorylation, which enhances the myosin–actin filament interaction and the formation of actin fibers.[120] Myosin has also been shown to be critical for AQP2 recycling.[121] In addition to acting as a barrier to prevent AQP2 trafficking, actin fibers may function as "cables" that promote and direct AQP2 transport. Dynamic actin reorganization may be responsible for the transformation of the actin barrier into actin cables. Further studies are necessary for understanding these sequential events.

Fusion of Aquaporin-2 Vesicles with the Apical Membrane

The docking and fusion of AQP2-containing vesicles with the apical membrane involves the action of N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins, including VAMP-2, SNAP-23, syntaxin-3, and syntaxin-4.[52] Syntaxin-binding protein 2 (also called Munc18b) is reported to function as a negative regulator of SNARE complex formation and AQP2-vesicle fusion to the apical membrane.[122]

Aquaporin-2 Recycling and Endocytosis

AQP2 is a recycling membrane protein. Upon vasopressin stimulation, AQP2 is transported to the apical membrane, rendering the cell water permeable, as described previously. After vasopressin stimulation is terminated, AQP2 is shuttled back to the cell cytoplasm, a process that restores the water-impermeability of the cell (Fig. 4.5). This internalization process consists of AQP2 retrieval into early endosomes that express early endosome antigen 1, and subsequent transferal of this water channel to storage vesicles that express Rab-11.[123] From Rab-11-positive vesicles, AQP2 is able to go again to the apical membrane. Actually, this recycling process occurs constitutively, and many signaling pathways are involved for the regulation of each part of this recycling itinerary. Vasopressin signaling is the most potent and most important factor that enhances the exocytotic process among the recycling itinerary.

During the endocytotic process of the AQP2 recycling pathway, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process.[124,125] Dynamin is a GTPase that is involved in the formation and pinching off of clathrin-coated pits to form clathrin-coated vesicles, and its dominant-negative mutant K44A renders the protein GTPase deficient and arrests clathrin-mediated endocytosis. This GTPase-deficient dynamin mutant K44A is shown to accumulate AQP2 in the plasma membrane even without vasopressin stimulation.[125] Furthermore, this dynamin mutant K44A or methyl-β-cyclodextrin is able to accumulate the phosphorylation-deficient mutant in the cell surface despite the fact that AQP2-S256A accumulation in the cell surface is not induced by vasopressin. Methyl-β-cyclodextrin depletes membranes of cholesterol and results in a rapid inhibition of endocytosis.[126] These data also support the constitutive recycling of AQP2 and the presence of the processes that are not dependent on phosphorylation of AQP2 at serine 256.

A heat shock protein hsc70, which is important for uncoating clathrin-coated vesicles, binds to the C-terminus of nonphosphorylated AQP2 and is required for AQP2 endocytosis.[127)] Kamsteeg et al.[128] reported that the myelin and lymphocyte protein (also known as MAL), which is involved in the organization of the glycosphingolipid-enriched membrane, interacts with AQP2 and enhances accumulation of AQP2 in the apical membrane by decreasing the level of internalization of the protein. Ubiquitination at lysine 270 of AQP2 is important for AQP2 endocytosis and degradation.[129] Furthermore, LIP5, which is involved in multivesicular body formation, interacts with AQP2 and facilitates its lysosomal degradation.[130]

Regulation of Water Transport Activity of Individual Aquaporin-2s

As described previously, AQP2 phosphorylation induces its apical membrane insertion, rendering the collecting duct cells water permeable. However, whether this phosphorylation regulates the water permeability of individual AQP2 water channels remained unclear until recently. Several groups had examined the role of phosphorylation on osmotic water permeability (P_f) of individual AQP2s. In the Xenopus oocytes expression system, one study showed that PKA phosphorylated AQP2 at serine 256, which increased the P_f of oocytes without a significant increase in the amount of AQP2 on the oocyte surface.[131] However, another study using a similar method showed that the P_f values that corrected for the plasma membrane abundance of AQP proteins were not different between WT-AQP2 and the nonphosphorylation-mimicking mutant S256A-AQP2 expressing oocytes, indicating a lack of an effect of phosphorylation at S256 on the P_f of individual AQP2 proteins.[99] Lande et al.[132] purified endosomes derived from the apical membrane of rat IMCD cells that were highly enriched for AQP2. Then, these endosomes were phosphorylated by an exogenous PKA catalytic subunit or were dephosphorylated by an exogenous alkaline phosphatase. There was no significant difference in P_f between these two samples, suggesting that the P_f of AQP2 is not changed by its phosphorylation. However, involvement of other proteins, such as endogenous phosphatases, cannot be neglected.[132]

To clarify whether the P_f of AQP2 is regulated by its phosphorylation event alone, the experimental system that does not contain any other regulatory proteins is required. Eto et al.[133] performed a large-scale expression of full-length recombinant human AQP2, purification and reconstitution in proteoliposomes, and examined the protein function.

The results showed that the P_f of proteoliposomes was enhanced approximately twofold by phosphorylation at serine 256. This observation indicates that AQP2 water channel activity is directly regulated by its phosphorylation, and the mechanism involved may be the channel gating that is well studied in plant AQPs by X-ray crystal structure analysis.[134] Thus, in addition to AQP2 translocation to the luminal membrane, the water transport activity of individual AQP2 proteins may be involved in the regulation of water reabsorption in kidney collecting ducts.

Actually, vasopressin increases the water permeability of the collecting ducts more than tenfold.[49,83] Thus, vasopressin-induced short-term regulation of P_f of the collecting ducts still seems to be mainly due to AQP2 translocation, and the altered water transport activity of individual AQP2s may have a role of doubling the effect.

Long-Term Regulation of Water Permeability of the Collecting Duct by Altered Aquaporin-2 Abundance

In addition to short-term regulation of collecting duct water permeability described previously, long-term regulation also plays an important role in body water balance. Long-term regulation of collecting duct water permeability is seen when water intake is restricted for 24 hr or more, resulting in an increased maximum concentrating ability. This response is mainly induced by an increase in AQP2 abundance due to increased transcription of the AQP2 gene.[135,136] An increased AQP2 expression level during water restriction is a downstream of vasopressin signaling.[137,138] It is known that the cAMP responsive element (CRE) is present in the 5′-flanking region of the AQP2 gene and regulates the transcription of the gene.[139,140] Hasler et al.[141] examined the AQP2 gene transcription in a cultured cell line, which endogenously expresses AQP2 and showed that the vasopressin-stimulated transcription of AQP2 was mediated through CRE.

Medullary collecting ducts are surrounded by interstitial hypertonicity and the hypertonicity may be another determinant of AQP2 transcription.[142,143] Hypertonicity affects transcription of many genes through the interaction between the tonicity-responsive enhancer (TonE) and its transcription factor TonEBP. TonE is observed in the AQP2 gene, suggesting that TonE/TonEBP contributes to AQP2 transcription. This possibility was examined in TonEBP gene knockout mice, which had mostly embryonic lethality or died within a few weeks after birth. However, the surviving mice showed atrophy of the kidney medulla, and the protein expression of AQP2 but not of AQP3 was clearly downregulated in the knockout mice, confirming the role of TonE/ToneEBP in AQP2 transcription.[144] Taken together, AQP2 protein abundance in long-term regulation is likely to be mediated by stimulated transcription of the AQP2 gene through CRE and TonE motifs. In addition, the presence of AP1 and GATA elements are known in the promoter region of the AQP2 gene, but their roles remain speculative (Fig. 4.5).

CHANNELS AND TRANSPORTERS CONTRIBUTING TO URINE CONCENTRATION AND DILUTION

Urine concentration and dilution are enabled by the specialized organization of the renal tubule and vasculature, and the presence of channels and transporters with their specific localizations. This section describes water channel aquaporin family members other than AQP2 and other channels and transporters involved in urine concentration and dilution. Table 4.1 summarizes the basic characteristics of AQP members in the kidney, and tissue localization and phenotypes of mice and humans caused by gene knockout or mutations. Figure 4.8 shows the localization of these channels and transporters along the nephron. Figure 4.9 shows the maximal urine osmolalities of the knockout mice in which channels and transporters involved in urinary concentration are deleted.

Aquaporin-1

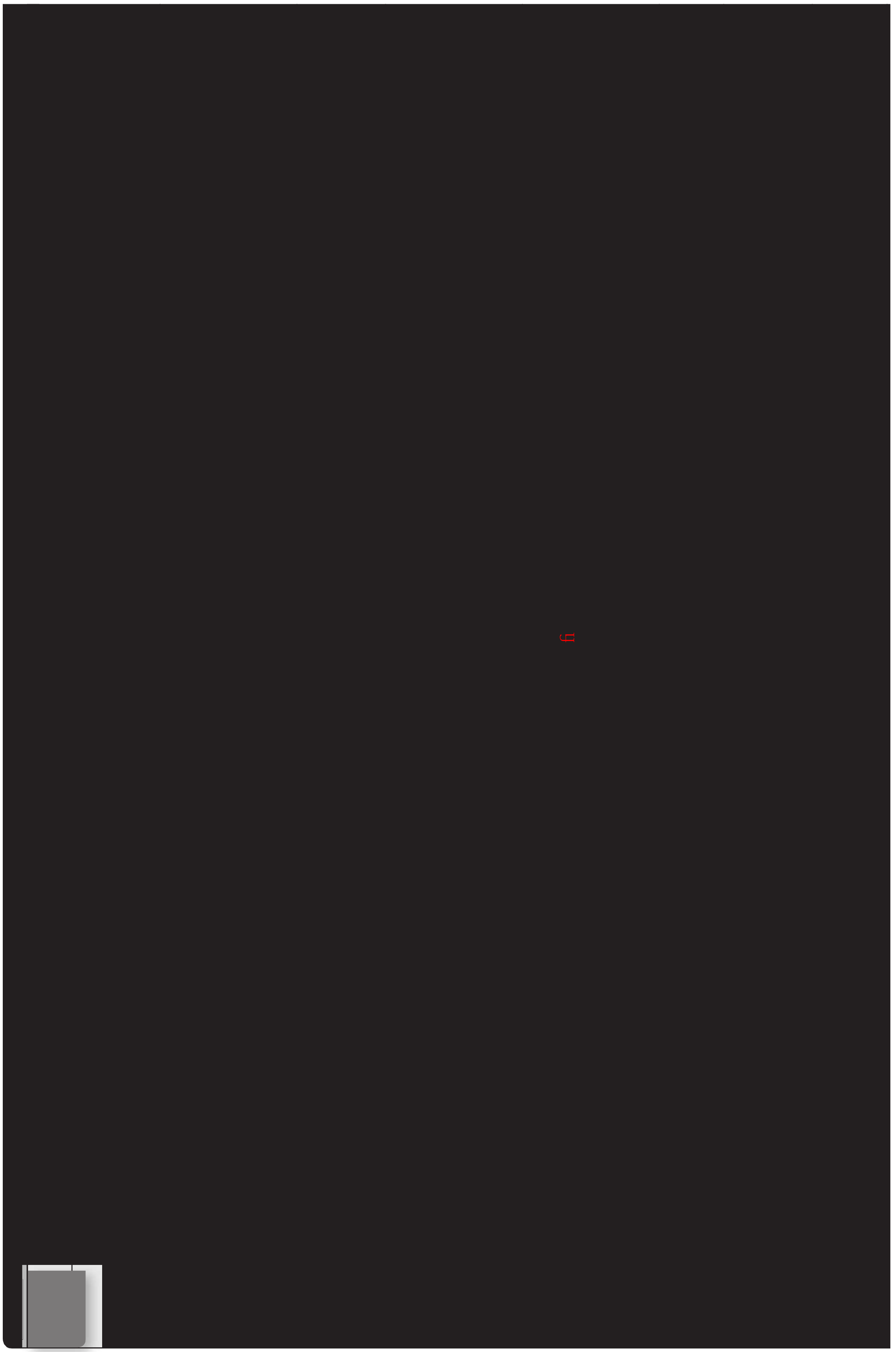
AQP1 is localized on the apical and basolateral membrane of the proximal tubules, the descending thin Henle limbs, and the outer medullary descending vasa recta.

AQP1 is constitutively active as a water-selective pore in these segments.[1,26]

AQP1-null mice show marked polyuria. Urinary osmolality in AQP1-null mice is low and unresponsive to vasopressin secretion or water deprivation.[11,145] As shown in Figure 4.9, maximal urine osmolalities of AQP1 knockout mice is 23% of that of wild-type mice under the same stimulated condition (i.e., 36 hr water deprivation). This phenotype was explained by two distinct mechanisms: impaired near iso-osmolar water reabsorption by the proximal tubule and reduced medullary hypertonicity resulting from impaired countercurrent exchange.[146] Analysis of AQP1-null mice clarified that AQP1 is the principal water channel in the proximal tubules and the descending thin Henle limbs and provides the major route for transepithelial water permeability in these segments.[146] In 2001, two unrelated individuals with a deficiency in AQP1 expression were reported.[147] One of these individuals was homozygous for a deletion of exon 1 of AQP1. The second individual was homozygous for a frame-shift mutation in exon 1 of AQP1. Although both patients seemed asymptomatic under normal conditions, they showed an impaired urinary concentrating ability following 24 hr of water restriction as compared with healthy controls (450 versus 1,000 mOsm per kilogram of water). Such a defect can become clinically meaningful in circumstances that require maximal urinary concentration; for instance, during vomiting and diarrhea.[148]

Aquaporin-3

The AQP3 water channel is present in the principal cells of the connecting tubule and the collecting duct, and enables water entry into the interstitium in these segments.[149] AQP3 is constitutively localized in the basolateral plasma



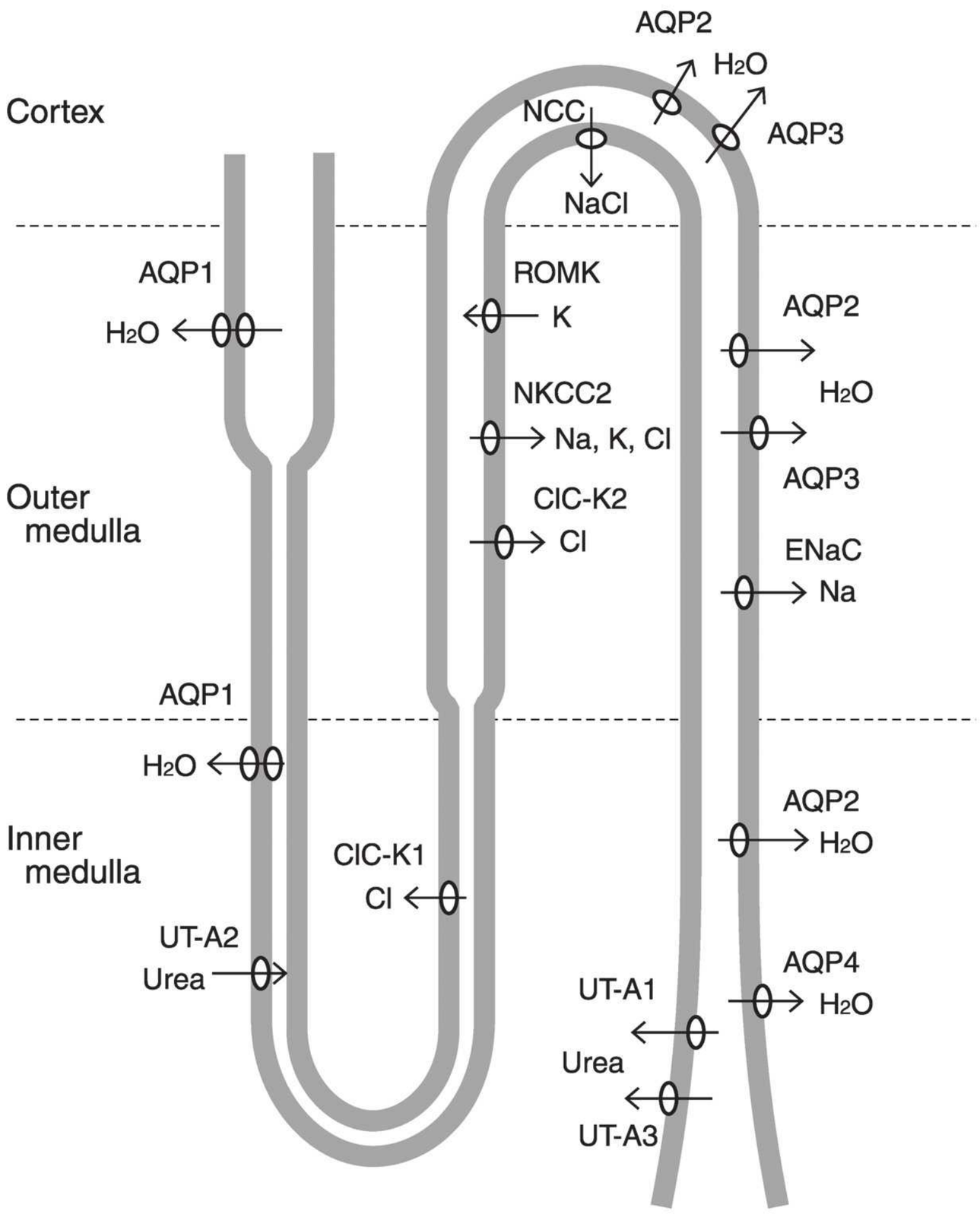


FIGURE 4.8 Transporters and channels involved in urine concentration along the nephron. Water is reabsorbed in the proximal tubule and the thin descending limb by AQP1. Chloride with sodium is passively reabsorbed in the thin ascending limb by the ClC-K1 chloride channel. NaCl is actively reabsorbed across the thick ascending limb by the apical plasma membrane Na-K-2Cl cotransporter (NKCC2). Potassium is recycled through an apical plasma membrane potassium channel, ROMK, and chloride is transported across the basolateral membrane by ClC-K2. Water is reabsorbed across the apical membrane of the collecting duct by AQP2 water channels. Water is reabsorbed across the basolateral membrane by AQP3 in the cortical and outer medullary collecting ducts and by both AQP3 and AQP4 in the inner medullary collecting duct. Urea is reabsorbed in the terminal inner medullary collecting duct by the urea transporters UT-A1 and UT-A3.

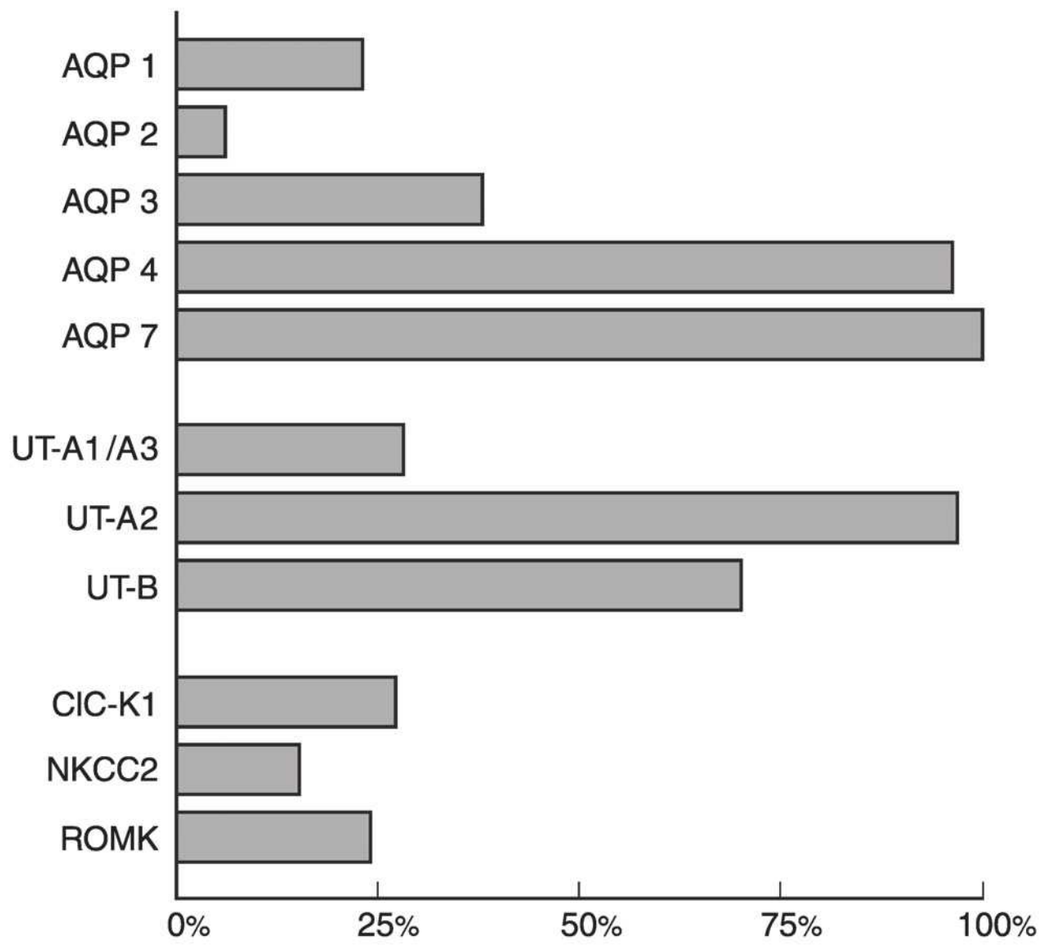


FIGURE 4.9 Maximal urine osmolalities of knockout mice in which transporters or channels involved in urine concentration are deleted. Data are expressed as a percentage of the maximal urine osmolalities of the wild-type mice in the same study. A maximally stimulated urine concentration was induced by water deprivation for 6 to 36 hr or by vasopressin administration. Data are taken from References 11, 13, 32, 42, 44, 78, 152, 158, 172, 180, and 186.

membrane. AQP3, together with AQP4, represents potential exit pathways from these cells for water entering the cell via AQP2. The AQP3 expression is shown to be increased by thirst and by vasopressin or aldosterone secretion.[150,151] AQP3 knockout mice exhibit an NDI-like phenotype, indicating that AQP3 has an important role in urine concentration (Fig. 4.9).[152,153] AQP3 deficiency was reported in humans.[154] This defect is caused by homozygous mutation affecting the 5′ donor splice site of intron 5 of AQP3. This mutation causes the skipping of exon 5 and generates a frameshift change and premature stop codon. However, phenotypes associated with this defect were not reported, possibly because the patients seemed normal.

Aquaporin-4

AQP4 is present in the principal cells of the collecting duct.[155] AQP4 is more abundant in the inner medullary collecting duct cells than is AQP3. The organization of AQP4 into orthogonal arrays of particles might enhance AQP4 water permeability,[156] and this process might be regulated by vasopressin.[157] Similarly to AQP3, AQP4 is constitutively localized in the basolateral plasma membrane. AQP4 knockout mice have a very little urinary concentrating defect (Fig. 4.9).[153,158] In contrast to AQP3, AQP4 expression is not regulated by thirst or by vasopressin secretion. AQP4 water permeability is decreased by high levels of protein kinase C and dopamine. This effect is mediated by phosphorylation at serine 180 of AQP4.[159]

Aquaporin-6

The water channel family member AQP6 is localized in the intracellular vesicles in intercalated cells in the collecting duct.[160–162] AQP6 has low water permeability and acts primarily as an anion transporter.[163] AQP6 is expressed in acid-secreting type-A intercalated cells and colocalizes with V-type H^+-ATPase; therefore, AQP6 is suggested to function to promote urinary acid secretion.

Aquaporin-7

AQP7 is localized in the brush border of proximal straight tubules (S3 segment)[12,164,165] and is able to transport glycerol as well as water.[12] AQP7 knockout mice showed marked glyceroluria,[13] indicating that glycerol can be reabsorbed through AQP7 in the proximal straight tubule, and that there might be no other glycerol reabsorbing system to compensate for this defect in the distal nephron segments.

In AQP7 knockout mice, water permeability of the brush border membrane vesicles was slightly but significantly lower than that in wild-type mice,[13] which suggests that AQP7 makes a small contribution to the water permeability of the proximal straight tubules. This contribution has been estimated to be one-eighth that of AQP1.[166] As expected from the small decrease in water permeability, AQP7 knockout mice did not show a urine concentrating defect (Fig. 4.9).[13]

The case of a man with a point mutation in AQP7 has been reported. However, no renal symptoms were mentioned, although he did have a defective glycerol metabolism.[167]

Aquaporin-11

AQP family members have two widely conserved, membrane-embedded NPA motifs that are essential for forming the water-permeable pore structure. However, AQP11, together with AQP12, have atypical NPA boxes, suggesting that their structure and function are unique.[168] In the kidney, AQP11 is present in the proximal tubular cells. Within the cells, AQP11 is localized in the endoplasmic reticulum (ER) membrane, but not localized in the cell surface plasma membrane. AQP11 knockout mice are born healthy but grow poorly and die before weaning because of uremia caused by enlarged multiple cysts in the kidney.[169] The cysts originate from kidney proximal tubule cells, inside which swollen ER are observed. The role of AQP11 is still unclear, although it seems to have an important role in ER membrane function in proximal tubular cells. Kidney cyst cells in AQP11 knockout mice showed an abnormal gene expression pattern, which is similar to that observed in polycystic kidney disease (PKD) model animals, suggesting that AQP11 knockout mice and PKD animals share common pathogenic mechanisms for cyst formation.[170] Yakata et al.[171] measured the Pf of the vesicles from the membrane fraction of Sf9 cells expressing AQP11 and showed that AQP11 has slow but constant water transport activities. Further studies are required for clarifying the role of AQP11.

Urea Transporters

In the kidney, there are four urea transporters: UT-A1, UT-A2, UT-A3, and UT-B (Figs. 4.4 and 4.8). UT-A1 and UT-A3 are expressed in the terminal IMCD,[32] and UT-A2 is present in the thin descending limb of the long-looped nephron in the inner medulla and the thin descending limb of the short-looped nephron in the outer medulla.[14] Their localization is essential for the urea recycling pathway as described in the section Urea Accumulation in the Inner Medulla, discussed previously. Vasopressin stimulation promotes the phosphorylation of UT-A1 and UT-A3 and the accumulation of UT-A1 and UT-A3 in the plasma membrane of the IMCD cells. This is responsible for the increased urea permeability of the terminal IMCD that results in greater urea reabsorption during antidiuresis.[50] Vasopressin stimulation also increases UT-A2 protein abundance.[14]

UT-A1/UT-A3 knockout mice show a large urinary concentrating defect (Fig. 4.9). This concentrating defect is ameliorated by a low protein diet, indicating that this defect is caused by a urea-dependent osmotic diuresis.[32] Another possibility may be that a low protein diet reduces urea concentrations in the medulla in both UT-A1/UT-A3 knockout mice and wild-type mice, and the difference of urine concentrating abilities of these two groups becomes small. Nevertheless, UT-A1/UT-A3 greatly contributes to urine

concentration by accumulating urea in the medulla when protein intake is normal.

On the other hand, in UT-A2 knockout mice, a small urinary concentrating defect and a reduction in medullary urea content are observed only on a low protein diet, but not on a normal diet (Fig. 4.9; a value on a normal diet is shown).[172] This finding indicates a relative role of recycling urea through the "tubular route"; urea leaves from IMCD to the interstitium, diffuses around, then enters into the tubular lumen at the thin descending limb (mediated by UT-A2), and again comes back the IMCD (Fig. 4.4), is small, and becomes significant when urea delivery to the IMCD is low.

UT-B is localized in the descending vasa recta endothelial cells.[46] UT-B knockout mice show a decrease in urea accumulation in the medulla and a urinary concentrating defect, indicating that the recycling of urea by a countercurrent exchange via the vasa recta is important for urine concentration abilities (Fig. 4.9).[44] Humans genetically lacking UT-B (Kidd blood group antigen) are identified and their maximal urinary concentrating ability is reduced (UOSM, max = 819 mOsm per kilogram of water), which is consistent with the results of the knockout mice.[173]

ClC-K1 and ClC-K2

The chloride channel ClC-K1 is mainly expressed in the thin ascending Henle limb and its expression is upregulated by water deprivation.[10,15,16] ClC-K2 is present from the thick ascending limb through to the collecting duct.[174,175] ClC-K1 knockout mice show a significantly reduced osmolality of the papilla and a severe defect in urinary concentrating ability (Fig. 4.9).[42] This finding indicates that the rapid chloride exit to the medullary interstitium is important for the maintenance of the high inner medullary interstitial osmolality and supports the passive mechanism as described in the section Countercurrent Multiplication in the Outer Medulla and Other Mechanisms in the Inner Medulla, discussed previously. Severities of impaired urine concentrating abilities are comparable in ClC-K1 and UT-A1/UT-A3 knockout mice (Fig. 4.9), implying a functional linkage between the chloride transport by ClC-K1 and the urea transport by UT-A1/UT-A3, which is again consistent with the operation of the passive model in the inner medulla.

The human orthologue of the ClC-K2 gene is one of the genes that, when mutated, causes Bartter syndrome in humans, which is a disease manifested by salt wastage, hypokalemia, metabolic alkalosis, and hyperaldosteronism.[176,177] Polyuria and polydipsia are commonly observed in Bartter syndrome and these symptoms are not relieved by vasopressin, thus showing the presence of NDI.[178] Thus, NaCl reabsorption in the thick ascending limb mediated by a basolateral chloride channel, ClC-K2, importantly contributes to urine concentration through the operation of the countercurrent multiplication system.

NKCC2 and ROMK

NKCC2 is expressed in the thick ascending limb and contributes to NaCl reabsorption in this segment.[56] The gene encoding NKCC2 is another gene, the mutations of which cause Bartter syndrome.[179] Different from NHE3, NKCC2 is also present in the macula densa.[56] NKCC2 knockout mice cannot survive to weaning because of severe dehydration and polyuria. This may be because the compensatory decrease in glomerular filtration does not occur in response to increased distal fluid delivery.[180] This finding indicates that NKCC2 plays an important role in the macula densa in the mediation of tubuloglomerular feedback and is critical for distal fluid delivery. Treatment with indomethacin rescues the knockout mice, and the surviving mice show a large vasopressin-resistant defect in urine concentrating ability (Fig. 4.9).[180] Thus, in the same mechanism as in ClC-K2, NKCC2 contributes to urine concentration.

Another Na-K-Cl cotransporter isoform, NKCC1, is expressed in the collecting duct and contributes to NaCl secretion.[181,182] NKCC1 knockout mice show a reduced capacity to excrete free water.[183] NKCC1 is present also in the juxtaglomerular afferent arteriole and the glomerular and extraglomerular mesangium, where it is thought to participate in the process of tubuloglomerular feedback and the regulation of blood pressure.

The ATP-sensitive, inwardly rectifying potassium channel, ROMK, is expressed in the apical membrane of the thick ascending limb and throughout the distal nephron segments and is involved in NaCl reabsorption in the thick ascending limb.[176,184] Mutations of the gene encoding ROMK are also found in patients with Bartter syndrome.[185] Knockout mice with ROMK have hydronephrosis, are severely dehydrated, and 95% die before 3 weeks of age. The urine concentrating ability of the surviving mice is severely impaired, as expected (Fig. 4.9).[186]

A Na-H exchanger isoform NHE3 is expressed in the thin descending Henle limb and the thick ascending Henle limb and contributes to sodium reabsorption in these segments.[57] NHE3 knockout mice show a marked reduction in proximal tubule fluid absorption. Nevertheless, these mice maintain a relatively normal distal delivery, and its urinary concentrating defect is mild.[187,188] This is due to a compensatory decrease in the glomerular filtration rate owing to an intact tubulo-glomerular feedback.[188] Furthermore, the concentrating defect of NHE3 knockout mice is explained by the decreased expression of NKCC2, a Na-K-Cl cotransporter isoform.[189]

NCC and ENaC

The Na-Cl cotransporter NCC is present in the distal convoluted tubule,[19] and the Na channel ENaC is present in the connecting tubule and the cortical collecting tubule (Fig. 4.8).[28,29] Both the proteins mediate sodium reabsorption beyond the macula densa and contribute to urine concentration by reducing fluid delivery to the medullary

collecting duct. Long-term vasopressin stimulation increases the abundance of NCC and ENaC.[190] Short-term vasopressin stimulation promotes ENaC accumulation in the apical membrane, resulting in increased sodium reabsorption.[191]

URINE CONCENTRATING DEFECTS

Central Diabetes Insipidus

Central diabetes insipidus is caused by inadequate secretion of vasopressin from the posterior pituitary in response to osmotic stimulation. In most cases, this is due to the destruction of the neurohypophysis by a variety of lesions, including granulomas (e.g., histiocytosis, sarcoidosis), neoplasms (e.g., craniopharyngioma, germinoma, lymphoma, leukemia, meningioma, pituitary tumor, metastasis), infections (e.g., meningitis, tuberculosis, encephalitis), trauma, and cerebrovascular diseases (e.g., cerebral hemorrhage, infarction). Several cases with inherited central diabetes insipidus are also reported, although it is a rare disorder.[192–194] Most of these cases show an autosomal dominant mode of inheritance and are caused by mutations in the gene coding for the vasopressin-neurophysin precursor. It is postulated that these mutations cause the production of an abnormal precursor protein that accumulates and damages the neurons.

Osmoreceptor Dysfunction

The osmoreceptors that control vasopressin secretion and thirst are located in the anterior hypothalamus. Osmoreceptor dysfunction is caused by their damage by a variety of brain lesions including granulomas, neoplasms, and cerebrovascular diseases. In contrast to central diabetes insipidus, osmoreceptor dysfunction-causing lesions usually occur more rostrally in the hypothalamus because of the location of the osmoreceptors. One lesion unique to this disorder is an anterior communicating cerebral artery aneurysm. In this disorder, osmoregulation of both vasopressin secretion and thirst is impaired.[195] Patients with osmoreceptor dysfunction typically have osmolalities in the range of 300 to 340 mOsm per kilogram of water, whereas patients with central diabetes insipidus maintain their plasma osmolality within the normal range by polydipsia. This underscores the importance of a normal thirst mechanism. On the other hand, in patients with osmoreceptor dysfunction, the baroreceptor mediated pathway[196] and responses to other nonosmotic stimuli such as nausea are intact. These patients have normal vasopressin and renal concentrating responses to hypovolemia and hypotension.

Nephrogenic Diabetes Insipidus

NDI is caused by the inability of the kidney to respond to vasopressin stimulation. Thus, plasma vasopressin levels in NDI patients are increased or normal depending on their plasma osmolality. Hyperosmolar patients with NDI have elevated vasopressin levels, whereas those with central NDI have an absence of blunted vasopressin responses relative to their plasma osmolality. There are congenital and acquired forms of NDI.

Congenital Nephrogenic Diabetes Insipidus

In more than 90% of cases of congenital NDI, the condition results from a loss of function mutations in the AVPR2 gene encoding the vasopressin V2 receptor, which makes the collecting duct cells insensitive to vasopressin. The AVPR2 gene is located in chromosome region Xq28, and its mutations cause the X-linked inheritable form of NDI. To date, over 200 mutations have been reported in the AVPR2 gene, which are categorized into five classes according to the cellular fate.[197–199] Class I mutations result in premature stop codons or unstable mRNA, and include promoter alterations, exon skipping, aberrant splicing, frameshift, and non-sense mutations that result in truncated proteins. On the other hand, mutations of class II, III, IV, and V result in fully translated proteins. Class II comprises the missense or insertion/deletion mutations that lead to the misfolding of this protein. Misfolded proteins are retained in the ER and, subsequently, are mostly targeted for proteasome degradation. Class II is most prevalent, comprising approximately 45% of all mutations in the AVPR2 gene. Class III and IV mutations do not interrupt the translocation to the plasma membrane. Class III mutations interfere with binding to the stimulatory Gs protein, leading to the reduced activation of adenylate cyclase, whereas Class IV mutations interfere with binding to vasopressin. Class V mutations result in trafficking defects beyond the early secretory pathway and misrouting to different organelles in the cell. Although most female carriers of the X-linked V2 receptor defect have no clinical disease, some females were reported with symptomatic NDI.[200] Rare female patients manifest severe NDI due to the inactivation of the normal X chromosome.[201,202] Furthermore, a significant number of de novo mutations have also been reported.[203]

Congenital NDI can also result from mutations of the autosomal gene that codes for AQP2. AQP2, the gene that encodes AQP2, is located in 12q13 and is comprised of four exons.[139,204,205] To date, over 43 mutations in the AQP2 gene have been reported (Fig. 4.10).[51,206,207] Two inheritance types are possible for the disease: Autosomal-recessive NDI is associated with 35 mutations, and autosomal-dominant NDI is associated with 8 mutations. Almost all of the mutations in recessive NDI are located in the core region of the protein, and they lead to misfolded proteins that become trapped in the ER and then are targeted for rapid degradation by the proteasome. On the other hand, AQP2 homotetramers composed only of wild-type proteins are properly translocated to the apical membrane. This effect explains the healthy phenotype of patients' parents.[206]

All mutations in autosomal-dominant NDI locate in the cytosolic C-terminus of AQP2 (Fig. 4.10). This region is important for AQP2 trafficking and these mutations impair trafficking to the apical membrane, although the water channel function of these mutants is preserved. Arg254Leu and

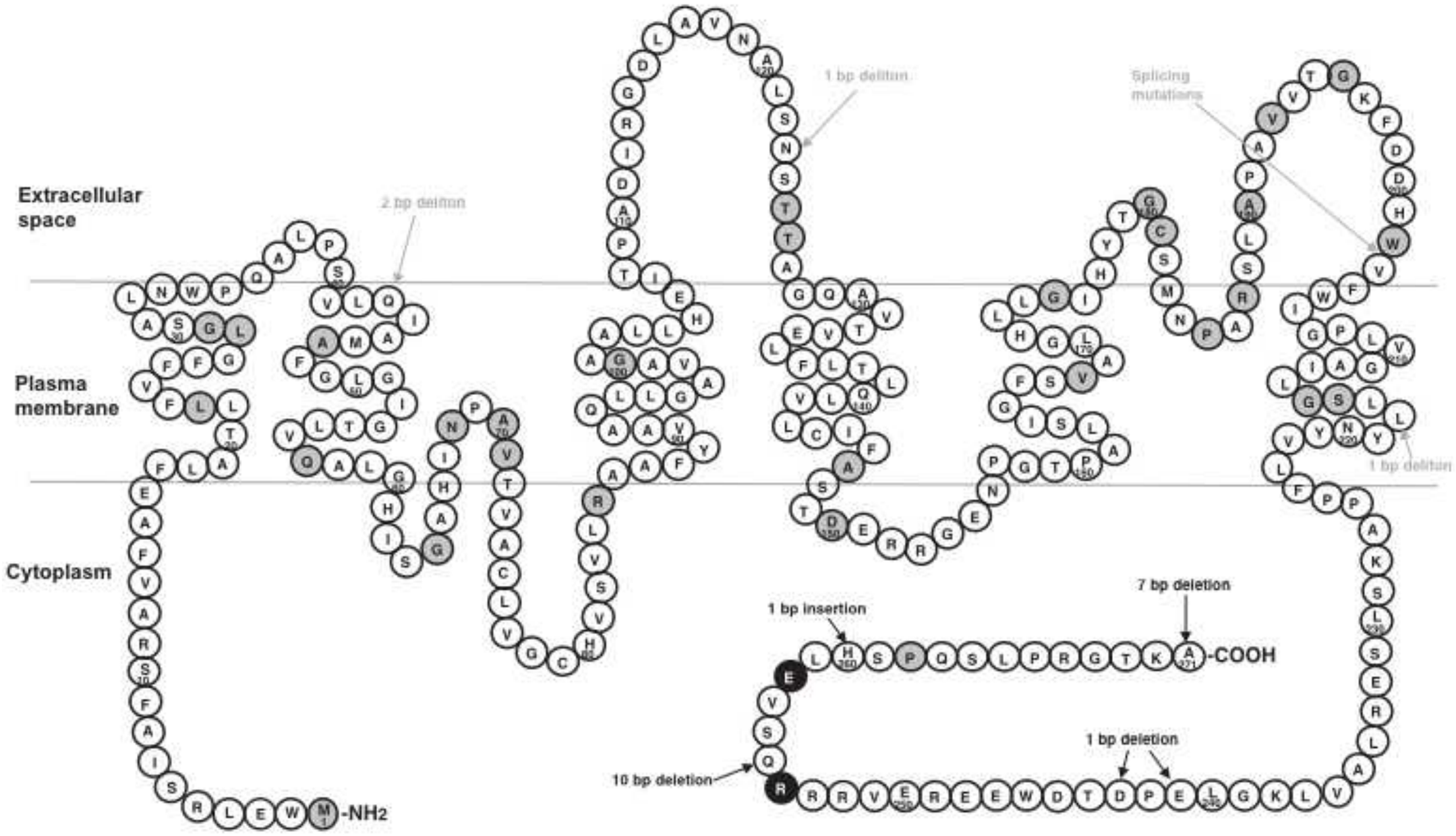


FIGURE 4.10 The AQP2 membrane topology and mutations causing nephrogenic diabetes insipidus. Amino acids that, when mutated, cause the autosomal-recessive form of nephrogenic diabetes insipidus are shown in grey. Also, *grey arrows* show AQP2 deletions and the location of the first amino acid of the protein affected by these nucleotide deletions, which causes the autosomal-recessive form of nephrogenic diabetes insipidus. Amino acids, when mutated, that cause the autosomal-dominant form of nephrogenic diabetes insipidus are shown in *black*. Similarly, in *black* are also reported AQP2 deletions or insertions, and the location of the first amino acid of the protein affected by these nucleotide changes, which cause the autosomal-dominant form of nephrogenic diabetes insipidus. *bp*, base pair.

Arg254Gly mutations destroy the site for PKA phosphorylation, so that forskolin-induced trafficking to the plasma membrane is impaired.[207,208] The Glu258Lys mutant of AQP2 is missorted to multivesicular bodies and/or lysosomes.[209] AQP2 mutants resulting from three gene deletions, 721delG, 763–772del, and 812–818del, have similar extended C-terminal tails, which contain the basolateral-membrane-sorting dileucine (LeuLeu) motif, so these mutated proteins are wrongly translocated to the basolateral membrane.[114,210] In contrast to the AQP2 mutants associated with the recessive form of the disease, AQP2 mutants associated with the dominant form of the disease are not misfolded, so they are able to form heterotetramers with wild-type AQP2. Because of the dominance of the missorting motif in the mutant proteins, tetramers composed of the mutant and wild-type forms are missorted, which leads to severely decreased amounts of AQP2 on the apical membrane. This effect explains the dominant mode of NDI inheritance in patients with these mutations. Sohara et al.[211] generated gene knockin mice with the heterozygous mutant AQP2 resulting from a gene deletion (763–772del) that produces a mouse model of dominant NDI. The mutant AQP2 is wrongly translocated to the basolateral membrane; it forms a heterotetramer with wild-type AQP2 and shows a dominant-negative effect on the normal apical sorting of wild-type AQP2. The urine concentrating ability of these gene knockin mice is severely reduced.

Several other mouse models of NDI caused by AQP2 mutations have also been generated. Yang et al.[21] created mice with a T126M knockin mutation in the AQP2 gene. These homozygous mutant mice died within 6 days after birth, suggesting that the mice may be highly sensitive organisms with regard to water homeostasis, and are unable to survive with polyuria. Lloyd et al.[212] generated mice with a F204V mutation in the AQP2 gene that survived beyond the neonatal period and had a much milder form of NDI.

Acquired Nephrogenic Diabetes Insipidus

Acquired NDI is more common than congenital NDI and is caused by various conditions, including drug treatments, electrolyte disturbances, and urinary tract obstruction. Dysregulation of AQP2 plays a fundamental role in many cases of acquired NDI.

Lithium is widely used for treating bipolar disorder and 20% to 30% of patients treated with lithium develop NDI.[213] In lithium-induced NDI, both AQP2 expression and its trafficking to the apical membrane are inhibited. Lithium enters cells expressing AQP2 via the epithelial sodium channel (ENaC) in the apical membrane and accumulates

intracellularly. This accumulation leads to the inhibition of signaling pathways that involve glycogen synthase kinase-3β (GSK3β). Although the mechanism by which AQP2 is dysregulated in this context is not established, the involvement of GSK3β is speculated. Inhibition of GSK3β by lithium increases the expression of cyclo-oxygenase 2 and the local excretion of prostaglandin E2.[214] Prostaglandin E2 is suggested to counteract vasopressin activity by causing an endocytic retrieval of AQP2 from the plasma membrane, thus impairing the urinary concentrating ability of the cell. Furthermore, lithium increases the intracellular accumulation of β-catenin,[215] which can serve as an activator of T-cell factor-dependent transcription. AQP2 downregulation may be achieved via this transcription mechanism. In addition, AQP3 expression is also decreased. Moreover, lithium treatment caused a marked reduction in the fraction of the principal cells in the collecting duct with a parallel increase in the population of intercalated cells.[216] This restructuring of the collecting duct, together with the downregulation of collecting duct AQPs, may be important in lithium-induced NDI.

Hypokalemia and hypercalcemia cause downregulation of AQP2 expression, which results in a vasopressin-resistant urinary concentrating defect. With regard to hypercalcemia, in addition to AQP2, the expression levels of AQP1 and AQP3 are also decreased.[217] In addition, hypercalcemia reduces the expression of NKCC2 and ROMK,[218] resulting in sodium absorption defects in the thick ascending limb, which would affect the countercurrent multiplication.

Ureteral obstruction decreases AQP2 expression and impairs urine concentrating capacity.[219] In addition to AQP2, AQP1, AQP3, and AQP4 are also decreased by ureteral obstruction.[219]

A urinary concentrating defect is also observed in patients with nephrotic syndrome.[220,221] AQP2 expression is decreased in nephrotic rats.[220,222] However, changes in AQP2 expression levels have not yet been confirmed in patients with nephrotic syndrome.

Mouri et al.[223] reported that AQP2 translocation to the apical membrane is inhibited by metabolic acidosis, a mechanism that might be responsible for the diuresis in patients with chronic renal failure.

Water Retention by Urine Diluting Defects

AQP2 has a critical role in the pathophysiology of many diseases associated with water balance disorders. The best known example is congestive heart failure (CHF). Water retention and hyponatremia are common and clinically important complications of CHF. Plasma vasopressin levels are suppressed by hyponatremia in healthy individuals; however, these levels are not suppressed in patients with hyponatremia who have CHF.[224,225] In CHF, effective blood volume and arterial filling are decreased, which is sensed by aortic and carotid baroreceptors, thus resulting in stimulation of vasopressin secretion. Upregulation of AQP2 expression and increased AQP2 trafficking to the apical membrane of principal cells of the collecting duct have been shown in rat models of cardiac failure.[226,227] Furthermore, water retention and hyponatremia in these rats are reversed by a V2 receptor antagonist.[227] These findings indicate that hyponatremia is caused by nonosmotic stimulation of vasopressin, which promotes the expression and trafficking of AQP2. In patients with heart failure, V2 receptor antagonists promote electrolyte-free water excretion and elevate serum sodium concentration.[228–230] In these patients, vasopressin antagonists also have been shown to improve several symptoms of heart failure, such as dyspnea.[231]

Water retention and hyponatremia are observed in patients with hepatic cirrhosis. In these patients, the nonosmotic secretion of vasopressin occurs secondary to splanchnic arterial vasodilatation and relative arterial underfilling.[225] In cirrhotic rats, AQP2 expression was increased and correlated with the volume of ascites.[232] In patients with hyponatremic cirrhosis, V2 receptor antagonists are effective at inducing free water diuresis and raising plasma sodium levels.[229,233]

During pregnancy, arterial underfilling secondary to systemic arterial vasodilatation with nonosmotic vasopressin secretion and upregulation of AQP2 is observed.[75,234] The administration of a V2 receptor antagonist increases electrolyte-free water excretion in pregnant rats.[234]

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a condition in which plasma vasopressin levels are not appropriately suppressed despite hypoosmolality. SIADH is the predominant cause of euvolemic hyponatremia and is a commonly encountered disorder.[235] SIADH occurs frequently in association with vascular, infectious, or neoplastic abnormalities in the lung or central nervous system. In patients with SIADH, a V2 receptor antagonist is shown to be effective in increasing urine volume and plasma sodium levels.[236] However, the long-term effects of its administration are limited in rats with SIADH.[237] Although the AQP2 protein expression is reduced shortly after the administration of the V2 receptor antagonist to rats with SIADH, it increases again in parallel with the decline of the therapeutic effects.

The nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is a rare hereditary disease caused by gain of function mutations of the V2 receptor gene. Two causative mutations at the arginine residue at the position 137, Arg137Cys and Arg137Leu, have been reported.[238] In patients with this disease, endogenous vasopressin is completely suppressed, whereas antidiuresis persists due to the constitutive activation of the receptor, showing the same clinical pictures as SIADH.[238] The clinical presentation of the disease starts from childhood, but the same mutations seem to explain some sporadic episodes of euvolemic hyponatremia in adults. To differentiate NSIAD from SIADH, it may be helpful to observe the responses to V2 receptor antagonists; the former does not respond to the antagonist, whereas the latter does.[239]

The urinary AQP2 excretion level is associated with vasopressin activity in the kidney and is, therefore, a clinically useful biomarker.[240] AQP2 is excreted into the urine through the secretion of exosomes originating from the internal vesicles of

multivesicular bodies (Fig. 4.5).[241] During this process, the outer membrane of multivesicular bodies fuses with the apical plasma membrane. Urinary AQP2 excretion is increased by dehydration or vasopressin and is decreased by hydration. Urinary AQP2 excretion is also increased in patients with CHF and hepatic cirrhosis and in pregnant women.[242–244] In patients with CHF, the administration of a V2 receptor antagonist produced a significant increase in urine flow and solute-free water excretion, and was accompanied with a dramatic decrease in urinary AQP2 excretion.[242] An increased urinary excretion of AQP2 is also found in SIADH and NSIAD.[245,246] Thus, the urinary excretion of AQP2 is a useful marker of the antidiuretic activity of the collecting duct.

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CHAPTER

5 Tubular Sodium Transport

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Na^+, the primary extracellular cation, is of critical importance to the maintenance of extracellular fluid volume. The kidneys play the dominant role in regulating Na^+ excretion. Each day, the glomeruli filter roughly 25,000 mEq of Na^+. From this quantity, almost 10 times the total exchangeable Na^+ in the body, the kidneys typically absorb over 99%. A remarkable feature of the Na^+ absorptive process is the precision with which it is regulated. An individual consuming a typical diet containing 6 g of Na^+ will excrete 260 mEq of Na^+ per day. The same individual placed on a 2-g Na^+-restricted diet will promptly reduce Na^+ excretion to 87 mEq per day. Although the fraction of filtered Na^+ absorbed by the kidney changes from 99.0% on a standard diet to 99.6% on a Na^+-restricted diet, this small change represents the addition or removal of over 1 L per day to extracellular fluid volume. Thus, the kidneys absorb large amounts of filtered Na^+ with remarkably precise control.

The exquisitely sensitive regulation of Na^+ absorption by the kidneys relies on sequential actions of the various nephron segments, each with highly specialized transport capabilities. Figure 5.1 provides an overview of Na^+ transport along the nephron. In general, the absolute rates of Na^+ reabsorption are greatest in the proximal tubule and fall as the tubular fluid proceeds from proximal to distal segments. Conversely, the ability to transport Na^+ against steep tubular fluid to blood gradients and its physiologic control increase along the nephron. For example, the proximal tubule reabsorbs the bulk (60% to 70%) of the filtered Na^+ load, but as will be detailed later, does so against at most small electrochemical gradients. Moreover, the ability to alter Na^+ transport in the proximal tubule, in relative terms, is rather limited, usually varying by less than 25%. The collecting duct, in contrast, reabsorbs only a minor fraction ($\sim$2% to 4%) of the filtered Na^+ load. However, the collecting duct can transport Na^+ against a large electrochemical gradient to produce urine, which is almost Na^+ free ($<$10 mEq/L). In addition, the rate of Na^+ transport in the collecting duct can vary over a wide range (tenfold) in response to physiologic stimuli. The different nephron segments thus permit both high rates of Na^+ transport (proximal segments) and highly regulated Na^+ transport (distal segments).

Substantial progress has been made recently in identifying the proteins that mediate Na^+ transport in each nephron segment and in defining their interactions and regulation within each segment. Many Na^+ transport proteins have been linked to specific genetic disorders (Table 5.1). Given the primacy of renal Na^+ transport to the control of extracellular fluid volume, it is not surprising that the majority of these genetic disorders are characterized by either hypotension or hypertension. An updated and curated database of these genetic disorders is maintained at the Online Mendelian Inheritance in Man website (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM).

This chapter considers the transepithelial transport of Na^+ by the various nephron segments. The discussion of each nephron segment begins with a description of the general features of Na^+ transport in that segment along with pertinent structure–function relations. The mechanism of Na^+ transport is then considered on a cellular or subcellular level, with emphasis on recent electrophysiologic, biochemical, and molecular findings. Finally, each section includes a consideration of the factors that regulate Na^+ transport in the individual segments.

PRINCIPLES OF MEMBRANE TRANSPORT

This section describes physical principles that underlie the movement of ions across individual membranes and epithelia. However, it is not intended to be an extended treatment of the thermodynamic aspects of membrane transport processes.

Diffusion Processes

Solute transport across membranes may occur by diffusion or convection, or by a mediated process. Diffusion is the random Brownian motion of a molecule with respect to adjacent molecules and occurs as the consequence of thermal energy.[1] Because the diffusional movement of an

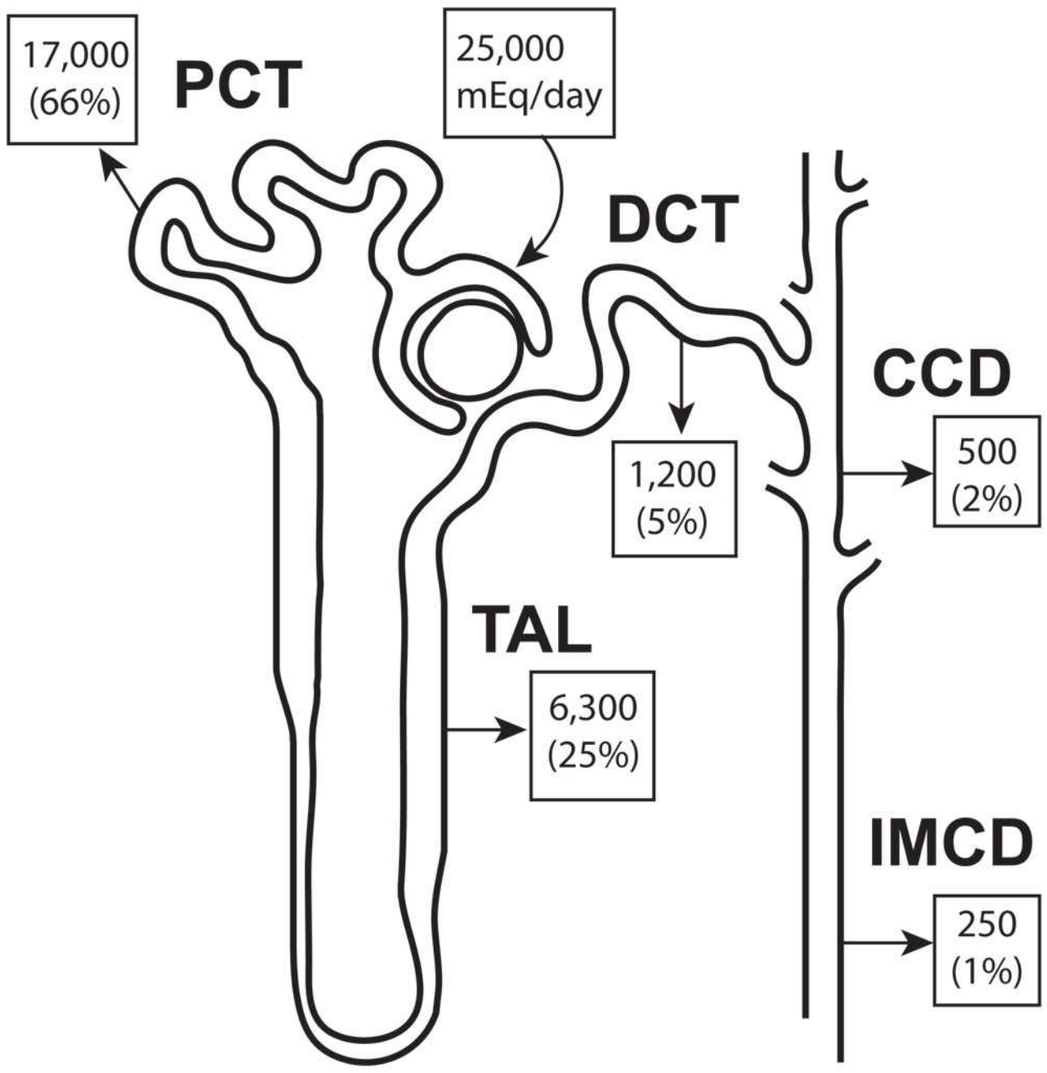


FIGURE 5.1 The contribution of various nephron segments to Na^+ transport. *PCT*, proximal convoluted tubule; *DCT*, distal convoluted tubule; *CCD*, cortical collecting duct; *TAL*, thick ascending limb; *IMCD*, inner medullary collecting duct.

individual molecule is random, a concentration gradient is required for any net transfer of molecules to occur across a membrane. Thus, the concentration gradient represents the driving force for net transport.

For charged solutes, the driving force for transport is the sum of the chemical and electrical potential gradients. The Nernst equation describes the equilibrium condition for a membrane permeable only to a single ionic species:

$$V_m = V_2 - V_1 = -(RT/ZF) \ln (C_2/C_1) \quad (1)$$

where R is the gas constant, T is the absolute temperature, Z is the valence of the solute, F is the Faraday constant, and C and V are concentration and electrical potential terms, respectively. At equilibrium, then, the voltage (V_m) across an ideally selective membrane is defined by the concentrations of the permeant ion on both sides of the membrane, C_2 and C_1, respectively. For systems containing more than one permeant ion, the equilibrium voltage can be described by the Goldman–Hodgkin–Katz (GHK) equation[2,3]:

$$V_m = -RT/F \ln [(P_{Na}C_{2Na} + P_KC_{2K} + P_{Cl}C_{1Cl})/(P_{Na}C_{1Na} + P_K C_{1K} + P_{Cl} C_{2Cl})] \quad (2)$$

where P_x is the permeability of the respective solutes, in this case, Na^+, K^+, and Cl^-. Thus, in a system containing multiple charged solutes, the transmembrane voltage is a function of the relative concentrations and permeabilities of each solute on the two sides of the membrane.

Convective Processes

Convection is the vectorial movement of an ensemble of molecules and is driven by an externally imposed force (e.g., hydrostatic pressure). Examples of convective transport include glomerular filtration and solvent drag, a process in which solute movement is coupled to water movement.

Bulk water flow may be driven by hydrostatic pressure and/or osmotic pressure. The familiar Starling equation:

$$J_v = K(\Delta P - \Delta\pi) \quad (3)$$

describes net volume flow (J_v) in response to hydrostatic (ΔP) and osmotic ($\Delta\pi$) pressure differences. The equivalence of osmotic and hydrostatic pressure is explicit in the Starling equation. The degree to which a solute exerts an osmotic pressure depends on the degree to which it permeates membranes. The ratio of the observed osmotic pressure to that predicted if a solute were excluded absolutely from a membrane is termed the reflection coefficient, σ:

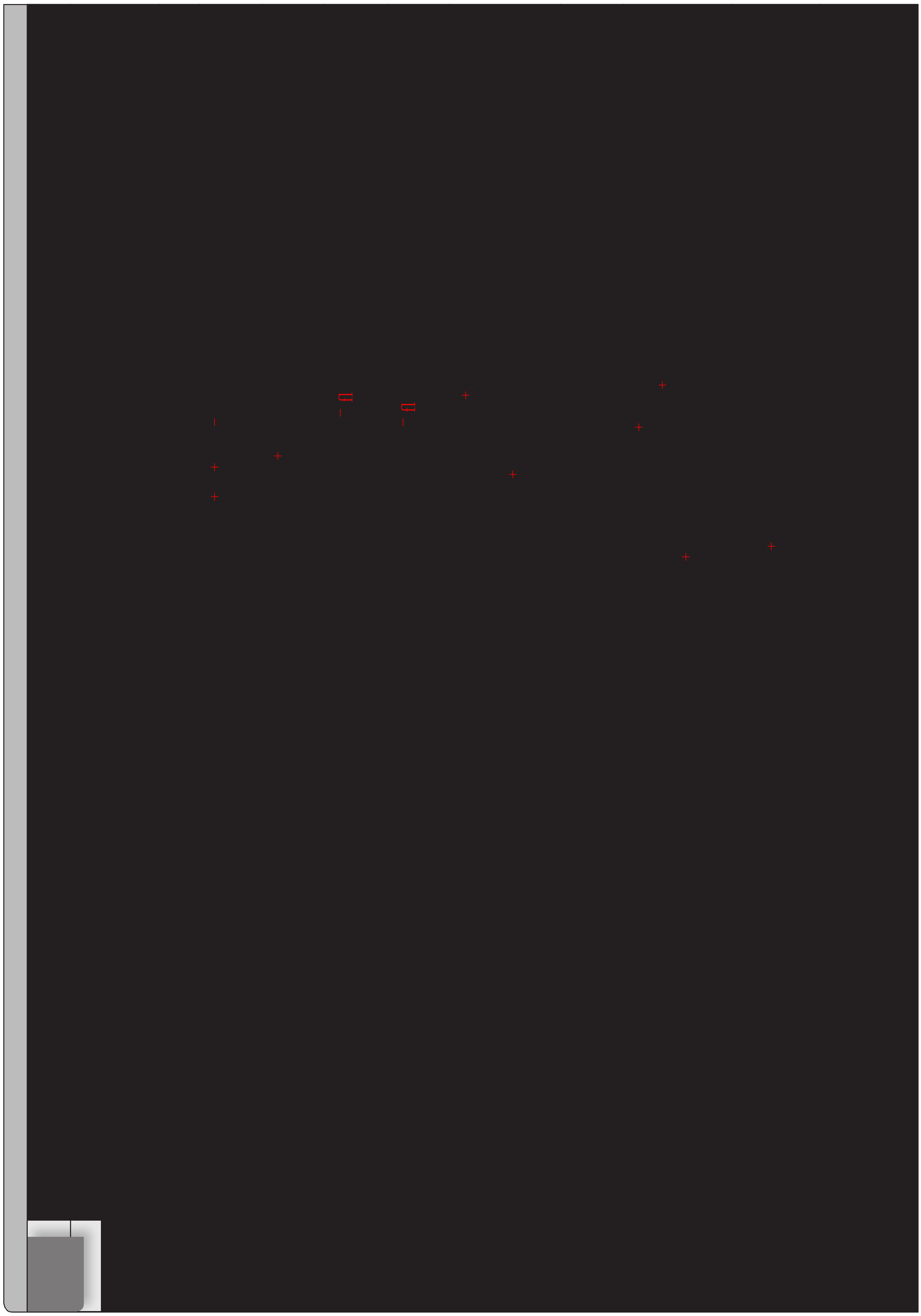
$$\sigma = \Delta\pi_{obs}/\Delta\pi_{theoretical} \quad (4)$$

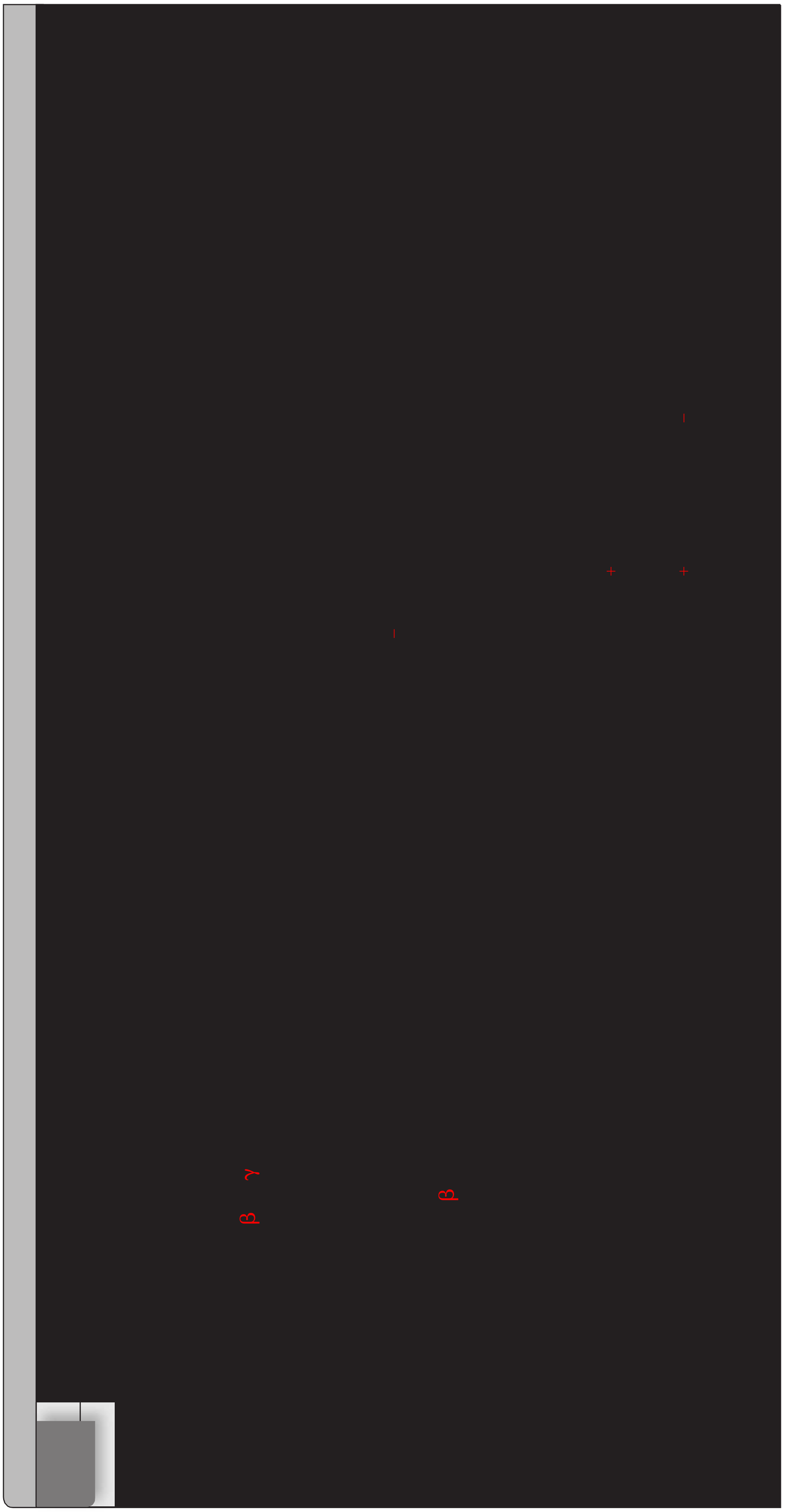
For impermeant solutes, $\sigma = 1$; for highly permeable solutes, σ approaches 0.

For solutes with $\sigma < 1$, transmembrane solute flux will be accelerated in the direction of volume flow. This acceleration is known as solvent drag.[4] Thus, the net passive flux of a permeable solute across a membrane may be driven by both diffusion and entrainment with solvent flow (i.e., solvent drag).

Facilitated Diffusion

Biologic membranes are composed primarily of lipid bilayers. Because the permeability of many hydrophilic solutes through lipid membranes is low, membranes contain proteins that facilitate the transport of certain solutes. Transport proteins, often termed carriers or transporters, have a high degree of specificity for the transported solute. Flux through the limited number of transporters saturates as the solute concentration is increased. An example of carrier-mediated facilitated diffusion is the entry of glucose into renal tubular cells mediated by the hexose transporter, GLUT-1.[5] The movement of ions through ion channels represents another form of facilitated diffusion. In this case, integral membrane proteins containing several membrane-spanning domains form pores in cell membranes through which ions permeate. Ion channels generally have a high degree of specificity for the ions being transported and very high transport rates. Facilitated diffusion mechanisms, like enzymes, serve only to accelerate the rate of transport, but do not affect the equilibrium distribution of solutes. In other words, facilitated diffusion, like simple diffusion and convection, is a passive process that tends to dissipate transmembrane gradients.

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Active Transport Processes

Active transport is a special case of facilitated transport in which chemical bond energy is supplied to the transport process so that the final distribution of the solute is remote from equilibrium. The coupling of solute transport to the energy source can take two forms. In primary active transport, solute transport is coupled directly to an energy-yielding reaction.

The most widely recognized example of primary active transport is the transport of Na^+ and K^+ by the Na^+,K^+-ATPase. This enzyme, often referred to as the sodium pump, couples the extrusion of cellular Na^+ to cellular K^+ uptake.[6] In renal tubules, this enzyme is localized to the basolateral membrane. In general, segments with high rates of active Na^+ transport have high Na^+,K^+–ATPase activity.[7] The hydrolysis of each ATP molecule ordinarily pumps three sodium ions out of the cell coupled to two potassium ions moving inward.[8] Therefore, the pump is electrogenic. The Na^+,K^+–ATPase is responsible for maintaining the cell Na^+ activity at a low level, which provides the energy for the Na^+-coupled transport of many other solutes. Thus, the inhibition of Na^+,K^+–ATPase (e.g., by peritubular ouabain addition) causes a significant rise in the cell Na^+ activity.[9] The affinity constant (K_m) of the pump for intracellular sodium, about 15 to 30 mM,[10] is similar to the intracellular sodium activity measured in proximal tubule cells.[9] Therefore, the pump is unsaturated with respect to sodium, and pump activity is very sensitive to changes in the intracellular sodium concentration activity.

In secondary active transport, solute movement against its electrochemical gradient is energized by the movement of another solute down its own gradient.[11] Na^+, because of its steep inward electrochemical gradient maintained by the sodium pump, often participates in the transport of other solutes, either in the same (cotransport or symport) or opposite (exchange or antiport) direction. Thus, by coupling solute transport with sodium movement into cells, cellular metabolic energy generated by the Na^+,K^+–ATPase is stored in the form of a Na^+ concentration gradient, analogous to a battery, and then dissipated in the transport of a variety of different solutes. Some examples of symport processes include Na^+-glucose and Na^+-amino acid cotransport; Na^+-proton and Na^+-calcium exchange are two examples of antiport processes.

PROXIMAL TUBULE

General Features

The proximal tubule is the major site for Na^+ absorption within the kidney and serves two major purposes. First, the proximal tubule protects the extracellular fluid volume by reclaiming the bulk, approximately 60% to 80%, of the glomerular filtrate.[12,13] The proximal tubule with its well-developed brush border membrane is optimally designed to perform the reabsorption of such a large fraction of the filtrate.[14] Second, the absorption of sodium in the proximal tubule provides, by way of coupled processes, the driving force for the absorption of other solutes, such as bicarbonate, glucose, phosphate, and amino acids.

Under most circumstances, fluid at any given point along the proximal tubule has virtually the same Na^+ concentration and osmolality as plasma.[13] The isosmotic nature of proximal tubule fluid absorption derives from the high water permeability of this segment,[15] which effectively clamps the osmolality of the tubular fluid at that of plasma. Although Na^+ transport in the proximal tubule occurs in the absence of large electrical or chemical gradients, the bulk of Na^+ absorption in the proximal tubule involves active transport. For example, Na^+ can be reabsorbed against both concentration[13,16] and electrical[17] gradients. In addition, fluid absorption and sodium transport cease when Na^+,K^+–ATPase activity is inhibited or when cell metabolism is slowed.[18,19]

A significant amount of proximal Na^+ transport also occurs passively.[20] For example, in the late convoluted and straight tubules (S2 and S3 segments), Na^+ diffuses passively out of the tubule driven by the lumen-positive electrical potential difference in those segments. This potential difference derives from a Cl^- concentration gradient across the tubule wall. Even in this case, however, it is the active transport of Na^+ in upstream portions of the proximal nephron that ultimately accounts for these gradients and potentials.

Nephron Heterogeneity

Analyses of proximal tubular Na^+ transport are complicated by two factors: a nonhomogeneous nephron population and axial changes in fluid composition. There is considerable heterogeneity of both morphologic and functional characteristics along the proximal tubule. The S1 segment cells have extensive basal interdigitations, numerous mitochondria, and a well-developed luminal brush border.[21] S3 segment cells, in contrast, are flatter and have fewer mitochondria, lower brush borders, and much less extensive basolateral membranes than S1 cells.[21] The Na^+,K^+–ATPase activity of the S3 segment is only 25% of that for the S1 segments.[22] As might be expected on the basis of these observations, the net rates of the Na^+ and fluid transport in the S3 straight segment are, in general, lower than in the S1 convoluted tubule.[23]

Juxtamedullary proximal convoluted tubules have higher rates of volume and bicarbonate absorption than their superficial counterparts,[23,24] although this disparity has not been noted between juxtamedullary and superficial straight segments.[25]

Glomerular ultrafiltrate in the early proximal tubule undergoes axial composition changes. Figure 5.2 illustrates that the chloride concentration rises as a consequence of the preferential absorption of $NaHCO_3^-$ over NaCl in this segment.[26] Glucose, amino acids, and other organic compounds are also absorbed avidly in association with Na^+ so that their luminal concentrations in this

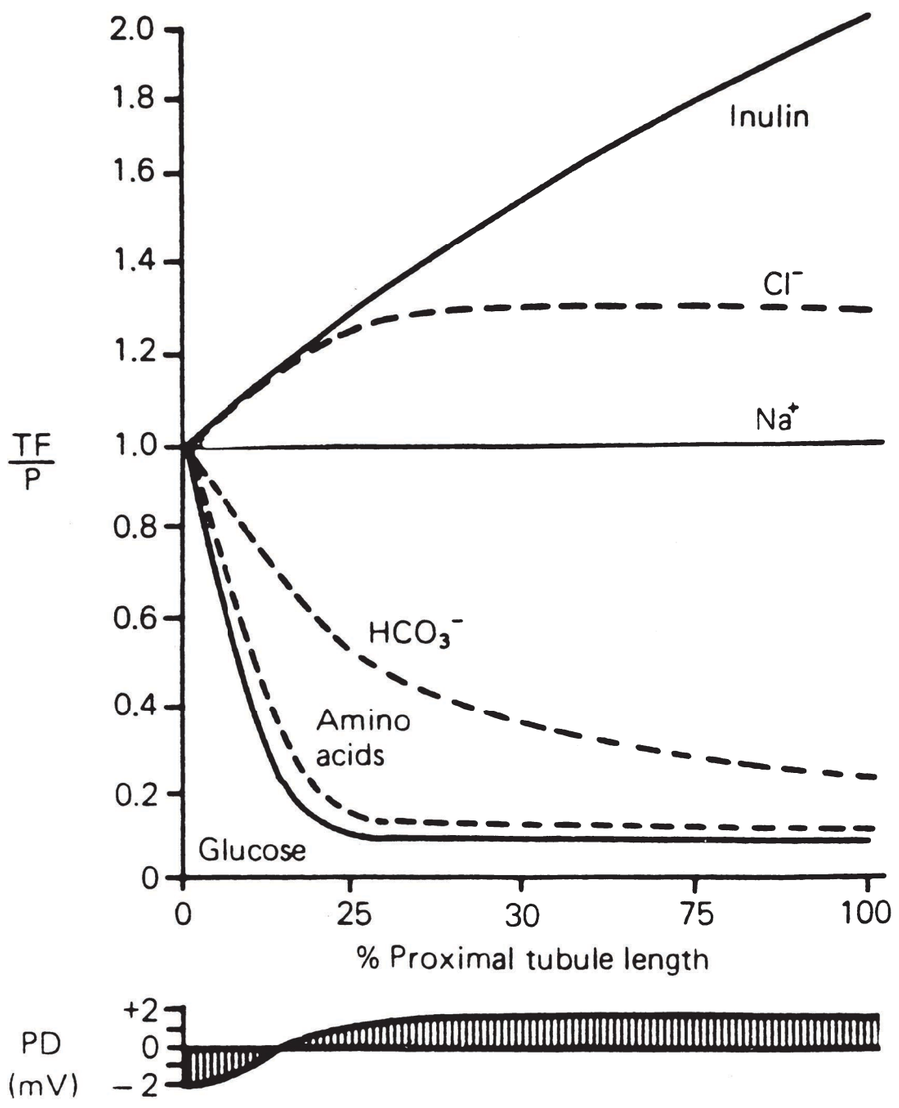

FIGURE 5.2 The profile of transepithelial voltage and solute concentrations along the mammalian proximal tubule. *TF/P*, tubular fluid/plasma concentration ratio; *PD*, potential difference. (From: Rector FC Jr. Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am J Physiol.* 1983;244:F461, with permission.)

segment approach zero.[26,27] The omission of glucose and amino acids from luminal fluids reduces both the potential difference and the volume absorptive rate.[24,28] In the S3 segment, on the other hand, the omission of glucose and alanine has no effect either on the potential difference or on the fluid absorptive rate,[25,29] although the deletion of all organic solutes does reduce volume absorption by 50%.[30] The rates of transport of glucose, amino acids, phosphate, and Na^+ in the early proximal convoluted tubule exceed those in the proximal straight tubule.[31–33] These rates correlate well with the relative basolateral membrane areas of the respective segments.[34]

Electrophysiology of the Proximal Tubule

Transepithelial Potential Difference

The electrogenic nature of the transport of Na^+ coupled to glucose and amino acids creates a lumen-negative transepithelial electrical potential difference in the early proximal convoluted tubule. The deletion of glucose and alanine from the luminal fluid reduces the potential difference from about −5.0 mV, lumen-negative, nearly to zero.[24,28] This transepithelial potential difference becomes lumen-positive (+2 to 4 mV) when the tubular fluid to plasma chloride concentration ratio is approximately 1.3.[35] This lumen-positive voltage is probably a diffusion potential arising from the Cl^- concentration gradient.

Electrical Resistance

The electrical resistance of the mammalian proximal tubule is remarkably low, making this tubule a classic example of a leaky epithelium, with resistances of 5 to 10 Ω-cm^2.[36,37] In the proximal tubule, the total cellular resistance (i.e., the sum of the apical and basolateral resistance) is 20- to 70-fold greater than the transepithelial resistance. This indicates that the paracellular resistance is low and that the predominant route for passive ion flows in the proximal tubule involves the paracellular pathway.

Ionic Selectivity

The initial convolution of the superficial proximal tubule is Na^+ selective; thereafter, the superficial convoluted and straight tubules are Cl^- selective.[29,38] In contrast, juxtamedullary proximal tubules are Na^+ selective throughout their course.[38] Because the Cl^- concentration rises as fluid flows along the convoluted tubule (Fig. 5.2), oppositely directed gradients for Cl^- and HCO_3^- in late convoluted and straight segments give rise to a lumen-positive transepithelial potential difference.[26] The latter indicates a higher permeability for Cl^- than HCO_3^-, both in superficial and juxtamedullary proximal tubules.[23,39]

MECHANISMS OF SODIUM REABSORPTION

Apical Membrane Sodium Entry

In the proximal tubule, Na^+ entry into the cell may be coupled to the movement of other solutes, such as glucose, chloride, or protons; or Na^+ may enter independently. In either case, the driving force for Na^+ entry is the steep electrochemical gradient favoring Na^+ influx.

The intracellular Na^+ activity ranges from 15 to 35 mM.[10,40] The entry of Na^+ into cells appears to be rate limiting for transepithelial Na^+ transport. Amphotericin B, a polyene antifungal, increases the permeability of the luminal membrane to Na^+ and causes a large rise in net sodium absorption.[41]

Na^+/H^+ Exchange

Directly coupled Na^+/H^+ exchange in the proximal tubular brush border is responsible for most proton secretion and for a large fraction of Na^+ reabsorption in the proximal tubule.[42] The mechanism whereby Na^+/H^+ exchange effects Na^+ reabsorption is presented in Figure 5.3. Briefly, entry of Na^+ is coupled to extrusion of a proton into the lumen. The proton titrates a filtered HCO_3^- molecule to form carbonic acid. Carbonic acid subsequently is dehydrated to CO_2 in a reaction catalyzed by carbonic anhydrase IV in the brush border membrane.[43] Within the cell, the reverse process occurs: carbonic acid formed by the hydration of CO_2 dissociates into H^+ and HCO_3^-. The H^+ is extruded into the lumen by Na^+/H^+ exchange or by the vacuolar H^+–ATPase to repeat another cycle while the HCO_3^- is transported into the blood via a $1Na^+$:$3HCO_3^-$ cotransport process (vide infra).

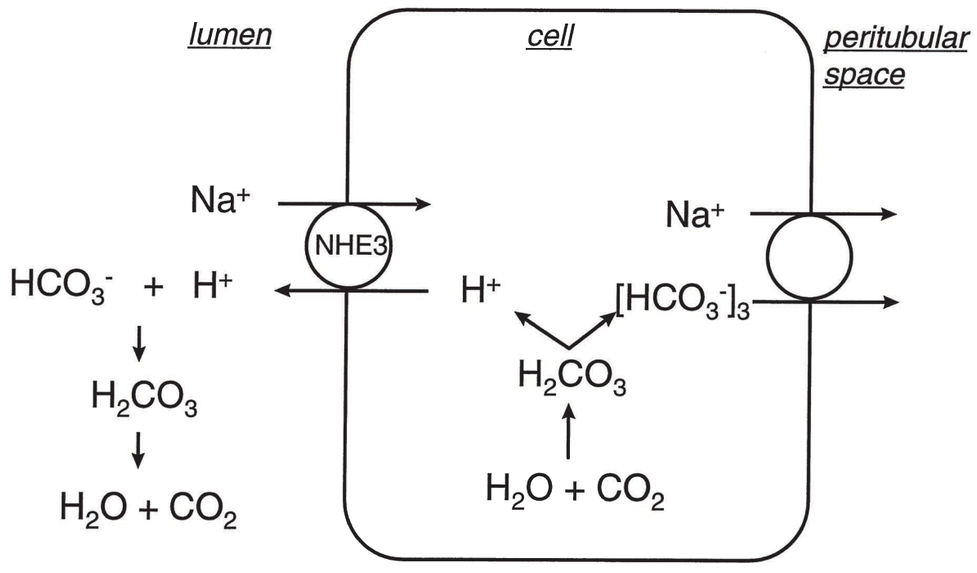

FIGURE 5.3 The scheme of $NaHCO_3$ transport mediated by Na^+/H^+ exchange. See text for explanation.

The mammalian Na^+/H^+ exchanger (NHE) is electroneutral with a stoichiometry of one proton for one sodium.[44] The exchanger is reversibly inhibited by high concentrations of amiloride.[45] Intracellular protons, via an internal activator site,[46] increase Na^+/H^+ exchange in response to intracellular acidosis.[47]

The apical and basolateral membranes of kidney cells contain different forms of NHEs with different affinities for amiloride.[48] The apical Na^+/H^+ exchanger is involved in urinary acidification and has a low amiloride affinity, whereas the basolateral exchanger has a high affinity for amiloride. The sensitivity of NHE1 to amiloride[49] and its basolateral localization[50] suggest that it represents the "housekeeping" NHE. In contrast, NHE3 is in the brush border membrane of proximal tubule cells.[51] NHE3 knockout mice have significantly reduced rates of Na^+ and HCO_3^- transport in proximal tubules.[52] In addition, pharmacologic inhibitors of NHE3 reduce proximal tubule Na^+ reabsorption by about one third.[53] However, a significant rate of amiloride-sensitive HCO_3^- transport still persists in the proximal tubules of NHE3 knockout mice,[54] indicating that the NHE3 is responsible for much, but not all, proximal tubular Na^+-coupled luminal acidification (vide infra). NHE8 is also expressed in the apical membranes of cortical tubules and may contribute to these processes.[55]

Sodium–Glucose Cotransport

Electrophysiologic studies in kidney proximal tubules show that apical membranes depolarize with addition of glucose to luminal fluids.[56] The depolarization occurs because the Na^+-glucose transporter is electrogenic. The Na^+-glucose transporter is specific for the D-stereoisomers of glucose, galactose, and α-methyl-D-glucoside.[57] The Na^+-glucose cotransporter has little affinity for cations other than Na^+.[58] Phlorizin inhibits Na^+-glucose cotransport by competing with glucose for its binding site.[59]

The rate of glucose transport by the early proximal tubule is greater than in late proximal segments.[60] In the proximal straight tubule, the K_m for D-glucose is 5 to 20 times lower than in the proximal convoluted tubule. Moreover, the transporter in the early cortical proximal tubule has a 1:1 Na^+ to glucose stoichiometry,[61] whereas the transporter in the straight medullary segment has a 2:1 stoichiometry.[62] By coupling the energy from two Na^+ ions moving down their electrochemical gradient to the transport of each glucose molecule, the medullary transporter is able to establish a much greater cellular to extracellular glucose concentration ratio than a 1:1 Na^+:glucose transporter.[27] The 2 Na^+:1 glucose transporter is, therefore, well suited to the straight segment, where tubular fluid glucose concentrations have already been reduced by glucose absorption in the more proximal segments.

A Na^+-glucose cotransporter (SGLT-1), which belongs to the SLC5 gene family,[63] mediates high affinity Na^+-glucose cotransport with a sodium-to-glucose coupling ratio of 2:1, whereas SGLT-2[64] shares 59% homology to SGLT-1 and mediates low-affinity Na^+-glucose cotransport with a sodium-to-glucose coupling ratio of 1:1.[65] In situ hybridization revealed high levels of a SGLT-2 message in the S1 segment of the proximal tubule.[66] Recently, better antibodies have confirmed that, in rat kidney, SGLT-1 immunolocalizes to the brush border membrane of all three segments of the proximal tubule.[5] By immunohistochemistry, SGLT-2 was detected at the brush border of the early proximal tubule in mice, which was absent in SGLT-2 knockout animals.[67] Thus, it appears that SGLT-2 may represent the low-affinity, high-capacity sodium–glucose cotransporter in the early proximal tubule, whereas SGLT-1 may represent the high-affinity, low-capacity transporter of the proximal straight tubule.[65]

Mutations in SGLT-2 form the basis for renal glycosuria (Table 5.1), an inherited condition characterized by a lowered threshold for tubular reabsorption of glucose.[68] In contrast, the dominant clinical manifestations of inactivating mutations of SGLT-1[69] relate to the failure to absorb sugars in the intestinal tract (glucose–galactose malabsorption). These findings suggest that SGLT-2 plays a much more significant role, quantitatively, than SGLT-1 in proximal tubule glucose reabsorption. SGLT-2 inhibitors are currently under investigation as potential therapeutic agents for the treatment of diabetes.[70]

Sodium–Amino Acid Cotransport

The proximal tubule reabsorbs amino acids from the tubular fluid via an active transport step at the luminal membrane.[31] Samarzija and Frömter,[71] using double-perfusion micropuncture techniques, observed a depolarization of the luminal membrane during amino acid transport, and they were able to identify five classes of amino acid transporters in the luminal membrane. Over the last decade, many of the transport proteins that mediate the different amino acid transport systems have been identified in kidney and intestine. This topic has been recently reviewed.[72]

Both Na^+-dependent and Na^+-independent amino acid uptake pathways have been characterized in the kidney.[72] Neutral amino acid transport appears to involve at least

three separate transport systems,[73] one that transports all neutral amino acids, one specific for imino acids, and one for the β-amino acids. Glycine may also have a specific transporter.[74] In the kidney, neutral amino acid transport is driven by a Na^+ gradient, as supported by experiments in slices, perfused tubules, and brush border membranes. The neutral amino acid transporter B^0AT1 (SLC6A19) cotransports one Na^+ per amino acid.[75] The K_m of the substrate decreases with an increasing cosubstrate concentration and vice versa. The initial step for transport involves the binding of the amino acid to B^0AT1, and this binding affinity increases under hyperpolarizing conditions.[76]

The acidic and basic amino acid groups each have their own transport systems.[77,78] At least one amino acid transporter, a Na^+-independent transporter for neutral and dibasic amino acids, has been cloned from the kidney.[79,80]

NaCl Transport

Two basic mechanisms account for NaCl reabsorption in the proximal tubule. In simple electrogenic Na^+ entry, sodium is transported actively through the cell, thereby creating a lumen-negative potential difference. Cl^- reabsorption then proceeds through the paracellular pathway driven by the lumen-negative potential difference. In electrically neutral NaCl transport, both Na^+ and Cl^- move through the cell at equal rates, such that no transepithelial potential and, hence, no driving force for paracellular Cl^- movement is generated.

Neutral NaCl Transport

Several lines of evidence indicate that a sizable fraction of proximal NaCl transport is transcellular and electroneutral.[81] First, by virtue of the coupling of Cl^- entry to apical Na^+ entry, the intracellular Cl^- activity of proximal tubule cells is greater than predicted from an equilibrium distribution.[82] Second, Cl^- absorption persists even when the driving force for passive, paracellular movement is abolished.[83] Conversely, Cl^- reabsorption is inhibited by cyanide in the absence of any change in the passive driving forces for Cl^- movement.[83] Finally, the luminal application of SITS,[84] an anion-exchange inhibitor, or removal of chloride from the tubule perfusate,[85] reduces net Na^+ reabsorption.

In principle, electroneutral NaCl transport across the apical membrane of proximal tubule cells could occur as directly coupled NaCl cotransport or as parallel Na^+/H^+ and Cl^-/base exchangers. There is no good evidence for the former process in the mammalian proximal tubule.[81] However, considerable evidence supports the view that electroneutral NaCl transport in the proximal tubule involves parallel exchangers. The coupling of Na^+ absorption to Cl^- absorption in this case occurs because of the relation between cell pH and concentration of base within the cell. With reference to Figure 5.4, the extrusion of H^+ in exchange for Na^+ results in the liberation of the base for participation in Cl^-/base exchange. The uphill entry of Cl^-, then, is indirectly coupled to the downhill entry of Na^+, because both are coupled to the transport of an acid–base pair. The model illustrated in Figure 5.4 uses formate/Cl^- exchange as the anionic component of electroneutral NaCl transport.

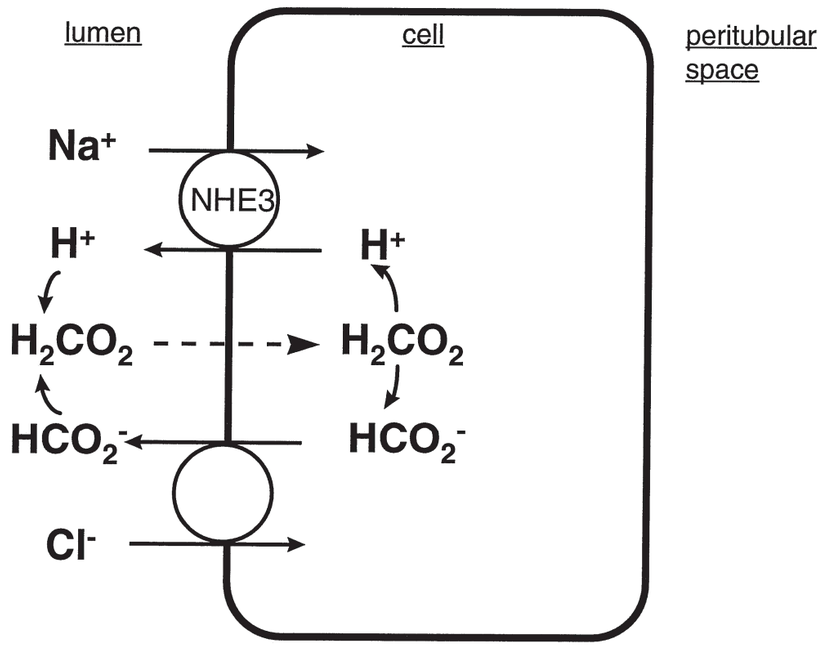

FIGURE 5.4 The scheme of neutral NaCl transport mediated by the parallel action of $Na^+ - H^+$ exchange and formate/Cl^- exchange. Formate (HCO_2^-) combines with H^+ in the tubular lumen to form formic acid (H_2CO_2), which reenters the cell by nonionic diffusion. A similar scheme applies for oxalate–Cl^- exchange.

As indicated previously, there is abundant evidence for a Na^+/H^+ exchanger in proximal tubule brush border membranes. With respect to NaCl transport, the inhibition of Na^+/H^+ exchange by high concentrations of amiloride[86] or more specific inhibitors of NHE3[53] results in a dramatic fall in transcellular NaCl transport. Likewise, knockout of NHE3 also reduces NaCl and fluid reabsorption in the proximal tubule.[52] Several Cl^-/base exchangers have been implicated in NaCl transport. Recent interest has focused on the role of Cl^-/formate (HCO_2^-) and Cl^-/oxalate ($C_2O_4^{2-}$) exchange in NaCl transport.

A Cl^-/formate exchanger is present in brush border membrane vesicles.[87] A role for Cl^-/formate exchange in neutral NaCl transport is suggested by the finding that the addition of formate to the luminal perfusate increases the rate of NaCl reabsorption in rabbit proximal tubules.[88] As depicted in Figure 5.4, formate is presumed to leave the cell in exchange for Cl^-. The secreted formate then combines with a proton, which was transported by the Na^+/H^+ exchanger to form formic acid. The formic acid then reenters the cell by nonionic diffusion and dissociates to supply substrate for the continuation of both exchange processes. CFEX (SLC26A6), a homolog of pendrin, is a protein capable of mediating Cl^-/formate exchange and is present in apical membranes of the proximal tubule.[89]

Cl^-/oxalate ($C_2O_4^{2-}$) exchange has also been demonstrated in brush border membrane vesicles.[90] It has been suggested that Cl^-/oxalate exchange may mediate neutral NaCl transport in a manner analogous to that described for Cl^-/formate exchange. It has also been suggested that NaCl absorption proceeds via the operation of three parallel transporters: the Na^+-sulfate cotransport, the sulfate/oxalate exchange, and the Cl^-/oxalate exchange.[91] Indeed, the CFEX protein, in addition to Cl^-/formate exchange, is also able to mediate Cl^-/oxalate, oxalate/formate, oxalate/oxalate, and oxalate/sulfate exchange.[92]

Other Na^+-dependent transport processes have been described in the apical membrane of the proximal tubule. However, they do not contribute significantly to Na^+ reabsorption because of the low concentrations of substrate present.

Simple Electrogenic Na^+ Entry

The classic Ussing model for salt reabsorption involves passive entry of Na^+ across apical membranes and extrusion across the basolateral membrane by Na^+,K^+–ATPase. A problem in assessing the contributions of electrogenic processes to Na^+ transport in the proximal tubule is the presence of other mechanisms of Na^+ entry. However, when the contributions of Na^+/H^+ exchange and Na^+ cotransport to net Na^+ absorption are minimized by deleting glucose, amino acids, and bicarbonate from the perfusate, a fraction of fluid absorption in isolated perfused straight segments persists and the transepithelial potential is −1.0 mV.[30] These results indicate that in the proximal straight tubule simple electrogenic Na^+ transport constitutes a mechanism for Na^+ absorption. A conductive Na^+ pathway has been demonstrated in brush border membrane vesicles.[93] Unlike the Na^+ channel found in the distal nephron segments, the Na^+ channel in the proximal tubule is not blocked by amiloride.[93]

In the proximal convoluted tubule, however, the deletion of glucose, bicarbonate, and amino acids completely abolishes fluid absorption.[23] Consequently, simple electrogenic proximal Na^+ transport may be limited to straight segments.

Passive NaCl Absorption

The rise in tubular fluid Cl^- concentration, and the attendant lumen-positive voltage (Fig. 5.2), provides a mechanism for passive NaCl absorption in late regions of the proximal nephron. In the Cl-selective superficial pars recta, approximately one-third of net NaCl absorption can be accounted for by this mechanism.[20]

Basolateral Membrane

The proximal tubule, particularly the S1 segment, possesses high Na^+,K^+–ATPase activity in the basolateral membrane. The Na^+,K^+–ATPase pumps Na^+, which entered cells apically, across basolateral membranes. In other words, the pump keeps the cell Na^+ activity low and maintains the electrochemical gradient for Na^+ entry across the apical membrane. Consequently, the inhibition of Na^+,K^+–ATPase activity with ouabain decreases transepithelial Na^+ reabsorption and increases the intracellular Na^+ activity in the proximal tubule.[94]

Na^+ also exits across the basolateral membrane in concert with HCO_3^-. Studies in intact tubules and in membrane vesicles have demonstrated an electrogenic, stilbene-sensitive Na^+-HCO_3^- cotransporter in the basolateral membrane of rat and rabbit proximal tubules.[95,96] The cotransporter transfers two net negative charges across the basolateral membrane. The stoichiometry of this process is 1 Na^+: 1 HCO_3^-: 1 CO_3^{2-} (or SO_3^{2-}).[97] Thus, this transport moiety for Na^+ extrusion, $[Na^+(HCO_3^-)_3]^{2-}$ is electronegative, with the lumen-negative cell interior providing a major driving for Na^+ extrusion. A number of Na^+-HCO_3^- cotransporters have been cloned, along with different splice variants.[98] One of these, NBC1 (encoded by SLC4A4 and subsequently renamed NBCe1-A) is localized to the basolateral membrane of the S1 and S2 segments of the proximal tubule.[99] As illustrated in Figure 5.3, the net result of these steps is the reabsorption of Na^+ and HCO_3^-, accounting for the bulk of HCO_3^- reabsorption and about 20% of Na^+ reabsorption in the proximal tubule. It is believed that this cotransport process accounts for the basolateral transport of most of the bicarbonate reclaimed from the luminal fluid.[97] Mutations in NBCe1-A cause proximal (type II) renal tubular acidosis and other defects in the eye, teeth, and mental development (Table 5.1). The regulation and role of NBC in acid–base transport in the proximal tubule is an area of active research that has been reviewed recently.[100]

The pathways for Cl^- exit across the basolateral membrane are less well defined. Several pathways for Cl^- exit across the basolateral membrane have been proposed: conductive Cl^- channels, KCl cotransport, and $Na^+(HCO_3^-)_2/Cl^-$ exchange (Fig. 5.5). Studies of rat proximal convoluted tubules[101] and rabbit convoluted and straight proximal tubule segments[102,103] using intracellular microelectrodes have indicated that the proximal tubule cell has a very low Cl^- conductance. Thus, in normal proximal convoluted tubule and proximal straight tubule segments, conductive Cl^- efflux across basolateral membranes appears to play a minor role in NaCl absorption. Under hypotonic conditions, however, cell swelling dramatically increases the basolateral membrane Cl^- conductance.[104]

Because the chemical gradient for K^+ to leave cells exceeds that for Cl^- entry, KCl cotransport can mediate basolateral Cl^- exit from proximal tubule cells. Ion-selective microelectrode studies have demonstrated KCl cotransport in basolateral membranes of rabbit proximal tubule cells.[103,105]

Stilbene-sensitive, Na^+-dependent Cl^-/HCO_3^- exchange has been demonstrated in rat[106] and rabbit[107,108] proximal tubules. In this case, the entry of 1 Na^+ and 2 HCO_3^- across the basolateral membrane is coupled to the efflux of Cl^-. The Na^+

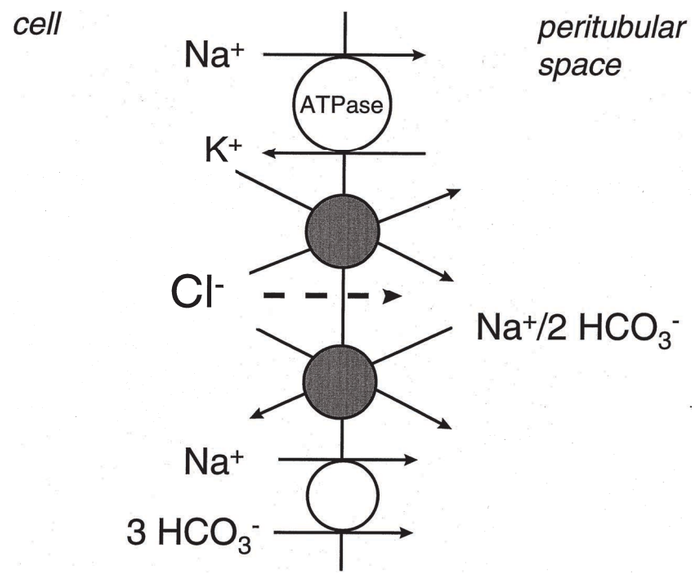

FIGURE 5.5 The transport pathways for Na^+ and Cl^- absorption across the basolateral membrane of proximal tubular cells. Cl^- can leave the cell via KCl cotransport, Na^+–$2HCO_3^-/Cl^-$ exchange, and Cl^- channels (minor). Na^+ exits via the Na^+,K^+–ATPase and Na^+–$3(HCO_3^-)$ cotransport.

and HCO_3^- that enter the cell are thought to be recycled through the $[Na^+(HCO^-_3)_3]^{2-}$ cotransporter (see previous). Indeed, $Na^+(HCO^-_3)_2/Cl^-$ exchange may account for much more Cl^- movement than KCl transport.[108] Na^+-independent Cl^-/HCO_3^- exchange is also present in the basement membrane of proximal tubules.[106–108] However, under physiologic conditions, this process mediates net Cl^- influx and does not contribute to net NaCl absorption.

CONTROL OF PROXIMAL TUBULAR SODIUM REABSORPTION

Glomerulotubular Balance (GTB)

The proximal tubule responds to an increase in glomerular filtration with an increase in the absolute rate of fluid absorption (APR) to minimize variations in the fractional proximal fluid absorption. This phenomenon is termed glomerulotubular balance (GTB). The efficiency of GTB—that is, the extent to which APR/GFR remains constant—is subject to physiologic and pathologic control. The prime factor modulating GTB in vivo is the effective circulating volume. Thus, at a constant or near constant GFR, volume expansion and volume contraction decrease and increase, respectively, the absolute rate of proximal Na^+ absorption. In other words, volume expansion and volume contraction reset GTB upward and downward, respectively.[109,110] This section considers some of the factors that modulate proximal Na^+ absorption.

Peritubular capillary oncotic pressure is one of the factors regulating the rate of salt and water absorption from the proximal tubule.[111] The oncotic pressure of the peritubular proteins favors the movement of fluid across the basement membrane, whereas capillary hydrostatic pressure retards this movement. Thus, at a given renal blood flow, an increase in the glomerular filtration rate and, hence, the filtration fraction, will cause an increase in the oncotic pressure in the postglomerular peritubular capillaries. At constant single nephron glomerular filtration rates (SNGFR), the perfusion of efferent capillaries with hypo-oncotic fluids decreases the absolute rate of proximal fluid absorption, whereas perfusion of the capillaries with hyperoncotic fluids increases proximal absorption.[112] The effects of the peritubular protein concentration can also be demonstrated in isolated perfused tubules.[113]

It is not precisely clear how the peritubular protein concentration modulates proximal fluid absorption. The effect is not simply because of the oncotic pressure exerted by the proteins, because changes in absorption do not occur when active transport is inhibited or from comparable changes in the transtubular hydrostatic pressure.[114] The prevailing views are that the peritubular protein may directly affect transcellular Na^+ transport[115] or the back leak of Na^+ through the paracellular pathway.[113,116]

Luminal factors also contribute to glomerulotubular balance. The flow dependence of proximal absorption has been investigated in the convoluted[15] and straight segments[30] of isolated, perfused rabbit nephrons. The key observations are (1) the volume absorptive rate is clearly dependent on the perfusion rate, (2) the flow dependence persists in the absence of active transport when anion gradients are present, and (3) the flow dependence is abolished in the absence of active transport and anion gradients.

An explanation for these results lies in a consideration of axial versus radial changes in fluid composition along the tubule.[117] The rate of passive Na^+ absorption in the late proximal tubule is dependent on the magnitude of the chloride gradient between the lumen and the bath. At low axial perfusion rates, the radial Cl^- gradient tends to dissipate as a function of distance along the tubule, but this dissipation is minimized at higher axial perfusion rates. Hence, the integrated driving force for passive NaCl absorption increases with the rate of tubule perfusion. In addition, the availability of solutes, such as glucose, amino acids, and bicarbonate, is also partly responsible for the flow dependence of proximal fluid absorption.[118]

The flow dependence of reabsorption in the proximal tubule has also been tested in the mouse proximal tubule. In this experimental model, which included the use of the NHE3 knockout mouse, the data supported the hypothesis that the "brush border" microvilli act as mechanosensors that transmit fluid dynamic torque to the actin cytoskeleton and thus modulate Na^+ absorption.[119]

Catecholamines

Renal denervation reduces proximal tubule Na^+ and fluid absorption.[120] Both α- and β-adrenergic receptors exist in the proximal tubule.[121,122] The rate of salt and water reabsorption in the proximal tubule is stimulated by α- and β-adrenergic agonists.[123] α-Adrenergic agonists increase apical Na^+ entry via the stimulation of Na^+/H^+ exchange[122] and also increase basolateral Na^+ efflux via Na^+,K^+–ATPase activity in rat proximal tubules by a pathway that involves the activation of calcineurin.[124] The effects of β-adrenergic agonists on Na^+,K^+–ATPase activity are less clear, with one study showing that they increase Na^+,K^+–ATPase activity via protein kinase C (PKC).[125] However, others have found that β-adrenergic agonists inhibit Na^+,K^+–ATPase activity via PKA.[126]

Dopamine, which is produced by proximal tubular cells,[127] inhibits Na^+ reabsorption. Dopamine inhibits Na^+,K^+–ATPase activity via its receptors DA-1 and DA-2.[128]

Parathyroid Hormone

Parathyroid hormone (PTH) causes a 30% to 50% reduction in proximal tubular Na^+ and phosphate absorption.[129] PTH stimulates adenylyl cyclase, cAMP production, and PKA, which in turn inhibits Na^+/H^+ exchange in several proximal tubule systems.[130] Weinman et al.[131] demonstrated that phosphorylation by PKA inhibits Na^+/H^+ exchange activity via a PDZ domain-dependent interaction with the NHE regulatory factor (NHERF). In the absence of NHERF, cAMP

does not inhibit the exchange activity of NHE3.[132] The current model for this inhibition is that NHE3 associates with PKA indirectly via NHERF and the cytoskeletal protein ezrin. PKA, when active, phosphorylates NHE3 at serines 552 and 605, which mediates the inhibition of the exchanger.[133] NHE3 is directly phosphorylated by other protein kinases, including calmodulin-dependent protein kinase II, which inhibits Na^+/H^+ exchange activity and PKC, which stimulates the exchanger.[134]

Angiotensin II

The systemic administration of low doses of angiotensin II (Ang II) inhibits the excretion of Na^+,[135] whereas inhibitors of Ang II increase Na^+ excretion.[136] Systemic Ang II causes changes in renal blood flow, aldosterone secretion, filtration fraction, and catecholamine release from renal sympathetic nerve endings.[137] Low concentrations of Ang II ($<10^{-9}$ M) cause an increase in proximal tubule fluid and bicarbonate reabsorption, effects that are mediated by the AT1 subtype of Ang II receptors present in both the brush border and basolateral membranes of the proximal tubule.[138] Higher concentrations ($>10^{-8}$ M) depress fluid and bicarbonate absorption, presumably via counterbalancing effects mediated by the lower affinity AT2 receptor.[138] Studies have demonstrated that the stimulatory effect of Ang II on fluid and bicarbonate reabsorption occurs via enhanced apical Na^+/H^+ exchange via NHE3 and basolateral Na^+-$(HCO_3^-)_3$ cotransport via NBC-1 in the proximal tubule.[139] The physiologic effects of Ang II may involve the coupling of these receptors to both phospholipase A_2 and inhibitory G proteins.[140,141]

Thyroid Hormone

On a clinical level, hypothyroidism is associated with a decreased cardiac output, renal blood flow, and glomerular filtration rate (GFR). Clearance studies in hypothyroid rats have documented decreases in GFR, renal Na^+ reabsorption, and renal Na^+,K^+–ATPase activity.[142] These changes are reversible after thyroid hormone replacement.[143] The thyroid hormone may exert direct effects to stimulate proximal tubular salt and fluid reabsorption via increased basolateral K^+ permeability[144] and/or direct stimulation of Na^+/H^+ exchange through an increase in NHE3 transcription.[145]

Corticosteroids

Although mineralocorticoids do not have an effect on proximal tubular sodium reabsorption,[146] there is evidence for glucocorticoid receptors in the proximal tubule.[147] Dexamethasone inhibits apical membrane Na^+-phosphate cotransport in cultured proximal tubular cells via PKC activation.[148] Dexamethasone also enhances the activity of apical NHE3 and the mRNA expression and functional activity of basolateral NBC-1.[149] The resulting increase in proximal tubule HCO_3^- reabsorption could contribute to the maintenance of the metabolic alkalosis that is associated with increased glucocorticoid production in vivo.

Nitric Oxide

Various forms of nitric oxide synthase (NOS) are expressed in the proximal tubule.[150] Low basal production of nitric oxide (NO) by the proximal tubule is boosted dramatically by lipopolysaccharide (LPS) and cytokines. Even under basal conditions, the proximal tubule may be affected by NO produced by adjacent cells, such as endothelium or other nephron segments. The overall effect of NO on proximal tubule Na^+ transport is controversial and may be biphasic, with acute inhibition and chronic stimulation of Na^+ reabsorption as assessed by pharmacologic agents and genetic knockouts of NOS, respectively.[151] In vitro, NO decreased Na^+/H^+ exchange and Na^+,K^+–ATPase activity in cultured proximal tubule cells.[152]

MECHANISM OF ISOTONIC FLUID ABSORPTION

Proximal tubule water absorption is coupled tightly to solute absorption, because the measured osmolality of the tubular fluid is generally identical to plasma. Three general mechanisms of solute–solvent coupling have been suggested to account for this isotonic absorption: lateral interspace hypertonicity, effective osmotic gradients because of different reflection coefficients for solutes in the tubular and peritubular fluids, and luminal fluid hypotonicity.

The standing gradient theory argues that active transport of salt into the lateral intercellular space raises the osmolality of the space, thus providing an osmotic gradient for fluid transport from the lumen to the interspace.[153] The tight junctions in this model are presumed to be impermeable to water, so that the osmotic flow of water from the cell into the hypertonic interspace raises the hydrostatic pressure in this compartment and forces fluid across the basement membrane.

An alternative explanation[117] proposes that an effective osmotic driving force for fluid absorption can exist between solutions of identical osmolalities if the reflection coefficients of the membrane for the solutes in the solutions differ. Specifically, the elevated tubular fluid-to-plasma Cl^- concentration found in the late proximal convolution and in the pars recta provides an effective osmotic driving force for fluid absorption because σHCO_3^- exceeds σCl^-. That is, the bicarbonate in the peritubular fluid is a more "effective" osmotic agent than is the chloride in the tubular fluid, and, thus, net water flows out of the tubule. Although this mechanism may be applicable to the proximal straight tubule, the reflection coefficients for NaCl and $NaHCO_3$ measured across the rabbit proximal convoluted tubule are virtually identical,[154] so oppositely directed Cl^- and HCO_3^- gradients may make only a negligible contribution to fluid absorption in convoluted segments.

Finally, because of the high osmotic water permeability of the proximal tubule, only small degrees of absolute luminal hypotonicity are needed to provide a sufficient driving

force to account for the observed rates of fluid reabsorption.[20] Experimental evidence supports the view that absolute luminal hypotonicity is a significant driving force for fluid reabsorption in the proximal tubule. Thus, when proximal tubules are perfused and bathed by symmetric NaCl solutions, the luminal fluid becomes slightly hypotonic.[155] The development of luminal hypotonicity can be amplified by maneuvers that decrease the water permeability of the proximal tubule. The aquaporin 1 (AQP1) water channel is abundantly expressed in the proximal tubule. In AQP1 knockout mice, the osmolality of tubular fluid at the end of the proximal tubule is significantly lower than in normal mice.[156] As the luminal fluid becomes more hypotonic, the resorbate becomes more hypertonic, and the degree of resorbate hypertonicity correlates with the rate of volume reabsorption by the tubules.[157]

THE LOOP OF HENLE

The dissociation of salt and water absorption by the loop of Henle is ultimately responsible for the capacity of the kidney, either to concentrate or to dilute the urine. The active absorption of NaCl in the water-impermeable thick ascending limb of Henle (TALH) serves both to dilute the urine and to supply the energy for the "single effect" of countercurrent multiplication. A functionally similar segment, known as the diluting segment, is found in amphibians and teleosts.[158]

The mammalian loop of Henle contains the descending thin limb (DTL), the ascending thin limb (ATL), and the thick ascending limb (TAL). The loop of Henle absorbs about 25% to 40% of the filtered Na^+ load.[159] Furthermore, the fluid leaving the loop is dilute, indicating that more NaCl is absorbed in the loop than water.

SALT TRANSPORT BY THE THIN DESCENDING AND THIN ASCENDING SEGMENTS

As the tubular fluid enters the descending thin limb and flows toward the tip of the renal papilla, it becomes more concentrated.[160] Passive models for urinary concentration indicate that this increase in osmotic pressure is attributable to water extraction rather than solute entry.[161] Current observations indicate that the aquaporin water channel AQP1 mediates water movement across the luminal surface of the DTL.[162] According to some reports, however, AQP1 expression is significantly lower in short loop nephrons than long loop nephrons,[163] suggesting that (1) not all DTLs in the kidney extract water to the same degree, and/or (2) water movement in short loop DTLs is facilitated by alternative water channels or via the paracellular route. In contrast to the DTL, in vitro microperfusion studies demonstrate that the ATL is relatively impermeable to water.[164]

The study of NaCl transport by the DTL and ATL has been complicated by the fact that the transport characteristics of these two nephron segments exhibit significant interspecies heterogeneity.[165] Although the Na^+ and Cl^- permeabilities appear to vary widely among rabbits, hamsters, and rats, one consistent finding was that the DTL is relatively less permeable to NaCl than the ATL. Coupled with the DTL's high permeability to water, the relative lack of solute permeability ensures that the osmotic pressure of fluid entering the renal papilla is greater than that of the fluid leaving it.[166] Thus, the formation of dilute urine by the loop of Henle begins in the ATL.

The decrease in osmolality in the ATL is due primarily to a fall in the NaCl content of the luminal fluid. The electroneutral transport of NaCl appears to occur through two key mechanisms. The transepithelial movement of sodium from the ATL lumen occurs via the paracellular pathway,[167] whereas Cl^- diffusion occurs through a transcellular route. Yoshitomi et al.[168] detected conductive pathways for Cl^- in both the apical and basolateral membranes of ATL cells. The transcellular movement of Cl^- in the ATL is regulated, because the basolateral Cl^- conductance is inhibited at low pH[168] and by low intracellular Ca^{2+} concentrations.[169] Uchida et al.[170] cloned a Cl^- channel in 1993 from the rat renal medulla, ClC-K1 (termed ClC-Ka in humans), which represents the major mediator of transcellular Cl^- movement in the thin ascending limb. This channel, which belongs to the ClC family of Cl^- channels, is expressed exclusively within the kidney and has been localized by immunohistochemistry to both the apical and basolateral membranes of the thin ascending limb of Henle.[171] Its activity is dependent on the coexpression of barttin, an accessory protein that forms a complex with ClC-K1, increases its abundance at plasma membranes, and modifies channel gating.[172–175] The expression of ClC-K1 is increased by dehydration.[170] Genetic knockout of the ClC-K1 gene in mice produced a urinary-concentrating defect, confirming the role of passive NaCl transport in the thin ascending limb in the urinary concentrating mechanism.[176]

NaCl ABSORPTION IN THE THICK ASCENDING LIMB

General Features

The studies of Rocha and Kokko[177] and Burg and Green[178] were the first to investigate salt absorption in the thick ascending limb, and their work defined several key features of this unique epithelium. First, salt absorption in the medullary and cortical TAL generates a lumen-positive transepithelial voltage, which is sensitive to furosemide. Second, the transport of Cl^- occurs against both electrical and chemical gradients and involves an active transport process that is dependent on intact basolateral Na^+,K^+–ATPase activity.[179] A final important feature of the TAL is that this segment consists of a tight epithelium, which despite its high ionic conductance, possesses a very low permeability to water. The apical membrane of the TAL constitutes the major barrier to transcellular and paracellular water flow.[180] The high

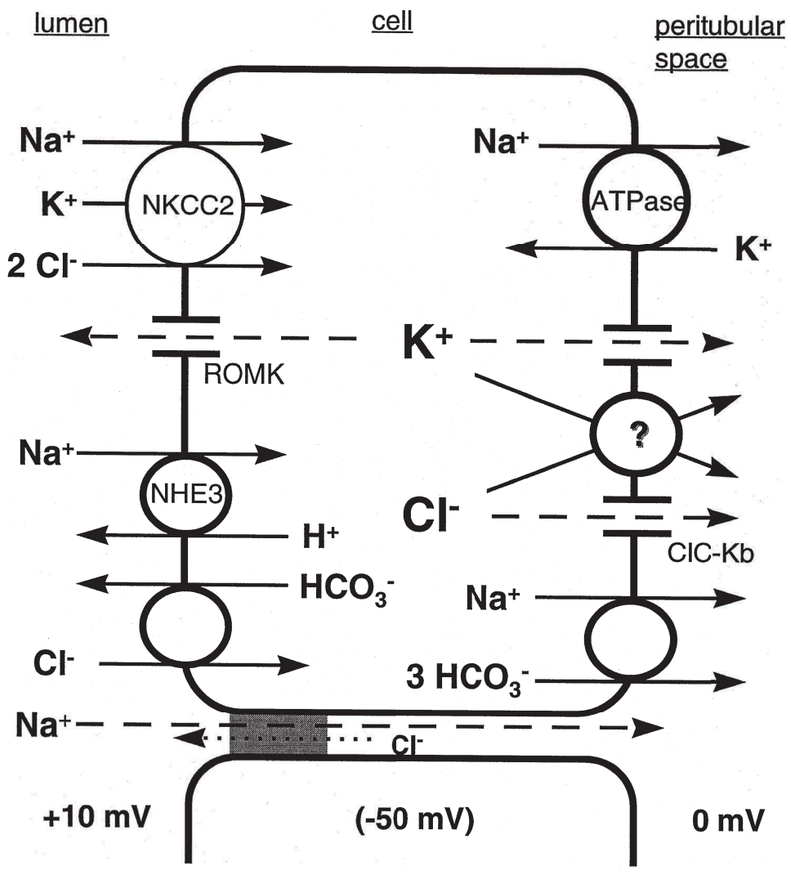

FIGURE 5.6 A model depicting the major elements of the mechanism of NaCl absorption by the thick ascending limb. *Dashed lines* indicate passive ion movements down electrochemical gradients. *ROMK*, renal outer medullary K^+ channel; *ClC-Kb*, chloride channel Kb.

ionic conductance and low water permeability effectively further dilutes fluid entering the TAL from the ascending thin limb.

Figure 5.6 integrates the results of several electrophysiologic and biochemical studies to provide a model of salt reabsorption in the thick ascending limb. According to this model, net Cl^- absorption by the TAL is a secondary active transport process. Luminal Cl^- entry into the cell is mediated by an electroneutral Na^+-K^+-$2Cl^-$ cotransport process driven predominantly by the favorable electrochemical gradient for Na^+ entry.[181] Because the Na^+ gradient is maintained by the continuous operation of the basolateral membrane Na^+,K^+–ATPase pump, the apical entry of Cl^- via the cotransporter ultimately depends on the operation of the basolateral Na^+,K^+–ATPase.

In contrast to the electroneutral entry of Cl^- across the apical membrane, the majority of Cl^- efflux across the basolateral membrane proceeds through conductive pathways.[182,183] A favorable electrochemical gradient for Cl^- efflux through dissipative pathways has been demonstrated by Greger et al.[184] in the rabbit cTAL. Intracellular Cl^- is maintained at concentrations above electrochemical equilibrium by the continued entry of Cl^- via the apical Na^+-K^+-$2Cl^-$ cotransporter.[185]

According to the model in Figure 5.6, K^+ that enters TAL cells via the Na^+-K^+-$2Cl^-$ cotransporter recycles, to a large extent, across the apical membrane via a K^+-conductive pathway. This apical K^+ recycling serves several purposes. First, it ensures a continued supply of luminal K^+ to sustain Na^+-K^+-$2Cl^-$ cotransport. Without recycling, the luminal K^+ concentration would fall rapidly as a consequence of K^+ entry via Na^+-K^+-$2Cl^-$ cotransport and would limit net NaCl absorption. Second, the apical membrane K^+ current provides a pathway for net K^+ secretion by the TAL. In mouse TAL, for example, the rate of K^+ secretion amounts to about 10% of the rate of net Cl^- absorption.[182] K^+ secretion in this segment is an active process, ultimately driven by the Na^+,K^+–ATPase, proceeding in the face of a lumen-positive transepithelial potential. Third, under open circuit conditions, the transcellular and paracellular pathways form a current loop in which the currents traversing the two pathways are of equal size, but which traverse in the opposite direction. The potassium current from cell to lumen polarizes the lumen and causes an equivalent current to flow from the lumen to the bath through the paracellular pathway.[186] Because the paracellular pathway is cation selective ($P_{Na}/P_{Cl} = 2$ to 6), the majority of the current through the paracellular pathway is carried by Na^+ moving from the lumen to the interstitium. This paracellular absorption of Na^+ increases the efficiency of Na^+ transport by the TAL.[187] With reference to Figure 5.6, for each Na^+ transported through the cell and requiring the use of ATP, one Na^+ is transported through the paracellular pathway without any additional energy expenditure. Finally, the apical K^+ current satisfies the continuity requirement imposed by a high degree of conductive Cl^- efflux across basolateral membranes.[182]

A small component of Na^+ transport by the TAL is accounted for by $NaHCO_3$ absorption.[188] In the rat TAL, the rate of $NaHCO_3$ absorption is roughly 5% to 10% of that for NaCl absorption. $NaHCO_3$ absorption appears to be mediated by an apical membrane amiloride-sensitive Na^+/H^+ exchanger and a basolateral membrane electrogenic Na^+-$3(HCO_3^-)$ cotransporter.[188]

The following sections will describe the individual components of the mechanism for TAL salt transport (Fig. 5.6) in greater detail.

Apical Na^+-K^+-$2Cl^-$ Cotransport

Studies of Cl^- transport across apical membranes of intact TAL segments[189] and in isolated membrane vesicle preparations[190] established that the predominant mode for Cl^- entry into the TAL cell is via a Na^+-K^+-$2Cl^-$ cotransporter. A characteristic feature of this transporter is its sensitivity to inhibition by furosemide, bumetanide, and other 5-sulfamoylbenzoic acid derivatives.[191] The measurement of isotope flux into TAL cells or membrane vesicles prepared from the inner stripe of the outer medulla yielded a stoichiometry of 1 Na^+:1 K^+:2 Cl^- cotransport.[190] K^+-independent NaCl cotransport has also been described under certain conditions.[192]

The proteins that mediate the Na^+-K^+-$2Cl^-$ cotransport have been cloned. An absorptive form of the Na^+-K^+-$2Cl^-$ cotransporter, referred to as NKCC2 or BSC1, was initially cloned by Gamba et al.[193] based on sequence homology to the thiazide-sensitive Na^+-Cl^- cotransporter (see the following). A second Na^+-K^+-$2Cl^-$ cotransporter, NKCC1, was cloned by Payne et al.[194] NKCC2 (BSC1) is the primary mediator of apical salt entry in the thick ascending limb. In situ hybridization and single-nephron reverse transcriptase polymerase

chain reaction (RT-PCR) studies demonstrated the expression of NKCC2 in the MTAL and CTAL,[195] and immunohistochemical studies indicate that NKCC2 is localized to the apical membrane of these nephron segments.[196] The importance of NKCC2 in mediating salt reabsorption in the TAL is illustrated by the fact that loss-of-function mutations of NKCC2 cause Bartter syndrome (Table 5.1),[197] a Mendelian salt-wasting disorder characterized by hypokalemia, metabolic alkalosis, hyperaldosteronism, and normal-to-low blood pressure, results from a defect in salt absorption by the thick ascending limb.

The NKCC2 cDNA encodes a glycoprotein containing ~1100 amino acids and having a predicted molecular weight of 115 to 120 kDa.[193] The full-length protein contains 12 transmembrane domains containing a sizable extracellular loop with N-glycosylation sites positioned between transmembrane segments 7 and 8, and large intracellular amino and carboxy termini flank the transmembrane regions. NKCC2 belongs to the SLC12A family of cation chloride cotransporters, which is part of the amino acid polyamine organocation cotransporter (APC) superfamily.[198] Based on the homology to other crystallized APC family members, the cotransporter structure probably consists of two clustered groups of five transmembrane helices that are positioned in a symmetric, inverted orientation.[199] The details regarding how this fold facilitates the three-ion cotransport remain obscure but surely will provide an initial framework for more detailed structure-function studies in the coming years.

The two cytoplasmic domains of NKCC2 mediate specific regulatory functions. The amino terminus is believed to be unstructured and contains several cytosol-exposed serines and threonines, which are phosphoacceptor sites. These residues are phosphorylated by at least three protein kinases that stimulate NKCC2 activity and/or plasma membrane expression (discussed in detail below).[200] The carboxy terminus is large and comprises ~40% of the total NKCC2 sequence. It may also contain phosphorylation sites, although to date this has not been explored in detail. It is clear, however, that the NKCC2 C-terminus serves as a hub for interactions with proteins that regulate its trafficking, including the glycolytic enzyme aldolase[201] and secretory membrane carrier protein 2 (SCAMP2).[202] It also serves as the interface for the formation of NKCC2 homodimers,[203] which was confirmed recently when the crystal structure of the C-terminus of the related prokaryotic cation chloride cotransporter MaCCC was solved.[199]

At least six isoforms of NKCC2 have been identified.[200] These isoforms are the result of alternative splicing of two regions of the NKCC2 gene: the first region is a 96 base pair region that encodes part of the second transmembrane domain, whereas the second region encompasses the extreme C-terminus. Three variants of the 96 base pair region are encoded by different versions of exon 4 of the NKCC2 gene. These exons are differentially spliced into NKCC2 pre-mRNAs to generate three distinct isoforms (A, B, and F), which alter the amino acid composition of the second transmembrane domain. Each of the A, B, and F isoforms can have either a long C-terminus, or a truncated C-terminus; although to date, the short isoforms have only been described in the murine TAL.[204] In addition, several "tandem" transcripts have been described in the human kidney; these contain combinations of exons 4A, 4B, and 4F spliced alongside one another into the NKCC2 pre-mRNA.[205] Transcripts containing exons 4A/4F, 4B/4A, and 4B/4A/4F have been reported. Because these tandem transcripts contain redundant sequences encoding for the second transmembrane domain, they probably cause the misfolding of NKCC2, resulting in the formation of nonfunctional isoforms. Because these isoforms may still form oligomers with NKCC2, they likely exert a dominant-negative effect on NKCC2 function and inhibit its activity.[206]

The A, B, and F isoforms show differential expression within the thick ascending limb. In the rat nephron, the A isoform was found in both the cortical and medullary TAL, the B isoform is restricted to the cortical TAL, whereas the F isoform is present in the medullary, but not the cortical, the TAL, and to a lesser extent, in the outer medullary collecting duct. Although some interspecies discrepancies have been noted, similar findings have generally been observed in the embryonic mouse and human kidney.[205,207,208]

When the 4A, 4B, and 4F exons are spliced into transcripts containing a long C-terminus, all three products are capable of mediating Na^+-K^+-$2Cl^-$ cotransport. However, the A, B, and F isoforms have different transport properties that may have physiologic relevance. Isoforms A and B have higher affinities for Na^+, K^+, and Cl^- than the F isoform. The A isoform possesses the highest transport capacity of all three isoforms. Based on the known distribution of the A, B, and F isoforms in the TAL, it is currently thought that the A isoform accounts for the high transport capacity of the medullary TAL, whereas the presence of the more active A and B isoforms in the cortical TAL allows for the continued reabsorption of salt to take place, even though the tubular fluid in this segment is more dilute than plasma. Supporting this is experimental evidence demonstrating that the NKCC2 A and B isoforms can both be strongly activated by Na^+, K^+, and Cl^- at concentrations that are much more dilute than the composition of tubular fluid in the cortical TAL.[208]

Apical K^+ Conductance

An important feature of the luminal membrane of the TAL is a barium-inhibitable potassium conductance.[185] This apical membrane K^+ conductance allows K^+ to be recycled from the cell back into the luminal fluid to support further NaCl absorption via the NaCl cotransporter. Using measured values of intracellular K^+ activity (rabbit cTAL),[209] apical membrane conductance, and intracellular voltage, it can be shown that the measured apical membrane conductance is sufficient to provide for the recycling of all of the potassium uptake via the Na^+-K^+-$2Cl^-$ cotransporter.

Three types of K^+ conductances have been characterized in the apical membrane of the thick ascending limb: a high-conductance (150 pS) calcium-activated K^+ channel, which

does not contribute to net K^+ transport,[210] an intermediate-conductance (70 pS) K^+ channel,[211] and a low-conductance (30 to 35 pS) K^+ channel.[211,212] The latter two conductances account for the majority of the apical K^+ secretion in the TAL.[213] Both the intermediate- and the low-conductance channels are inhibited by intracellular ATP and are increased by high dietary potassium.[214]

A major breakthrough in elucidating the molecular mechanism of K^+ secretion in the TAL was made when Ho et al.[215] cloned the ATP-dependent K^+ channel ROMK from rat kidney. This channel is the prototype for a large family of inward-rectifying K channels (Kir channels), and hence, is also called Kir 1.1. Based on its biophysical properties and regulation, investigators had long suspected that ROMK was the sole mediator of the 30-pS K^+ conductance in the TAL. This hypothesis was confirmed when studies of the thick ascending limb in the ROMK knockout mouse revealed the absence of a 30-pS channel.[216] Subsequent studies of the ROMK knockout mouse demonstrated that these mice also lack a 70pS conductance.[214] Thus, it is likely that ROMK also comprises at least a portion of the intermediate K^+ secretory conductance in this nephron segment. It has been proposed that the intermediate-conductance channel may be a heteromeric protein containing ROMK and other, as yet, unidentified subunits.

The notion that ROMK is the predominant channel responsible for apical K^+ recycling in the thick ascending limb is also supported by the finding of mutations in the ROMK gene in families with type II Bartter syndrome.[217,218] These mutations have generally been confirmed to result in defective K^+-channel function.[219] As noted, Bartter syndrome results from a defect in thick ascending limb salt transport. Thus, the presence of ROMK mutations as a cause of Bartter syndrome indicates the important role of ROMK in net salt absorption by the thick ascending limb (Table 5.1).

Basolateral Membrane Cl^- Transport

Cl^- exit across the basolateral membrane of TAL cells is largely conductive, proceeding down its electrochemical gradient through Cl^--selective channels in the basolateral membrane.[220] The primary mediator of basolateral chloride transport in the TAL in humans is thought to be the chloride channel ClC-Kb, the sequence of which is closely related to the aforementioned human ClC-Ka channel expressed in the ATL (see previously). The name for the corresponding rodent ortholog of ClC-Kb is ClC-K2. In contrast to the relatively narrow expression of ClC-K1, ClC-K2 is expressed broadly throughout the basolateral membranes of multiple segments in the rodent nephron, including the TAL, the distal convoluted tubule, the connecting tubule, and the collecting duct.[171,221,222] Inherited mutations of the ClC-Kb cause type III Bartter syndrome, which can manifest as a mixed Bartter/Gitelman phenotype, possibly owing to the expression of ClC-K2/ClC-Kb in the distal convoluted tubule (DCT) and TAL.[223]

Similar to ClC-K1/ClC-Ka channels in the ATL, ClC-K2/ClC-Kb channels require barttin accessory subunits to be fully functional. Barttin was originally identified by positional cloning of the BSND gene, which is responsible for type IV Bartter syndrome (Table 5.1), a severe form of hereditary salt wasting that is accompanied by sensorineural deafness.[224] Barttin is located in the basolateral membranes of the thin and thick ascending limb, distal nephron, and also in the stria vascularis of the inner ear, where it is believed to play a role in K^+ secretion into the endolymph. The regulation of ClC-K channels by barttin appears to be multifaceted, because the accessory subunit influences channel protein stability, subcellular localization, and gating.[172,173,175] The mutations of barttin identified in Bartter syndrome patients generally impair the ability of barttin to produce a Cl^- conductance when expressed with ClC-Kb.[172,173]

Although less completely studied, there do appear to be additional transport pathways that mediate basolateral Cl^- flux in the TAL. For example, a barium-sensitive transcellular K^+-Cl^- cotransport mechanism has been proposed (Fig. 5.6).[225] The expression of two KCl cotransporters that belong to the same family of SLC12 electroneutral cotransporters as NKCC2, KCC1,[226] and KCC4[227] have been observed. Thus, both of these cotransporters may participate in the extrusion of K^+ and Cl^- from cells in the cortical and medullary TAL. Studies in knockout animals, however, indicate that KCC4 is not the primary mediator of basolateral Cl^- flux in the TAL, because these mice do not develop a salt-wasting phenotype. Rather, the KCC4 knockout mouse develops metabolic acidosis and sensorineural deafness, suggesting that KCC4 plays important physiologic functions in the acid-secreting intercalated cells of the collecting duct and in the cells of the inner ear.[228]

Synchronous Na^+/H^+:Cl^-/HCO_3^- Exchange

Friedman and Andreoli[229] found that net Cl^- absorption and the transepithelial voltage were doubled when CO_2 and HCO_3^- were added to the external solutions bathing cortical TAL segments. Because the (CO_2 + HCO_3^-)-stimulated rate of NaCl absorption did not result in net CO_2 transport and could be abolished by the lipophilic carbonic anhydrase inhibitor ethoxyzolamide or by the luminal addition of the anion-exchange inhibitor SITS or DIDS, it was proposed that the apical membrane of the mouse cortical TAL contains parallel, near synchronous Na^+/H^+:Cl^-/HCO_3^- exchangers in addition to a Na^+-K^+-$2Cl^-$ cotransporter. The addition of CO_2 and HCO_3^- to the bathing solutions had no effect on net NaCl transport in either the rabbit cTAL or the mouse mTAL. Both the rat and mouse medullary TAL do contain Na^+/H^+ exchangers in their apical membranes. However, in these segments, Na^+/H^+ exchange plays a role in net HCO_3^- transport and cell pH regulation rather than transcellular NaCl absorption.[230] The NHE3 isoform of the Na^+/H^+ exchanger is the major isoform expressed in the apical membrane of the thick ascending limb.[231,232]

REGULATION OF SALT ABSORPTION IN THE TAL

Vasopressin

The peptide hormone arginine vasopressin (AVP, also known as antidiuretic hormone, ADH) remains the most extensively characterized stimulatory hormone for NaCl reabsorption in the TAL. The reabsorption of NaCl in the TAL is crucial for efficient urinary concentration, because it is this process that plays a key role in maintaining a hypertonic medullary interstitial solute gradient for water reabsorption in more distal portions of the nephron.[233] Thus, from a teleologic perspective, vasopressin ought to be an important regulator of this process. The cognate receptor for vasopressin, the V2 receptor (V2R), is expressed in both the cortical and medullary TAL, where it participates in signaling cascades that stimulate NKCC2 activity.[234]

Binding of AVP to V2R results in increased intracellular levels of cAMP in the TAL. The increase in cAMP levels ultimately drives the translocation of NKCC2 from intracellular subapical vesicles to the luminal plasma membrane. In addition, cAMP serves as a signal that increases the phosphorylation of residues in the NKCC2 amino terminus that stimulate cotransporter activity in vitro.[235,236] Although this process may be mediated upstream by the cAMP-dependent protein kinase A (PKA), it is unclear whether PKA directly phosphorylates the N-terminal residues that stimulate its activity. Rather, it appears that two other kinases in particular are important downstream mediators of this process. These kinases, the Ste20 SPS1-related proline alanine-rich kinase (SPAK) and oxidative stress responsive kinase 1 (OSR1), are structurally homologous, activated by vasopressin, bind to a defined docking site harbored within the NKCC2 N-terminus, and directly phosphorylate these previously identified stimulatory residues (Fig. 5.7).[237–239]

The importance of SPAK and OSR1 in NKCC2 function has recently been established in transgenic and knockout models.[237,240,241] A complete knockout of SPAK mRNA and protein expression yields a mild salt-wasting phenotype that approaches Gitelman syndrome, likely owing to the elimination of its activity in the DCT (see the following). However, the absence of a Bartter-like phenotype in these animals does not refute the importance of SPAK and OSR1 in the TAL. Rather, the Gitelman-like salt-wasting phenotype in these animals appears to be due to two factors. First, knockout of the SPAK gene not only ablates full-length kinase-active SPAK, but also eliminates the expression of a truncated kidney-specific kinase-defective SPAK isoform (KS-SPAK) that suppresses baseline SPAK and OSR1 activity in the TAL.[237] Second, in the SPAK knockout mouse, TAL-expressed OSR1 appears to compensate for the absence of SPAK activity. Both of these effects ultimately result in increased rather than decreased NKCC2 abundance and phosphorylation, which probably compensates for the lack of DCT salt reabsorption and gives rise to a relatively mild salt-wasting phenotype. The dominance of OSR1 over SPAK in the TAL was recently verified in studies of the OSR1 knockout mouse, which, in contrast to the SPAK knockout animal, exhibits a Bartter-like phenotype.[241] On the other hand, knock-in mice bearing a mutation that ablates a key catalytic activation site in SPAK do exhibit decreased NKCC2 phosphorylation; presumably, this inactivating mutation exerts a dominant-negative effect on NKCC2 phosphorylation by preventing the binding of stimulatory kinases such as OSR1 to the NKCC2 N-terminal docking site.[242] Although it is clear that SPAK and OSR1 are important kinases that are linked to tubular salt reabsorption in the TAL, it is unclear how they directly connect to the well-established upstream cAMP-dependent signaling cascades, which are mediated by vasopressin. Nevertheless, given the fact that vasopressin activates SPAK and OSR1 and stimulates NKCC2 phosphorylation at key SPAK/OSR1 phosphorylation sites, it would appear that these kinases are important downstream intermediaries of the vasopressin signaling pathway.

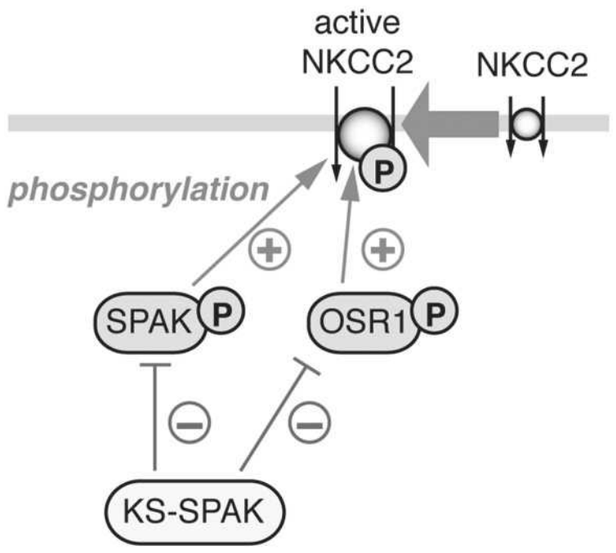

FIGURE 5.7 The current model for SPS1-related proline alanine-rich kinase /oxidative stress responsive kinase 1 (SPAK/OSR1) and kidney-specific (KS)-SPAK regulation of NKCC2 via phosphorylation in the thick ascending limb of the loop of Henle.

In addition to its effects on apical Na^+-K^+-$2Cl^-$ cotransport, vasopressin increases the transcellular electrical conductance of the mouse medullary TAL, and this conductance increase is a major element in the mechanism for the hormone-dependent increase in the rate of net salt absorption.[243,244] The available evidence suggests that both the apical and basolateral membrane conductances are increased by vasopressin. In apical membranes, AVP increases conductance by increasing the functional number of K^+ channels[182]; this increase occurs even when net salt absorption is abolished by furosemide. Ecelbarger at al.[245] showed that this increase in apical K^+ conductance was at least in part due to a dramatic upregulation in ROMK abundance and apical localization. Thus, the machinery for apical recycling of K^+ is increased in the TAL, which would aid in augmenting NKCC2-mediated NaCl reabsorption.

The predominant portion of the ADH-induced increase in cellular conductance is accounted for by an increase in the basolateral membrane Cl^- conductance.[182] Two mechanisms have been suggested for the hormone-

dependent increase in basolateral Cl^- conductance. Schlatter and Greger[183] have proposed that the ADH-induced increase in intracellular cAMP results in a direct increase in Cl^- channel activity. Such a mechanism has been amply demonstrated in Cl^--secreting epithelia, such as the trachea and intestine. Alternatively, ADH might enhance Cl^- conductance indirectly by increasing apical membrane Cl^- entry.[182] According to this proposal, an ADH-dependent activation of apical membrane NKCC2 and ROMK leads to an increase in intracellular Cl^- concentration. Because the activity of basolateral Cl^- channels is exquisitely sensitive to changes in intracellular Cl^- concentration,[246] this increase will translate into an increase in the basolateral membrane Cl^- conductance.

Prostaglandins

Prostaglandin 1 (PGE_2), the major product of prostaglandin synthesis in the renal medulla, participates in a local negative-feedback system that modulates the rate of NaCl absorption by the TAL. PGE_2 resulted in a 50% reduction in ADH-stimulated net Cl^- absorption in isolated perfused mammalian mTAL segments, but had no effect in the absence of ADH.[247] It is likely that PGE_2 inhibits ADH-stimulated generation of cAMP in the mTAL by activating an inhibitory G protein, G_i.[247] Although interstitial cells are a major source of PGE_2 production in the renal medulla, thick ascending limb cells can metabolize arachidonic acid through at least two pathways. Escalante et al.[248] demonstrated that purified medullary thick ascending limb cells produce 20-hydroxyeicosatetraenoic acid (20-HETE) via the cytochrome P450 enzyme, ω-hydroxylase. 20-HETE was subsequently shown to inhibit NaCl transport in the thick ascending limb at steps, which include the apical K^+ channel[249] and the Na^+-K^+-$2Cl^-$ cotransporter.[248] Thick ascending limb cells, particularly in the macula densa (MD), also express COX-2.[250] COX-2 expression in these cells may be coupled to renin secretion. Thus, COX-2 expression is increased by salt restriction, diuretics, and, in Bartter syndrome, all conditions characterized by hyperreninemia.[251] Moreover, COX-2 inhibitors reduce renin secretion in these settings.[252]

Osmolality

Peritubular osmolality is a third factor regulating mTAL salt absorption. In isolated mouse and rabbit TAL segments, increases in peritubular osmolality rapidly and reversibly inhibit the ADH-stimulated rate of net Cl^- absorption.[243] Molony and Andreoli[253] determined that hypertonicity inhibits the basolateral membrane Cl^- conductance. This inhibition of transcellular salt absorption occurs at a locus beyond the generation of cAMP, because supramaximal concentrations of either ADH or cAMP are unable to reverse the hypertonicity-mediated effect.[254] Thus, increasing the absolute magnitude of interstitial osmolality provides a negative feedback signal, which can reduce ADH-dependent salt absorption by the medullary TAL.

Hypercalcemia

Hypercalcemia often results in an ADH-resistant urinary concentrating defect, that is, nephrogenic diabetes insipidus.[255] At least part of this concentrating defect results from the inhibition of ADH-stimulated cAMP production in the TAL by calcium.[256] Preincubation of tubule segments with pertussis toxin abolishes the effect of hypercalcemia on cAMP generation, indicating that the inhibition of cAMP generation is mediated through the activation of G_i.[257] The effects of hypercalcemia are mediated by a G protein–coupled calcium-sensing receptor (CaSR) present on the basolateral membrane of TAL cells.[258] Activation of this receptor also inhibits the activity of the 70-pS apical K channel (ROMK, see previous) via the production of 20-HETE, a P450 metabolite of arachidonic acid.[259,260] Even changes in serum calcium within the physiologic range can alter NaCl absorption via the CaSR.[261] This may help to explain the hypotensive effect of high calcium intake in salt-sensitive hypertensive individuals.[262]

Modulation of NaCl Absorption by Other Peptide Hormones

In addition to ADH, a number of other peptide hormones stimulate adenylate cyclase activity in the TAL. In the mouse and rat, glucagon stimulates NaCl reabsorption and increases the transepithelial potential in isolated microperfused mTAL segments. Calcitonin and PTH stimulate sodium transport in the cortical, but not the medullary, portions of the TAL.[263]

Ang II receptors are present in the thick ascending limb.[264] Ang II has been reported to both stimulate and inhibit sodium transport in the thick ascending limb.[265,266] Chronic infusion of Ang II increased the abundance of NKCC2 in the rat outer medulla by 87%.[267] In a model of heart failure, in which Ang II levels are elevated, NKCC2 expression was also increased and this increase could be prevented by treatment with an angiotensin receptor antagonist.[268]

Adrenergic Agents

β-Agonist-sensitive adenylate cyclase activity is present in the rat, but not the rabbit TAL.[269] Likewise, β-adrenoceptors have been detected along the rat TAL by autoradiographic localization.[270] The physiologic effects of adrenergic agents have been tested in micropuncture and in in vitro microperfusion studies. DiBona and Sawin[271] demonstrated an enhancement of loop NaCl absorption during low-frequency renal nerve stimulation. Acute renal denervation, on the other hand, depressed NaCl absorption by the loop of Henle.[272]

Micromolar concentrations of isoproterenol stimulate net Cl^- absorption by in vitro perfused mouse medullary and cortical TAL.[273] The effects of isoproterenol on NaCl absorption in these segments can be blocked by propranolol.

Nitric Oxide

Acute administration of nitric oxide donor or L-arginine, the substrate for NOS, decreases NaCl absorption in isolated perfused thick ascending limb segments.[274] The effect of

L-arginine on NaCl transport can be blocked by L-NAME, an inhibitor of NOS, indicating that endogenous production of NO mediates the effect of L-arginine. The inhibitory effect of NO on net NaCl absorption appears to involve, at least in part, the inhibition of NKCC2 activity.[275] The thick ascending limb expresses all three isoforms of NOS.[276] Plato et al.[277] used mice deficient in the various NOS isoforms to determine that the effect of L-arginine is mediated by eNOS rather than iNOS or nNOS. In contrast to its effect on NaCl absorption, NO stimulates $NaHCO_3^-$ absorption in the thick ascending limb.[266] Finally, although short-term exposure to NO inhibits NaCl absorption, chronic exposure increases NKCC2 expression,[278] which could translate into increased NaCl absorption.

Sodium Balance

Dietary Na^+ restriction in rats results in a transient decrease in NKCC2 expression,[279] whereas high Na^+ intake has no major effect on NKCC2 expression.[280] Chronic treatment with furosemide in conjunction with a high sodium diet increases NKCC2 expression.[281] The latter phenomenon may account for the development of diuretic resistance and the interdose rebound in sodium absorption in patients chronically treated with loop diuretics.

THE DISTAL NEPHRON

Anatomic Considerations

The distal nephron may be divided into three segments: the DCT, the connecting tubule (CNT), and the collecting duct. Perhaps owing to the nature of the original micropuncture studies characterizing the distal nephron, the DCT was initially thought to be a segment consisting of a homogeneous population of epithelial cells. However, more recent work clearly indicates that the DCT can be further divided into two functionally distinct subsegments, referred to as the "early" and "late" DCT (DCT1 and DCT2, respectively).[282] One of the primary features that distinguishes between the early and late portions of the DCT is the differential sensitivity of these segments to the mineralocorticoid hormone aldosterone. More specifically, the late DCT expresses the enzyme 11beta-hydroxysteroid dehydrogenase 2 (11β-HSD2), which metabolizes cortisol, thereby rendering mineralocorticoid receptors sensitive to aldosterone.[283] For this reason, the late DCT, CNT, and the cortical collecting duct (CCD) are collectively termed the aldosterone-sensitive distal nephron (ASDN).

Na^+ Transport in the Distal Convoluted Tubule and the Connecting Segment

General Characteristics

The DCT absorbs roughly 5% to 10% of the filtered Na^+ load.[284] Fluid enters the DCT with a Na^+ concentration of 25 to 30 mM, but salt is added along the initial 20% of the DCT, so that the Na^+ concentration averages 50 mM at a point 200 to 300 μm from the macula densa.[285] From there, tubular Na^+ concentration decreases along the DCT to a value of approximately 30 mM at the end.[286] Tubular fluid to plasma Na^+ ratios as low as 0.10 have been observed during stationary microperfusion.[287] This finding, together with the presence of the lumen-negative potential difference (see the following), clearly establishes the active nature of Na^+ absorption in this segment.

Na^+ absorption by the DCT is load dependent. That is, over a wide range of delivery rates, the proportion of Na^+ absorbed by the DCT remains constant at 80%.[286] At high tubular fluid flow rates, the fall in luminal Na^+ concentration along the tubule is attenuated; thus, more Na^+ is available to distal Na^+ absorptive sites at high rather than at low flow rates.

Electrophysiologic Considerations

Depending on the type of electrodes that were used, measurements of the transepithelial voltage in the earliest loops of the DCT vary from slightly lumen negative to slightly lumen positive.[288–291] Consistent among all measurements, however, is the observation that the transepithelial voltage becomes progressively more lumen negative as tubular fluid passes to the end of the DCT and into the CNT and CCD.

The progressively lumen-negative transepithelial electrical potential is primarily due to a change in Na^+ transport pathways from the early DCT to more downstream nephron segments. As shown previously by Ellison et al.,[292] Na^+ reabsorption in the early DCT is largely mediated by an electroneutral, thiazide-sensitive NaCl cotransporter, whereas Na^+ absorption in the late DCT also involves an amiloride-sensitive electrogenic pathway.[282] These and other mechanisms of Na^+ absorption are discussed in detail in the following sections.

The Mechanism of Na^+ Absorption

A general model for these mechanism by which Na^+ is reabsorbed in the early and late DCT is presented in Figure 5.8.

The Apical NaCl Cotransport. The absorption of Na^+ and Cl^- in the early DCT are coupled.[293] The coupling of Cl^- to Na^+ entry provides a mechanism to maintain the intracellular Cl^- activity above its electrochemical equilibrium.[294] The early distal tubule is the site of action of thiazide diuretics.[295] Autoradiographic studies[296] and immunocytochemical studies[297] have demonstrated thiazide-binding sites in the apical membranes of DCT and connecting tubule cells.

The thiazide-sensitive NaCl cotransporter (NCC, TSC, SLC12A3) mediates electroneutral NaCl reabsorption in the early and late DCT (Fig. 5.8).[298] The cotransporter shares considerable sequence homology to the bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) present in the TAL,[193] yet exhibits markedly different inhibitor sensitivity and ionic requirements. NCC transports NaCl with a

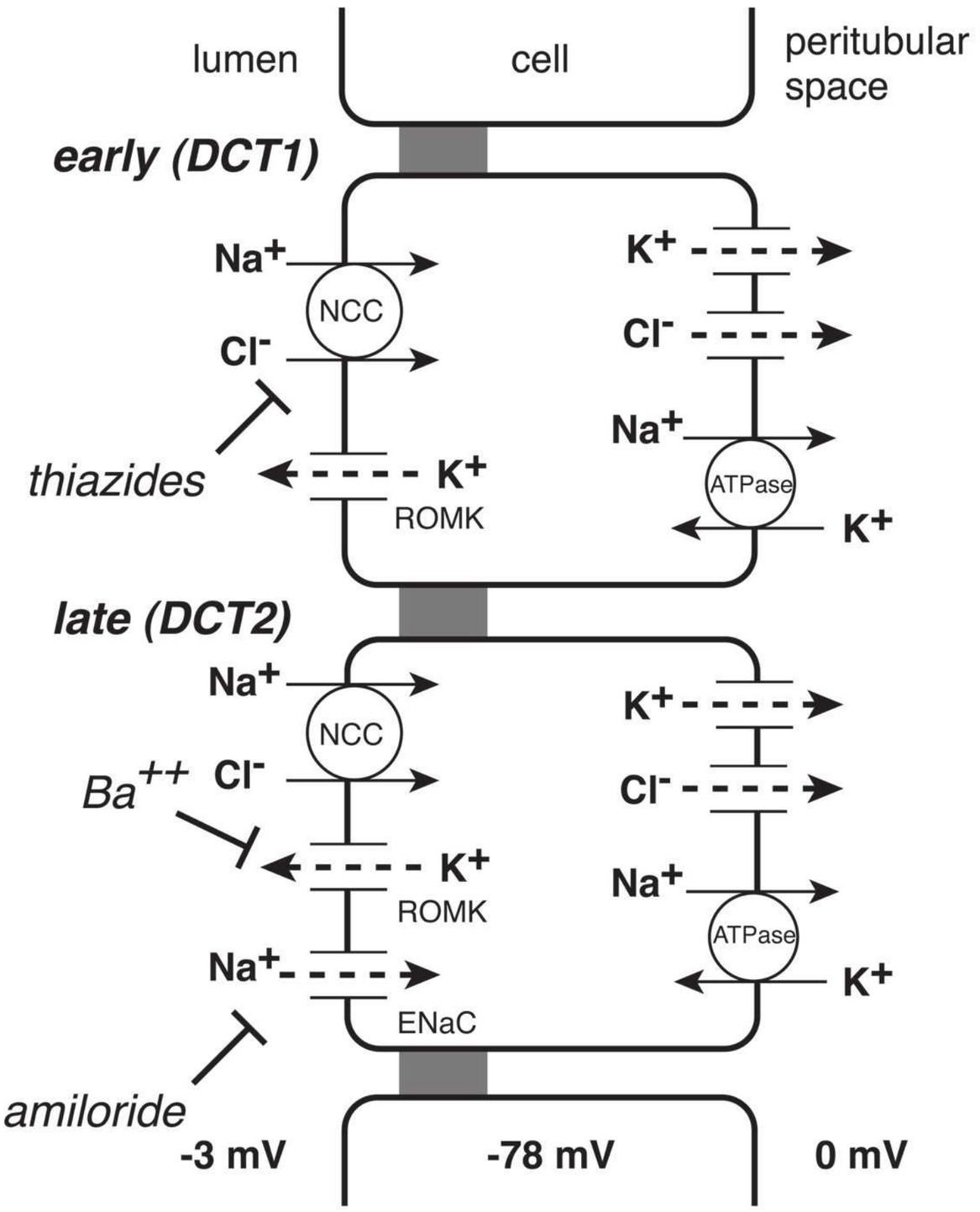

FIGURE 5.8 A model of NaCl absorption by cells of the early and late distal convoluted tubule (DCT). The early and late DCT express NCC, a thiazide-sensitive, electroneutral NaCl cotransporter. The late DCT also expresses the amiloride-sensitive Na^+ channel ENaC. Basolateral K^+ channels include Kir4.1, mutated in EAST/SeSAME (epilepsy, ataxia, sensorineural deafness, and tubulopathy/seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) syndrome, a hereditary salt-wasting disorder. *ROMK*, renal outer medullary K channel; *NCC*, thiazide-sensitive NaCl cotransporter.

1:1 stoichiometry, is K^+-independent, and is inhibited by thiazide diuretics.[299] NCC is expressed in the apical membrane of DCT cells and extends, in most species, into the connecting segment.[300]

Gitelman Syndrome. Loss-of-function mutations in the NCC gene have been linked to the pathogenesis of Gitelman syndrome.[218] This syndrome resembles Bartter syndrome (hypokalemia, alkalosis, sodium wasting), except that urinary calcium excretion is reduced in Gitelman syndrome and is elevated in most cases of Bartter syndrome. Thus, physiologically, Gitelman syndrome mimics the effects of thiazide diuretics. Several studies have evaluated the consequences of mutations causing Gitelman syndrome on NCC function, and in most cases, these mutations alter the NCC coding sequence in such a way that they reduce NCC expression and trafficking to the apical plasma membrane of the DCT.[301,302] In this regard, most of these disease-causing mutations result in the conformational misfolding of NCC, resulting in the recognition of mutant NCC by chaperone-dependent endoplasmic reticulum (ER) quality control machinery that targets the cotransporter for proteasomal degradation.[301,303]

Familial Hyperkalemic Hypertension. Studies of another inherited disorder of distal Na^+ transport, familial hyperkalemic hypertension ([FHHt] type 2 pseudohypoaldosteronism, Gordon syndrome), have yielded additional insights into the regulation of NCC function. FHHt is the phenotypic opposite of Gitelman syndrome and is characterized by hypertension, hyperkalemia, and metabolic acidosis (Table 5.1). The disorder is largely corrected by the infusion of sodium with poorly reabsorbable anions, or by treatment with thiazide diuretics.[304] These features suggested that the hypertension in FHHt is chloride dependent, and that an increase in NCC activity may be involved in the pathogenesis of PHA II. Positional cloning demonstrated that FHHt is caused by mutations in either of two with-no-lysine (WNK) serine-threonine kinases, WNK1 or WNK4.[305] These kinases have been intensely studied since their linkage to FHHt in 2001, and are well-established regulators of NCC, but the mechanisms by which they affect NCC function are complex. Both WNK1 and WNK4 have been shown to regulate NCC plasma membrane trafficking and activity (Fig. 5.9). In the current model it is believed that WNK4 acts as an inhibitor of NCC trafficking at the baseline state because it prevents the cotransporter from trafficking from the biosynthetic pathway to the cell surface.[306–308] In contrast to this inhibitory effect on NCC traffic, it has been hypothesized that under certain physiologic states that favor NaCl reabsorption, WNK4 stimulates NCC phosphorylation. Because NCC is structurally similar to NKCC2, it is activated by the serine–threonine kinases SPAK and OSR1 (see previous section). WNK4 and WNK1 activate SPAK and OSR1, which then can

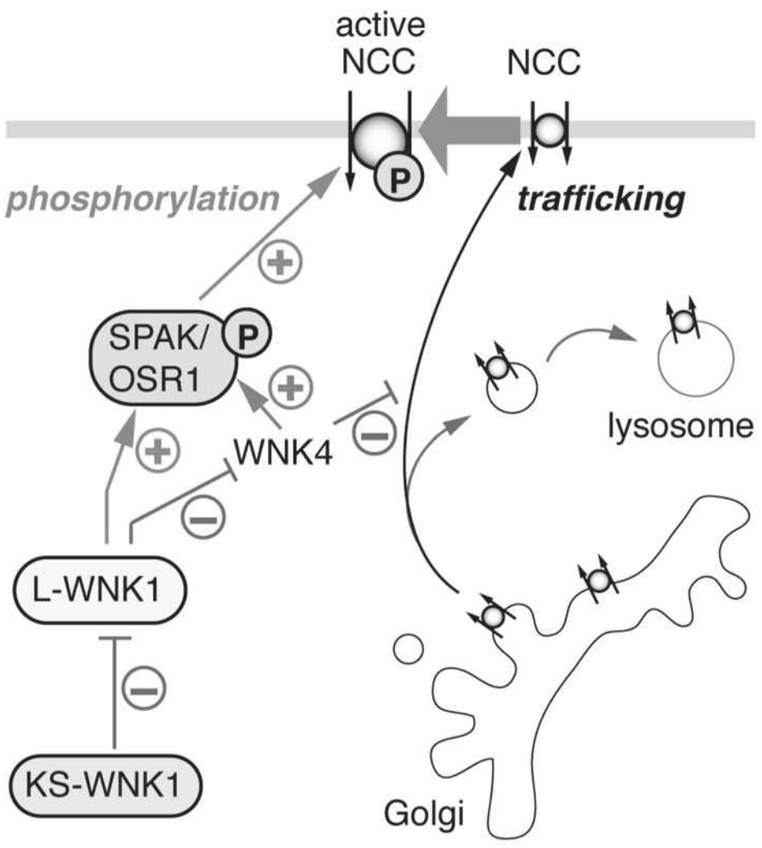

FIGURE 5.9 A model of thiazide-sensitive cotransporter (NCC) regulation by the with-no-lysine SPS1-related proline alanine-rich kinase/oxidative stress responsive kinase 1 (WNK-SPAK/OSR1) signaling pathway. With-No-Lysine (WNK) kinases regulate NCC trafficking and phosphorylation. *L-WNK1*, full-length kinase-active ("long") WNK1; *KS-WNK1*, short kinase-defective "kidney-specific" WNK1.

bind to NCC and stimulate its activity by phosphorylating a cluster of serines and threonines in its NH_2-terminus.[307] In contrast to the TAL, only the full-length kinase active SPAK is expressed in the DCT (i.e., no inhibitory KS-SPAK isoforms are expressed).[237] Due to the observations that WNK4 inhibits NCC under some experimental conditions and stimulates it under others, several have proposed that the WNK signaling pathway acts as a "switch" that converts the DCT from a salt-wasting to a salt-reabsorptive segment, although more work is needed to confirm this hypothesis.[309–311] In FHHt, missense mutations of WNK4 stimulate NCC activity through two mechanisms. First, mutant WNK4 is incapable of reversing the inhibitory effect on NCC at the baseline; this results in increased NCC surface expression.[308,312] Second, the FHHt-associated mutant increases NCC phosphorylation, presumably through enhanced SPAK/OSR1 activity. Thus, through incompletely defined mechanisms, mutant WNK4 appears to "lock" the DCT into a high Na^+ reabsorptive state by increasing the total number of active NCC molecules at the cell surface, resulting in thiazide-sensitive hypertension.[313]

In contrast, WNK1 appears to influence NCC traffic indirectly by suppressing the inhibitory effect of WNK4 (Fig. 5.9). This effect requires intact WNK1 kinase activity and releases NCC from intracellular retention, thereby facilitating NCC delivery to the cell surface.[307,308] Unlike WNK4, WNK1 mutations that cause FHHt do not alter the kinase's coding sequence. Rather, WNK1 mutations are large intronic deletions that increase the mRNA abundance of WNK1 isoforms.[305] Kinase-active WNK1 is capable of phosphorylating and activating SPAK and OSR1.[314] Thus, the WNK1 gene mutations that cause FHHt presumably do so by reversing the inhibitory effect of WNK4 on NCC traffic and by stimulating NCC phosphorylation.[315] As with SPAK gene expression in the TAL (see previous), long and short WNK1 isoforms are expressed in the DCT. The long form of WNK1 (L-WNK1) is kinase active and stimulates NCC activity and trafficking through the mechanisms described previously. A short kidney-specific kinase-defective product (KS-WNK1), in contrast, suppresses L-WNK1 activity (Fig. 5.9).[316] The balance of these two isoforms has been postulated to act as a "switch" that controls overall L-WNK1 kinase activity in the DCT, which should in turn regulate NCC activity.[310] Consistent with this idea, selective knockout of KS-WNK1 expression increases NCC activity, whereas overexpression of the kidney-specific isoform in KS-WNK1 transgenic mice causes salt-wasting through NCC inhibition.[317]

The Basolateral Electrogenic Na^+ Pump. In both subsegments, the basolateral extrusion of Na^+ from the cytosol into the peritubular space (and eventually, the plasma) occurs via the electrogenic Na^+,K^+–ATPase. The activity of this pump results in the generation of a constant transepithelial voltage across the basolateral membrane of −60 to −90 mV.[318,319] A reduction in the luminal sodium concentration causes V_{bl} to depolarize, whereas increases in the sodium concentration hyperpolarizes V_{bl}. In addition, V_{bl} depolarizes after ouabain treatment.[320] These observations are consistent with the notion that apical Na^+ entry stimulates the electrogenic Na^+,K^+–ATPase system in the basolateral membrane.

Basolateral K^+ Efflux. Recent insights from rare Mendelian diseases highlight the importance of basolateral K^+ transport in tubular sodium transport in the distal nephron. In 2008, two groups identified that patients with mutations in the K^+ channel gene KCNJ10 (Kir4.1) develop hereditary salt wasting.[321,322] Patients with these mutations develop a complex constellation of neurologic defects in addition to the renal salt wasting. The disease has been named EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) by some investigators and SeSAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) by others (Table 5.1). The salt-wasting phenotype in these patients is reminiscent of Gitelman syndrome, suggesting that the disorder results in an impairment of salt transport in the DCT. Indeed, Kir4.1 is expressed on the basolateral membrane of DCT cells, and patients with EAST/SeSAME syndrome mutations develop markedly reduced infoldings of the DCT basolateral membrane. This deficiency in total basolateral membrane content results in a decrease in the number of surface Na^+,K^+–ATPase molecules, resulting in decreased sodium pump activity and impaired salt reabsorptive capacity.[323] Additionally, it is thought that loss-of-function mutations of Kir4.1 impair K^+ recycling across the basolateral membrane, which reduces the efficacy of Na^+,K^+–ATPase (Fig. 5.8).[321]

Basolateral Cl^- Transport. As mentioned previously, ClC-Kb channels and barttin are both expressed in the DCT, where they mediate basolateral Cl^- reabsorption.[172] Because the apical transport mechanism in the DCT via NCC occurs through the coupled reabsorption of Na^+ and Cl^-, basolateral Cl^- transport is essential to the development of a gradient for Na^+ entry and to reduce the intracellular Cl^- concentration, which stimulates SPAK/OSR1–mediated NCC phosphorylation.[324] Mutations in either ClC-Kb or the accessory subunit result in impaired distal salt reabsorption. Because barttin is required for adequate basolateral Cl^- reabsorption in the loop of Henle and DCT (see previous), patients with barttin mutations develop a more severe form of Bartter syndrome.[172]

Apical Conductive Na^+ Channels. The entry of sodium into the Amphiuma distal tubular cell[325] and late rat DCT cell[326] is inhibited by amiloride, a Na^+ channel blocker. A Na^+ channel in the apical membrane would serve to depolarize the membrane and create the observed lumen-negative transepithelial potential. This transepithelial voltage, in turn, is a driving force for passive Cl^- reabsorption. Na^+ channel subunits have been found by immunolocalization in the late DCT in mouse and rat kidney,[327,328] but not the human kidney.[329]

The Regulation of NaCl transport in the Distal Convoluted Tubule

Na^+ Delivery. NaCl reabsorption in the DCT is dependent on the delivered load of NaCl.[286] The DCT responds to chronic increases in the delivery of NaCl with an increase in the capacity for NaCl transport,[330] as well as marked ultrastructural changes in the DCT cell. These morphologic changes include an increase in the size of the DCT cell, an increase in the basolateral membrane surface area, and an increase in the size of mitochondria.[330] Accompanying the functional and morphologic changes are an increase in Na^+,K^+–ATPase activity and an increase in thiazide-binding sites.[331] These effects appear to result from an increase in Na^+ entry into the DCT cell rather than the increase in distal NaCl delivery or changes in plasma aldosterone or ADH levels that occur with chronic furosemide treatment. Inhibition of NaCl entry into DCT cells with chronic thiazide treatment resulted in a loss of cell height, loss of normal polarity, and apoptosis of the DCT cells.[332] The cellular mechanisms whereby NaCl entry affects transport function and morphology are not known.

Dietary Na^+. Studies in rats and rabbits have yielded conflicting results regarding the effects of increased dietary Na^+ on DCT morphology and Na^+ transport. In rats, no consistent effect of a high Na^+ diet on either cell morphology, transport rates, or thiazide-receptor density could be demonstrated.[330,331,333] Rats fed a low Na^+ diet[279] or treated with thiazide diuretics,[281] however, demonstrated a significant increase in NCC expression, which may have been because of the stimulation of mineralocorticoid secretion.[334] In contrast, rabbits fed a high Na^+ diet developed an increased rate of DCT Na^+ reabsorption and an increase in Na^+,K^+–ATPase activity.[335]

Steroid Hormones. Currently available evidence suggests that adrenal steroid hormones regulate Na^+ transport in the DCT. The presence of both mineralocorticoid and glucocorticoid receptors in the DCT has been demonstrated by immunohistochemistry and by hormone binding.[283,336,337] In addition, an adrenalectomy resulted in a decrease in Na^+,K^+–ATPase activity in the DCT.[338] The Na^+,K^+–ATPase activity could be restored by replacement doses of glucocorticoids, but not by mineralocorticoids.[338,339] Microperfusion studies of superficial distal tubules (containing both DCT and CNT), however, demonstrated an increase in Na^+ transport in animals receiving aldosterone infusions.[340,341] Both the thiazide-sensitive and the thiazide-insensitive components of Na^+ transport were increased by aldosterone.[341] The former may reflect neutral NaCl cotransport in the DCT, whereas the latter reflects electrogenic Na^+ absorption in the late DCT or CNT. Aldosterone infusion also resulted in an increase in thiazide-binding sites in the renal cortex, as determined by [^{3}H]metolazone binding, an increase in the natriuretic response to thiazide diuretics, and a large increase in NCC protein.[341–343] These findings establish NCC as an aldosterone-regulated transporter. By combining immunohistochemical and in situ hybridization techniques, Bostanjoglo et al.[283] determined that DCT cells coexpress NCC, mineralocorticoid receptors, and 11beta-hydroxysteroid dehydrogenase type 2, an enzyme typically found in mineralocorticoid target sites. Thus, DCT cells, particularly those in the late portions of the DCT, express the key elements required for selective mineralocorticoid actions.

In 1998, Kim et al.[343] showed that aldosterone increases the total protein abundance of NCC. This effect did not correlate with changes in NCC mRNA abundance, suggesting that aldosterone regulates NCC abundance by posttranslational mechanisms. Recent work indicates that at least two molecular mechanisms account for this effect. Aldosterone rapidly induces and activates the serine–threonine kinase serum and glucocorticoid-regulated kinase 1 (SGK1), which directly phosphorylates and inactivates two inhibitors of NCC trafficking, WNK4 (described previously), and neural precursor cell-derived, developmentally downregulated 4-2 (Nedd4-2), an E3 ubiquitin ligase.[344,345] SGK1-mediated phosphorylation of two residues located at the C-terminus of WNK4 releases NCC from inhibition. This results in WNK4 inactivation and diverts the cotransporter away from intracellular degradation pathways, allowing NCC to traffic directly to the plasma membrane from the biosynthetic pathway.[344] By phosphorylating Nedd4-2, SGK1 suppresses the ability of the E3 ligase to attach ubiquitin molecules to NCC; this

reduces NCC degradation and increases its plasma membrane expression.[345] Both of these effects provide an explanation for the aldosterone-induced increase in NCC total protein abundance that was initially seen by Kim et al[343] Aldosterone also appears to stimulate NCC transport activity by promoting NCC phosphorylation, an effect that occurs independently of the effects of mineralocorticoids on NCC traffic.[346,347] The mechanism by which this effect occurs remains unclear but appears to correlate directly with increased SPAK/OSR1 activity, suggesting that additional connections between aldosterone and the WNK/SPAK/OSR1 signaling pathway may exist.

As mentioned previously, glucocorticoids increase Na^+,K^+–ATPase activity following an adrenalectomy in the DCT.[338,339] This effect was not blocked by spironolactone, a mineralocorticoid receptor antagonist, suggesting that glucocorticoids were acting via glucocorticoid receptors rather than mineralocorticoid receptors.[339] In addition, dexamethasone infusions increased thiazide-sensitive NaCl transport and [^{3}H]metolazone binding sites in adrenalectomized rats.[341,342] Nevertheless, the role of glucocorticoids in the physiologic regulation of Na^+ transport in the DCT remains unclear.

Gonadal steroid hormones may also influence NaCl transport in the DCT. Chen et al.[348] reported gender differences in the density of thiazide receptors and in the natriuretic response to thiazides in rats. Female rats had higher levels of thiazide-binding sites in the renal cortex than males. The levels in females fell following ovariectomy, whereas levels rose in males following orchiectomy. Moreover, the increase in urinary Na^+ excretion caused by thiazides was greater in females than in males, suggesting that the differences in thiazide-binding sites were reflective of differences in thiazide-sensitive salt transport in vivo. Likewise, using antibodies against NCC, Verlander et al.[349] found that estrogen treatment increased NCC expression in the DCT. These results are consistent with the view that male sex hormones (e.g., testosterone) may downregulate NCC expression and salt transport, whereas estrogens increase NCC expression and salt transport in the DCT. The authors are not aware of gender differences in the response of humans to thiazide diuretics.

Vasopressin. Recent work indicates that vasopressin is a potent activator of the thiazide-sensitive cotransporter.[350,351] Two groups have shown that the vasopressin analog deamino-Cys-1, d-Arg-8 vasopressin (dDAVP) stimulates increases in NCC abundance, trafficking to the plasma membrane, and activation through SPAK/OSR1-mediated phosphorylation of its amino terminus. This effect is probably dependent on cyclic AMP and protein kinase A (PKA), well-established intermediaries of vasopressin-dependent signaling.[352] The effect on NCC abundance may be a Nedd4-2-dependent process, because PKA can phosphorylate and inactivate Nedd4-2 through mechanisms similar to SGK1[353]; however, this hypothesis is yet to be tested.

Na^+ TRANSPORT IN THE CORTICAL COLLECTING DUCT

General Considerations

The transport processes in the collecting duct mediate final adjustments in urinary composition. The collecting duct is a major locus of action of mineralocorticoid hormones and plays a major role in K^+ homeostasis and acid–base balance. Quantitatively, it is a minor site of Na^+ absorption, reclaiming only about 2% to 4% of the filtered Na^+ load.[354]

Electrophysiologic Aspects

The transepithelial voltage in the CCD varies widely from +10 to −100 mV,[295,355,356] which largely results from differences in mineralocorticoid levels at the time of measurement in the animals. The reported values for transepithelial resistance also vary widely depending on apical Na^+ and K^+ channel activities, as shown by the effects of luminal amiloride and barium, respectively, on transepithelial resistance.[356,357] The basolateral membrane is conductive to K^+ and, at least in rabbits, Cl^-.[357]

Mechanisms of Salt Absorption in Collecting Ducts

A proposed model for Na^+ absorption and K^+ secretion in the CCD is presented in Figure 5.10. As indicated previously, apical membranes of CCD principal cells possess conductive pathways for Na^+ and K^+.[358,359] Na^+ enters principal cells through Na^+ channels in the apical membrane down its electrochemical gradient. Na^+ is then pumped across the basolateral membrane by the Na^+,K^+–ATPase in exchange for K^+. The Na^+ current across the apical membrane depolarizes

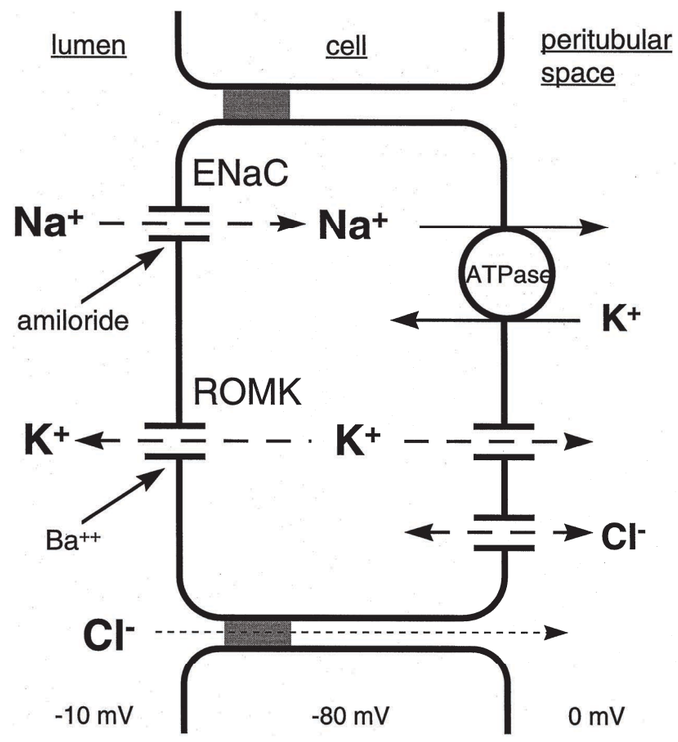

FIGURE 5.10 A model of salt transport by the principal cell of the cortical collecting duct. Apical Na^+ entry proceeds via amiloride-sensitive Na^+ channels (ENaC). Apical K^+ channels (ROMK) mediate K^+ secretion by this segment. Cl^- absorption is driven by the lumen-negative voltage through the paracellular pathway.

the cell, so that the cellular K^+ is above its equilibrium concentration, and thus leaves the cell through conductive pathways in the apical or basolateral membrane.[360]

Apical Na^+ Channels

Apical Na^+ entry depolarizes the apical membrane relative to the basolateral membrane, causing a lumen-negative transepithelial voltage, which in turn provides the driving force for Cl^- reabsorption through the paracellular pathway. Patch-clamp studies of collecting duct cells and urinary bladder cells provided some details regarding the electrophysiologic properties and regulation of apical membrane Na^+ channels.[361] The amiloride-sensitive epithelial Na^+ channel (ENaC) has been cloned,[362,363] has a single-channel conductance to Na^+ of 4 to 5 pS, and is highly selective for Na^+ over K^+ ($P_{Na}/P_K > 20$). ENaC exhibits slow gating, with openings and closings lasting several seconds. Amiloride blocks ENaC at submicromolar concentrations.[364]

ENaC consists of three homologous subunits (α, β, and γ).[363] The subunits share a common structure consisting of two transmembrane domains, intracellular N- and C-termini, and a large extracellular loop.[365,366] Although the α subunit of ENaC can form Na^+ channels on its own,[362] coexpression of all three subunits dramatically increases the membrane Na^+ conductance.[363] Based on homology to the related acid-sensing ion channel (ASIC1), the crystal structure of which was recently solved,[367] native ENaC likely exists as an α, β, and γ heterotrimer. The single channel properties of the expressed channel closely resemble those of the 4 to 5 pS highly selective channel studied in native tissues.[363] The large extracellular domains of the α and γ subunits can get proteolytically cleaved by furin in the biosynthetic pathway.[368,369] Other proteases like prostasin and plasmin may cleave the γ subunit.[370] These cleavage events release small inhibitory fragments of the α and γ subunits, causing an increase in the open probability of ENaC.[370] ENaC activity is also enhanced by increasing luminal flow rates, as the extracellular domain of ENaC subunits respond to laminar shear stress.[371,372] Thus, collecting duct Na^+ absorption is enhanced with increased distal fluid delivery via ENaC, a mechanosensitive channel.[373]

The key importance of ENaC for Na^+ reabsorption in the collecting duct is highlighted by naturally occurring mutations in ENaC that are responsible for Liddle syndrome, an autosomal dominant form of hypertension, and pseudohypoaldosteronism type 1, an autosomal recessive form of salt wasting (see Table 5.1).[374] In Liddle syndrome, mutations in the ENaC subunits result in an increase in amiloride-sensitive Na^+ channel activity with a consequent increase in sodium reabsorption and volume-mediated hypertension.[375–377] Most Liddle mutations occur in the cytoplasmic C-termini of the γ (SCNN1G) and β (SCNN1B) subunits.[375] These mutations affect a conserved PY motif in the C-terminus, which is necessary for interaction with the ubiquitin ligase Nedd4-2 and subsequent internalization and degradation of ENaC.[378] Liddle syndrome mutations cause excessive ENaC accumulation at the apical plasma membrane, increasing Na^+ absorption. Pseudohypoaldosteronism type 1 (PHA-1), the clinical opposite of Liddle syndrome, is caused by homozygous inactivating mutations in the ENaC channel, resulting in a syndrome of Na^+-wasting, hypotension, and hyperkalemia.[379] The majority of mutations causing PHA-1 are frameshift or nonsense mutations that result in truncated, nonfunctional ENaC proteins.[374]

Apical Electroneutral Na^+ Transport

It was first observed in rats that a portion of Na^+ entry across the apical membrane in perfused CCD segments was sensitive to luminal hydrochlorothiazide, which inhibited Na^+ and Cl^- absorption without changing the transepithelial voltage.[380] In addition, amiloride inhibited Na^+ transport in this segment by only 50%, and the effects of amiloride and hydrochlorothiazide were additive. It was thus concluded that the CCD may possess two parallel transport pathways for Na^+: an electrogenic pathway involving amiloride-sensitive Na^+ channels and a thiazide-sensitive neutral NaCl cotransport pathway.[380] More recently, a Na^+-dependent Cl^-/HCO_3^- exchanger (NDCBE/SLC4A8) was identified in intercalated cells and found to mediate the amiloride-resistant, thiazide-sensitive electroneutral Na^+ reabsorption in the CCDs of mice.[381] That NCC knockout mice also exhibited significant natriuresis following treatment with thiazide diuretics suggests the importance of this pathway. The mechanism for net NaCl reabsorption in this setting involves the parallel activity of the NDCBE and Na^+-independent Cl^-/HCO_3^- exchange via pendrin/SLC26A4 at the apical membrane of type B intercalated cells. The basolateral reabsorption pathways for the Na^+ and Cl^- that enter at the apical membrane in this model are the Na^+,K^+–ATPase and ClC-K Cl^- channels. Thus, a novel target of thiazide diuretics distinct from NCC has been identified in the collecting duct with the implication that Cl^- transport by intercalated cells plays an important role in blood pressure regulation.[382] Because pendrin is a key mediator of bicarbonate secretion, and thus acid–base regulation, these findings also suggest a strong link between acid–base and volume/blood pressure regulation.

Control of Na^+ Absorption in the Cortical Collecting Duct

Aldosterone

Aldosterone is one of the key regulators of Na^+ transport in the collecting duct, where it increases the rates of Na^+ absorption and K^+ secretion.[383,384] The major target site for mineralocorticoid effects is the principal cell of the CCD, although actions in the DCT have also been documented (see previous). Mineralocorticoid effects are produced by the binding of either mineralocorticoids or glucocorticoids[385] to mineralocorticoid receptors found predominantly in the CCD.[336,337] In addition, the binding of glucocorticoids to glucocorticoid receptors can also produce mineralocorticoid responses.[385] This lack of specificity results from two

factors: first, mineralocorticoid receptors do not discriminate between aldosterone and glucocorticoids,[386] so that either class of steroids can bind to and activate the receptor. Second, the DNA binding domains of mineralocorticoid and glucocorticoid receptors are highly conserved such that both receptors can activate many of the same genes.[387] The specificity for mineralocorticoids in vivo is provided by the selective degradation of glucocorticoids, but not mineralocorticoids, by the enzyme 11 β-hydroxysteroid dehydrogenase.[386] 11β-Hydroxysteroid dehydrogenase activity is high in CCD segments.[388,389] Illustrating the important role of this enzyme in regulating access of hormones to the mineralocorticoid receptor, genetic deficiency of this enzyme produces a syndrome, apparent mineralocorticoid excess, resembling hyperaldosteronism (hypertension, hypokalemia, metabolic alkalosis), except that aldosterone levels are low.[390] The clinical manifestations result from the stimulation of mineralocorticoid receptors by circulating glucocorticoids.

In isolated perfused CCDs from mineralocorticoid-treated rabbits, there is an increase in both the Na^+ and K^+ conductance of the apical membrane and an increase in basolateral Na^+,K^+–ATPase activity.[356] The functional changes after aldosterone treatment are accompanied by morphologic changes in principal cells. The basolateral membrane length of principal cells falls by 35% after an adrenalectomy, and the administration of aldosterone, but not dexamethasone, restores the membrane length to control levels.[391]

An early effect of aldosterone, occurring within a few hours of exposure, is an increase in the sodium permeability of the apical membrane of the CCD principal cell. Results from electrophysiologic and immunologic studies support the view that the early aldosterone-induced increase in apical sodium permeability is because of the activation of quiescent Na^+ channels rather than the synthesis and/or the insertion of new Na^+ channels into the membrane.[392–394] Several pathways have been implicated in the aldosterone-mediated increase in Na^+ channel activity. An important downstream mediator of aldosterone is the serum and glucocorticoid-regulated kinase SGK1.[395] SGK1 is expressed in the thick ascending limb, DCT, connecting segment, and cortical collecting tubules.[396] Aldosterone increases the transcription of SGK1 in vitro,[395] although the effects of aldosterone on SGK1 protein expression in vivo appear to be minor.[396] Coexpression of SGK1 with ENaC results in markedly greater sodium currents than seen with ENaC alone.[395,397] SGK1 is required for the stimulation of sodium transport by aldosterone both in vitro[397] and in vivo.[398] SGK1 increases sodium currents by increasing cell surface expression of ENaC[399] and also increasing the open probability of individual ENaC channels.[400] The effect of SGK1 on cell surface expression of ENaC is largely mediated by Nedd4-2. Specifically, Nedd4-2 is a substrate for SGK1. Upon phosphorylation by SGK1, the affinity of Nedd4-2 for the PY domains of ENaC is diminished,[401] whereas Nedd4-2 binding to 14-3-3 scaffolding proteins is increased.[402,403] Thus, 14-3-3 proteins act to sequester Nedd4-2 and prevent it from binding to ENaC. As discussed earlier, the binding of Nedd4-2 to ENaC induces internalization and degradation of the channel.

Recently, additional aldosterone-induced proteins have been discovered, including the glucocorticoid-induced leucine zipper protein (GILZ1), the transcription of which is rapidly induced by aldosterone in collecting duct principal cells. GILZ1 stimulates ENaC-mediated Na^+ transport and ENaC surface expression by inhibiting Raf-1 in the extracellular signal-regulated kinase (ERK) signaling pathway.[404,405] Soundararajan and colleagues[406] have also identified another aldosterone-induced protein, CNK3 (connector enhancer of kinase suppressor of Ras 3), which also stimulates ENaC and appears to be a scaffolding protein. The emerging story is that a multiprotein complex exists in CCD cells that promotes context-specific aldosterone signal transduction to induce ENaC activity and apical Na^+ conductance. Specifically, aldosterone stimulates ENaC activity through synergistic aldosterone-dependent activation by SGK1 via Nedd4-2 and by GILZ1 via Raf-1 and CNK3.

Nongenomic effects of aldosterone (rapid effects that are mineralocorticoid receptor-independent) have also been described in many tissues, including the kidney.[407] Elucidating the mechanisms of such effects is an area of active investigation and appears to include the mitogen-activated protein kinase (MAPK) pathway and the methylation of proteins and lipids. Aldosterone also increases the activity of a number of cellular methyltransferase enzymes.[408] Moreover, the β subunit of ENaC itself is a substrate for methylation, and, when methylated, exhibits increased Na^+ transport activity.[409] The effects of aldosterone on Na^+ channel activity may also involve small GTP-binding proteins, for example, Ras,[410] and phosphatidylinositol 3-kinase.[411] SGK1 is a downstream mediator of phosphoinositide-3-kinase.[412]

Sometime after the increase in apical membrane Na^+ conductance occurs, the basolateral membrane Na^+,K^+–ATPase activity and pump current increase.[413] This increase is due initially to the effect of increased cell sodium activity on existing pump units.[414] Later, aldosterone induces the synthesis of additional Na^+,K^+–ATPase pump subunits.[415] Increased apical membrane Na^+ entry may promote the late synthesis of Na^+,K^+–ATPase, because the inhibition of sodium entry by amiloride markedly reduced the aldosterone-induced increase in Na^+,K^+–ATPase activity.[416]

Aldosterone also exerts an additional, late effect on the amiloride-sensitive Na^+ conductance. Patch-clamp studies in rats exposed to high levels of aldosterone for several days demonstrated a large increase in the amiloride-sensitive whole cell Na^+ conductance,[417] which correlated with an increase in the number of active Na^+ channels in the apical membrane.[418] Increases in ENaC mRNA[419] and protein[334] levels in aldosterone-treated tissues suggest that the synthesis of new ENaC channels may contribute to the late aldosterone-induced increase in Na^+ conductance. SGK1, in addition to increasing the cell surface expression of ENaC, also regulates the transcription of ENaC subunits, principally α.[420]

Antidiuretic Hormone (ADH)

Exposure of rat CCD segments in vitro to ADH results in a sustained stimulation of Na^+ absorption.[421] ADH increases the transepithelial potential, depolarizes the apical membrane, and increases the conductance of the apical membrane of principal cells.[422] These changes are entirely reversed by luminal amiloride, indicating that ADH increases the apical membrane sodium conductance of the principal cell. These effects of ADH are mediated intracellularly by cAMP.[423] Moreover, the effects of ADH on Na^+ transport in the rat CCD are enhanced by prior treatment of the animals with mineralocorticoids.[421]

ADH and aldosterone increase apical membrane ENaC activity through both overlapping and distinct mechanisms of action. Like aldosterone via SGK1, ADH induces enhanced PKA-dependent phosphorylation of Nedd4-2 at phosphorylation sites that overlap those of SGK1.[353] As described previously, these phosphorylation events promote sequestration of Nedd4-2 through enhanced binding to 14-3-3 proteins. In contrast to aldosterone, which can activate quiescent channels, ADH, via cAMP, promotes the insertion of additional Na^+ channels into the apical membrane.[424] In addition, PKA-mediated phosphorylation may also directly stimulate the activity of ENaC channels already present in the apical membrane, potentially both directly[425] and indirectly via Nedd4-2 inhibition, causing enhanced channel residency time at the membrane and thus proteolytic cleavage.[370] Long-term exposure to ADH may also increase the capacity of the collecting duct for Na^+ transport by increasing the expression of both Na^+,K^+–ATPase and ENaC.[426]

Other Agents

Bradykinin is produced in the connecting duct[427] and binds to specific receptors in the CCD.[428] An intrarenal infusion of bradykinin produces a diuresis. Bradykinin has been reported to reduce Na^+ absorption in rat CCD segments.[429] More recently, bradykinin was shown to acutely and reversibly decrease the open probability of ENaC in patch-clamp studies performed ex vivo on split open CCDs isolated from rats. This effect appears to be mediated specifically through the bradykinin B_2 receptor.[430]

As will be discussed in the following section, the major site of action of atrial natriuretic peptide (ANP) is the inner medullary collecting duct. ANP stimulates cGMP production in the CCD,[431] and inhibits the hydro-osmotic actions of ADH in the CCD.[432] Biphasic effects of ANP on conscious, sedated rats have been observed with acute water and sodium excretion within minutes, followed by retention after 90 minutes, which was associated with increased apical membrane expression of α- and γ-ENaC. These delayed effects may represent a compensatory response to increase sodium and water reabsorption and prevent volume depletion in response to prolonged ANP infusion.[433]

α2-Adrenergic agonists inhibit sodium reabsorption in the rat CCD.[434] This inhibition is associated with an increase in the apical membrane resistance, reflecting decreased Na^+ entry through Na^+ channels. These changes appear to result from an inhibition of adenylate cyclase and antagonism of ADH by α2 agonists.[435]

Prostaglandin E_2 exerts diuretic and natriuretic effects on the kidney. Part of this action is mediated by an inhibition of Na^+ absorption in the CCD.[436] The application of PGE_2 to the basolateral surface of perfused rabbit CCD segments reversibly inhibits the negative transepithelial voltage and net sodium absorption.[436] The effect of PGE_2 on sodium transport is coupled to a rise in intracellular [Ca^{2+}] and is dependent on the activation of PKC.[437] Four PGE_2 receptor subtypes, designated EP1, EP2, EP3, and EP4, have been characterized. Studies using receptor subtype–specific agonists and antagonists suggest that the EP1 receptor mediates PGE_2-dependent inhibition of Na^+ transport in the CCD.[438] This inhibition of Na^+ transport is associated with depolarization of the apical membrane voltage, consistent with inhibition of the basolateral Na^+,K^+-ATPase.[439] In contrast to the inhibitory effect of basolateral PGE_2 on Na^+ transport, luminal PGE_2 increases transepithelial voltage and presumably Na^+ absorption via the EP4 receptor.[440]

Epidermal growth factor (EGF) reduces Na^+ absorption in rabbit CCD by about 50% via the inhibition of apical electrogenic Na^+ entry via ENaC.[441,442] More recent studies performed on cultured mouse CCD cells suggest that the effects are mediated by the ErbB2 EGF receptor and that there is actually a biphasic effect of EGF on ENaC activity.[443] Acutely (<4 h), EGF treatment increases the ENaC current, an effect that appears to be mediated via the PI-3-kinase pathway. Chronically (>8 h), the ENaC current was inhibited via effects through the MEK/ERK pathway.[443]

Endothelin-1 (ET-1) is heavily secreted from the basolateral aspect of CD epithelial cells where it binds to basolateral ET_B receptors and may thus act in an autocrine fashion to inhibit both Na^+ and water reabsorption in this segment, as shown in vitro.[444] The physiologic relevance of these findings has been confirmed in vivo, because mice with CD-specific knockout of ET-1 or ET receptors are hypertensive on a normal Na^+ diet and have exacerbated Na^+ retention and hypertension when placed on a high-Na^+ diet. ET-1 inhibits ENaC activity through Src- and MAPK-dependent pathways.[444]

Nitric oxide is also a downstream mediator of ET-1,[444] where it decreases Na^+ transport in rat CCD segments by 40% to 80%.[445] The addition of nitric oxide to tubules decreased the intracellular [Na^+], but did not affect the activity of basolateral Na^+,K^+–ATPase, suggesting that the primary effect of nitric oxide is to inhibit apical Na^+ entry via ENaC. The inhibitory mechanism involving NO remains to be determined.[444]

Dopamine inhibits ADH-dependent Na^+ transport and transepithelial voltage in rat and rabbit CCD segments, although the particular basolateral dopamine receptor subtype may be species specific.[446,447]

Ang II stimulates sodium channel activity in rabbit and mouse CCDs.[448] Chronic Ang II infusion also increases the abundance of the α subunit of ENaC, the rate-limiting subunit for ENaC assembly.[449] Both the acute effects of Ang II on ENaC activity and the chronic effects on ENaC abundance are mediated by the AT1 receptor.[448,449] A more recent study performed on rats suggests that the Ang II-induced ENaC stimulation downstream of the AT1 receptor involves a Ca^{2+}-independent PKC pathway that induces superoxide generation. The blocking of arachidonic acid–induced inhibition of ENaC may also play a role.[450]

Bicarbonate (HCO_3^-) changes in the CCD lumen, which may reflect changes in total-body acid–base status or local HCO_3^- secretion from neighboring intercalated cells via the apical Na^+-independent Cl^-/HCO_3^- exchanger, pendrin, have been shown recently to modulate Na^+ reabsorption in the CCD.[451,452] Specifically, the bicarbonate-stimulated soluble adenylyl cyclase (sAC) appears to stimulate both basal and agonist-stimulated Na^+ reabsorption in the kidney collecting duct, acting to enhance Na^+,K^+–ATPase catalytic activity.[451] Another study suggests that pendrin increases ENaC abundance and activity, at least in part by increasing luminal [HCO_3^-].[452]

The elucidation of other cellular signaling pathways and kinases that are important in the regulation of ENaC, and Na^+ transport in the CCD is a very active area of research and involves signaling pathways downstream of diverse stimuli, including inflammation (IkappaB kinase/NF-kappaB/NF-κB pathway)[453] and metabolic stress (AMP-activated protein kinase).[454] A full treatment of these signaling pathways is beyond the scope of this chapter, but the interested reader is referred to a recent review.[455]

NA^+ TRANSPORT IN THE OUTER MEDULLARY COLLECTING DUCT

The transport properties of the outer medullary collecting duct (OMCD) have been studied by in vitro perfusion of isolated tubule segments. The functional properties of the OMCD differ depending on the location of the segment within the outer medulla. Segments within the outer stripe of the outer medulla ($OMCD_o$) exhibit electrophysiologic properties resembling the cortical collecting duct; that is, a lumen negative transepithelial voltage and electrogenic apical Na^+ entry.[456] Compared to the CCD, the $OMCD_o$ displays a less negative transepithelial voltage, much lower ionic permeabilities, and a lower rate of active reabsorption of Na^+.[457] As the collecting duct descends into the medulla, principal cells, which mediate Na^+ and K^+ transport in the CCD (see previous), are replaced by cells the electrical properties of which are similar to intercalated cells of the CCD (i.e., the apical membrane lacks a demonstrable Na^+ or K^+ conductance).[458] Within the inner stripe of outer medulla ($OMCD_i$), principal cells are virtually absent and no net Na^+ absorption occurs.[457]

NA^+ TRANSPORT IN THE INNER MEDULLARY COLLECTING DUCT

Mechanism of Na^+ Transport

The analysis of salt transport by the inner medullary collecting duct (IMCD) has been confounded by problems of axial tubule heterogeneity, species variability, and differences in experimental approach. Based on morphologic factors, the IMCD has been divided into three subsegments: $IMCD_1$, $IMCD_2$, and $IMCD_3$.[459] This morphologic heterogeneity is paralleled, to some extent, by functional heterogeneity. For example, the urea permeability and its responsiveness to ADH increases from $IMCD_1$ to $IMCD_3$ with increasing medullary depth.[460] Further complicating the analysis is the observation that similar subsegments from different species exhibit different properties relating to salt transport.[461,462] Finally, for unclear reasons, studies examining IMCD function in vivo (e.g., by microcatheterization)[463] have yielded markedly different results than have in vitro studies of isolated perfused tubules.[461,464]

Microelectrode impalement studies of IMCD segments from rats demonstrated that the apical membrane constituted the major cellular resistance, and the luminal application of amiloride increased the apical membrane voltage and resistance and decreased the transepithelial voltage.[461] These results, and others,[465] are consistent with the presence of an amiloride-sensitive sodium conductance in the apical membrane of IMCD cells. Patch-clamp studies of cultured rat IMCD cells indicate that Na^+ entry is mediated by a 20 to 30 pS amiloride-sensitive, nonselective, cGMP-gated cation channel in the apical membrane.[465,466] The basolateral membrane of IMCD cells contains the Na^+,K^+–ATPase, a K^+ conductance and a HCO_3^- conductance.[461]

The results discussed previously can be combined into a model for sodium transport in the IMCD (Fig. 5.11). Na^+

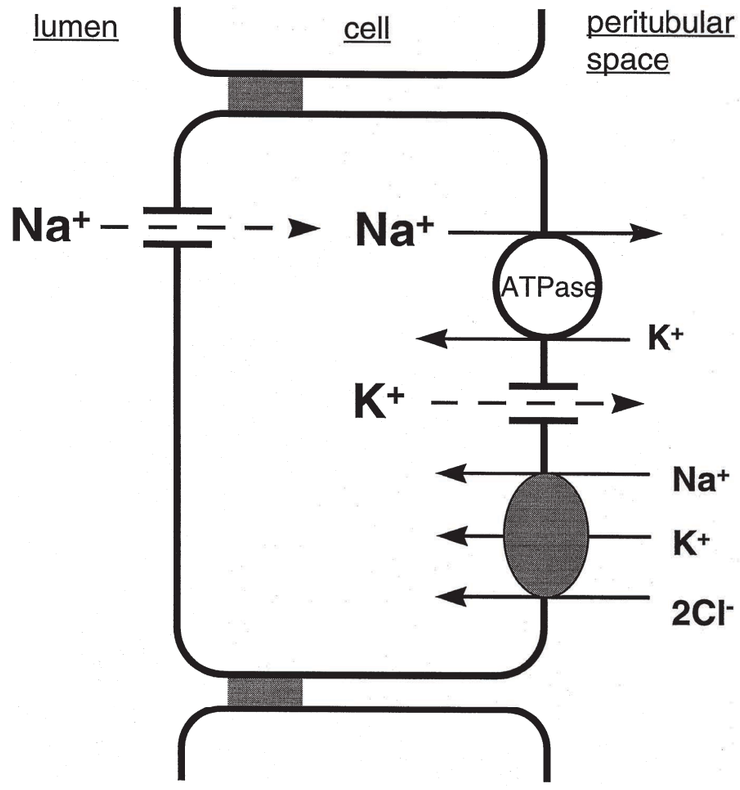

FIGURE 5.11 A model of Na^+ transport in the inner medullary collecting duct. Apical Na^+ entry proceeds via a nonselective amiloride-sensitive cation channel. Basolateral Na^+-K^+-$2Cl^-$ cotransport may be involved in Na^+ secretion.

entry across the apical membrane occurs down its steep electrochemical gradient through amiloride-sensitive Na^+ channels. Na^+ is extruded across the basolateral membrane by the Na^+,K^+–ATPase. The basolateral membrane K^+ conductance serves to recycle the K^+ that enters via the Na^+,K^+–ATPase. The K^+ conductance also hyperpolarizes the cell, thereby favoring Na^+ entry across the apical membrane.

There is some evidence for additional electroneutral Na^+ entry pathways in rat IMCD cells, although their importance in net Na^+ absorption by the IMCD in other species remains unclear. Furosemide and thiazide diuretics both inhibit a portion of Na^+ absorption by rat IMCD segments in vivo.[467,468] However, Zeidel and colleagues[469] failed to demonstrate sensitivity of rabbit IMCDs to loop diuretics and found that conductive Na^+ entry at the apical membrane predominated. A Na^+-K^+-$2Cl^-$ cotransporter appears to be present in the basolateral membrane of terminal IMCD segments.[470] The cloned Na^+-K^+-$2Cl^-$ cotransporter from cultured IMCD cells represents the "secretory" isoform of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) rather than the absorptive isoform present in the apical membrane of the thick ascending limb.[471] Finally, the Na^+-HCO_3^- cotransporter NBCn1 has been found in the basolateral membrane of rat IMCDs, which may contribute to cellular defense against both acidification and volume changes in this segment.[472]

Regulation of Na^+ Transport

The IMCD appears to be the major target site for the potent diuretic hormone ANP.[473] This hormone, working through cGMP,[474] inhibits Na^+ entry via apical Na^+ channels. In cell-attached and excised patches of cultured inner medullary cells, both ANP and dibutyryl cGMP inhibited the activity of the cation channel.[475]

In addition to inhibiting Na^+ reabsorption, ANP may stimulate Na^+ secretion in the IMCD.[470] In isolated perfused IMCD segments, ANP increased the bath to lumen flux rate of Na^+ and Cl^-, an effect that was inhibited by peritubular furosemide and by omission of either counterion, thus suggesting the role of a basolateral membrane Na^+-K^+-$2Cl^-$ cotransporter.[476] Sands et al.,[464] however, found no effect of ANP on the Na^+ permeability of the rat IMCD.

In one study,[477] ADH stimulated amiloride-sensitive Na^+ absorption by terminal IMCD segments perfused in vitro. Other studies, however, failed to find an effect of ADH or cAMP on the amiloride-sensitive cation channel in apical membranes of cultured IMCD cells.[464,465]

Micropuncture studies by Ullrich and Papavassiliou[478] demonstrated that mineralocorticoids increased net Na^+ absorption in the terminal IMCD, which was attributed to a decrease in the passive Na^+ permeability of the tubule leading to a decrease in back leak of NaCl into the lumen. Mineralocorticoids may also increase active Na^+ reabsorption in the IMCD. For example, aldosterone produced a three- to seven-fold stimulation of electrogenic Na^+ transport in cultured rat IMCD cells,[479] and chronic mineralocorticoid exposure in vivo increased the activity of Na^+,K^+ ATPase in the IMCD.[480]

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CHAPTER

6 Tubular Potassium Transport

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TUBULAR POTASSIUM TRANSPORT

Potassium (K^+) functions in a diversity of physiologic processes. Changes in intracellular K^+ affect cell volume, intracellular pH, enzymatic function, protein synthesis, DNA synthesis, and apoptosis. Changes in the ratio of intracellular to extracellular K^+ affect the cellular resting membrane potential, causing depolarization in hyperkalemia and hyperpolarization in hypokalemia; as a consequence, potassium disorders primarily affect excitable tissues, chiefly heart and muscle. Hypokalemia and hyperkalemia also have a variety of renal and cardiovascular consequences. A large body of experimental and epidemiologic evidence thus implicates hypokalemia or reduced K^+ intake in the pathogenesis of hypertension, heart failure, and stroke.[1] Hypokalemia also causes a host of structural and functional changes in the kidney, whereas hyperkalemia in turn has a significant effect on the ability to excrete an acid urine due to interference with the urinary excretion of ammonium (NH_4^+).

Potassium is almost exclusively an intracellular cation, with only 2% of total body K^+ contained within the extracellular fluid. Extracellular K^+ is maintained within a very narrow range by three major mechanisms. First, the distribution of K^+ between the intracellular and extracellular space is determined by the activity of a number of widely expressed and/or ubiquitous transport pathways. A net increase in cellular uptake can thus cause transient hypokalemia, whereas impairment of cellular uptake can lead to hyperkalemia. Second, the colon has the ability to absorb and secrete K^+, with significant mechanistic and regulatory similarities to renal K^+ transport. However, the colon has a relatively limited capacity for K^+ excretion, and a third mechanism, changes in renal K^+ excretion, plays the dominant role in responding to changes in K^+ intake. Regulated increases in K^+ secretion by the connecting tubule (CNT) and the cortical collecting duct (CCD) play a critical role in the response to hyperkalemia and K^+ loading, whereas inhibition of K^+ secretion and increases in the reabsorption of K^+ by the CCD and the outer medullary collecting duct (OMCD) function in the response to hypokalemia or K^+ deprivation.

This chapter reviews the renal and extrarenal mechanisms of K^+ homeostasis, with primary emphasis on the physiology of renal K^+ transport.

Extrarenal Potassium Homeostasis

The intracellular accumulation of K^+ against its electrochemical gradient is an energy-consuming process, mediated by the ubiquitous Na^+/K^+–ATPase. The Na^+/K^+–ATPase functions as an electrogenic pump, with a transport stoichiometry of three intracellular Na^+ ions to two extracellular K^+ ions. The enzyme complex is made up of a tissue-specific combination of multiple α, β, and γ subunits, which are further subject to tissue-specific patterns of regulation. Cardiac glycosides (i.e., digoxin and ouabain) bind to the α subunits of Na^+/K^+–ATPase at an exposed extracellular hairpin loop that also contains the major binding sites for extracellular K^+.[2] The binding of digoxin and K^+ to the Na^+/K^+–ATPase complex is thus mutually antagonistic, explaining in part the potentiation of digoxin toxicity by hypokalemia.[3] Although the four α subunits have equivalent affinity for ouabain, they differ significantly in the intrinsic K^+/ouabain antagonism.[4] Ouabain binding to isozymes containing the ubiquitous α1 subunit is relatively insensitive to K^+ concentrations within the physiologic range, such that this isozyme is protected from digoxin under conditions wherein cardiac α2 and α3 subunits, the therapeutic targets of digoxin, are inhibited.[4] Notably, the digoxin/ouabain binding site of α subunits is highly conserved, suggesting a potential role in the physiologic response to endogenous ouabain/digoxinlike compounds. Consistent with this hypothesis, a mouse strain that expresses α2 subunits with engineered resistance to ouabain is strikingly resistant to ouabain-induced hypertension and to adrenocorticotrophic hormone-dependent hypertension,[5] the latter of which is known to involve an increase in circulating ouabainlike glycosides.

Potassium can also accumulate in cells by coupling to the gradient for Na^+ entry, entering via the electroneutral Na^+-K^+-$2Cl^-$ cotransporters NKCC1 and NKCC2. The NKCC2 protein is found only at the apical membrane of

the thick ascending limb (TAL) and the macula densa cells, where it functions in transepithelial salt transport and tubular regulation of renin release (see Potassium Transport in the Thick Ascending Limb).[6] NKCC1 is widely expressed in multiple tissues,[6] including muscle, where it modulates extrarenal K^+ homeostasis.[7] The cotransport of K^+-Cl^- by the four K^+-Cl^- cotransporters (KCC1 to 4) can also function in the transfer of K^+ across membranes; although the KCCs typically function as efflux pathways, they can mediate influx when extracellular K^+ increases.[6]

Skeletal muscle contains approximately 75% of the body's potassium and exerts considerable influence on extracellular K^+. Skeletal muscle Na^+/K^+–ATPase activity in particular is a major determinant of the capacity for extrarenal K^+ homeostasis. Hypokalemia induces a marked decrease in muscle K^+ content and Na^+/K^+–ATPase activity, an altruistic[8] mechanism to regulate plasma K^+. This adaptation is primarily mediated by dramatic decreases in the protein abundance of the α-2 subunit of Na^+/K^+–ATPase. In contrast, hyperkalemia due to potassium loading is associated with adaptive increases in muscle K^+ content and Na^+/K^+–ATPase activity.[9] These interactions are reflected in the relationship between physical activity and the ability to regulate extracellular K^+ during exercise[10]; exercise training is associated with increases in muscle Na^+/K^+–ATPase concentration and activity, with reduced interstitial K^+ in trained muscles and an enhanced recovery of plasma K^+ after defined amounts of exercise.[10]

Several hormones have been implicated in the control of extrarenal K^+ homeostasis. In particular, increases in plasma K^+ have a stimulatory effect on insulin levels,[11] which in turn stimulates the uptake of K^+ by muscles, the liver, and other tissues. In contrast, insulin-stimulated K^+ uptake is rapidly reduced by 2 days of K^+ depletion, prior to a modest drop in plasma K^+,[12] and in the absence of a change in plasma K^+ in rats subject to a lesser K^+ restriction for 14 days.[13] Insulin activates muscle Na^+/K^+–ATPase, inducing the translocation of the Na^+/K^+–ATPase α-2 subunit to the plasma membrane with a lesser effect on the α1 subunit.[14] This translocation is dependent on the activity of phosphoinositide-3 (PI-3) kinase,[14] which itself also binds to a proline-rich motif in the N-terminus of the α subunit; the activation of PI3-kinase by insulin induces phosphatase enzymes to dephosphorylate a specific serine residue adjacent to the PI3-kinase binding domain. Trafficking of Na^+/K^+–ATPase to the cell surface also requires phosphorylation of an adjacent tyrosine residue, perhaps catalyzed by the tyrosine kinase activity of the insulin receptor itself.[15] In addition, the serum and glucocorticoid-induced kinase 1 (SGK1) plays a critical role in insulin-stimulated K^+ uptake via stimulatory effects on Na^+/K^+–ATPase activity and/or the Na^+-K^+–$2Cl^-$ cotransport.[16] The hypokalemic effect of insulin plus glucose is thus blunted in SGK1 knockout mice, with a marked reduction in hepatic insulin-stimulated K^+ uptake.[16]

The sympathetic nervous system also affects the balance between extracellular and intracellular K^+. The uptake of K^+ by the liver and muscles is stimulated via β_2 receptors. This hypokalemic effect of catecholamines is independent of changes in circulating insulin and has been reported in nephrectomized animals.[17] The cellular mechanisms whereby catecholamines induce K^+ uptake in muscles include an activation of the Na^+/K^+–ATPase, likely via increases in cyclic-AMP (cAMP).[18] Skeletal muscle β-adrenergic receptors also activate NKCC1, which may account for as much as one third of the uptake response.[7] In contrast to β-adrenergic stimulation, α-adrenergic agonists impair the ability to buffer increases in K^+ induced via intravenous loading or by exercise[19]; the transport mechanisms whereby this occurs are not known. β-adrenergic stimulation increases K^+ uptake during exercise to lessen exercise-induced hyperkalemia, whereas α-adrenergic mechanisms help blunt the ensuing postexercise nadir.[19]

The efflux of K^+ out of cells is primarily mediated by K^+ channels, which comprise the largest group of ion channels in the human genome. There are three major subclasses of mammalian K^+ channels: the six-transmembrane domain (TMD) family, which encompasses both the voltage-sensitive and Ca^{2+}-activated K^+ channels; the two-pore, four TMD family; and the two TMD family of inward rectifying K^+ (Kir) channels. There is tremendous genomic variety in human K^+ channels. Further complexity is generated by the presence of multiple accessory subunits and alternative patterns of mRNA splicing. Not surprisingly, an increasing number and variety of K^+ channels have been implicated in the control of K^+ homeostasis and the membrane potential of excitable cells such as muscle and heart, with important, evolving roles in the pathophysiology of potassium disorders. Adrenal K^+ channels have also been implicated in the adrenal release of aldosterone induced by hyperkalemia.[20,21] The emphasis in this chapter is on renal K^+ channels, particularly those in the distal nephron that mediate K^+ secretion.

Potassium Transport in the Proximal Tubule

The proximal tubule reabsorbs 50% to 70% of filtered K^+ (Fig. 6.1). Proximal tubules generate minimal transepithelial K^+ gradients, and the fractional reabsorption of K^+ by this nephron segment is similar to that of Na^+.[22] K^+ absorption thus follows that of fluid, Na^+, and other solutes,[23] such that proximal tubules do not play a direct role in regulated renal K^+ excretion. However, changes in Na^+-Cl^- reabsorption by the proximal tubule have considerable effects on distal tubular flow and distal tubular Na^+ delivery, with attendant effects on the excretory capacity for K^+. In addition, K^+ loading has significant effects on proximal tubular Na^+-Cl^- reabsorption. Thus, the intravenous infusion of K^+-Cl^- or increases in the peritubular K^+ concentration causes an inhibition of proximal tubular Na^+-Cl^- reabsorption[24]; this serves to increase both distal tubular flow and distal tubular Na^+ delivery, thus increasing distal K^+ secretion after K^+ loading.

The mechanisms involved in transepithelial K^+ transport by the proximal tubule are not completely clear, although

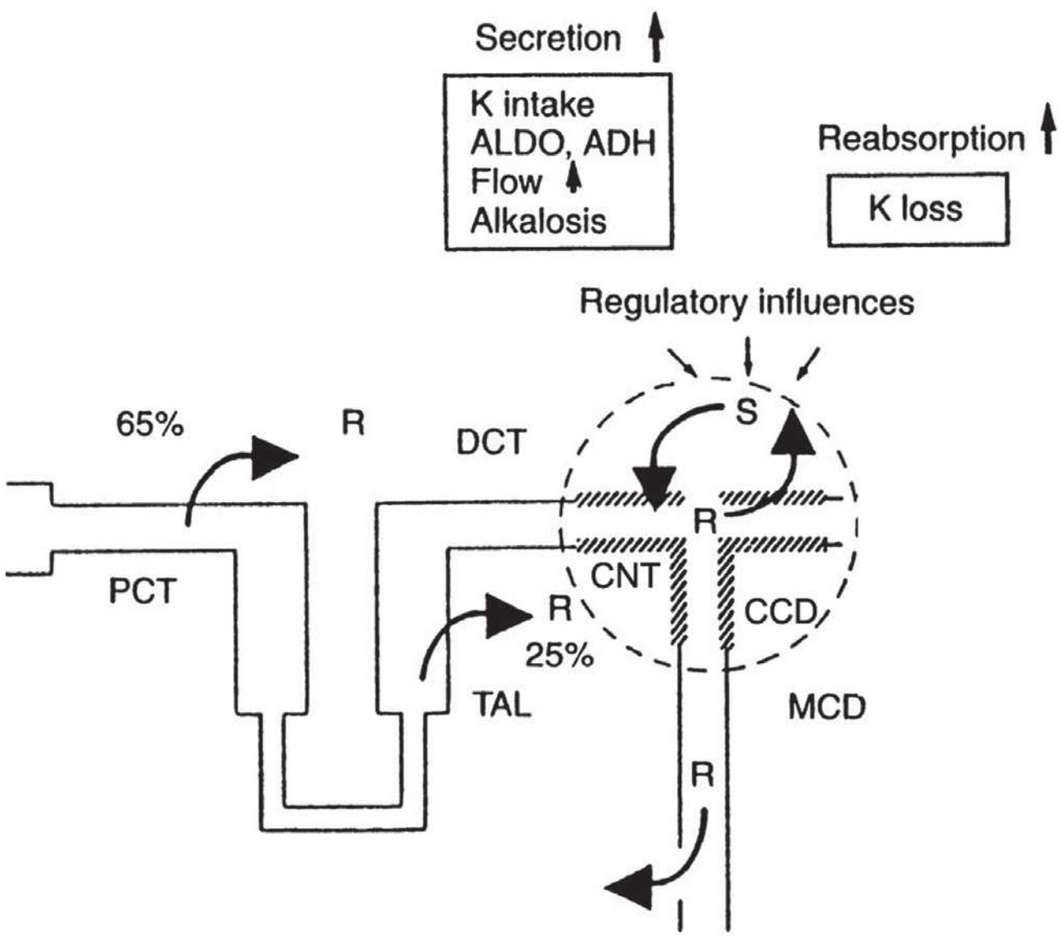

FIGURE 6.1 Potassium transport along the nephron. Approximately 90% of filtered K^+ is reabsorbed by the proximal tubule and the loop of Henle. K^+ is secreted in the connecting tubule and the cortical collecting duct; net reabsorption occurs in response to K^+ depletion, primarily within the medullary collecting duct. *PCT*, proximal tubule; *TAL*, thick ascending limb; *DCT*, distal convoluted tubule; *CNT*, connecting tubule; *CCD*, cortical collecting duct; *S*, secretion; *R*, reabsorption; *ALDO*, aldosterone; *ADH*, antidiuretic hormone; *MCD*, medullary collecting duct.

active transport does not appear to play a major role.[23,25] Luminal barium has modest effects on transepithelial K^+ transport, suggesting a component of transcellular transport via barium-sensitive K^+ channels.[26] However, the bulk of K^+ transport is thought to occur via the paracellular pathway,[26,27] driven by the lumen-positive potential difference in the mid-to-late proximal tubule. The total K^+ permeability of the proximal tubule is thus quite high due to the high permeability of the paracellular pathway.[26, 27] The combination of luminal K^+ concentrations that are ~10% higher than that of plasma, a lumen-positive potential difference of ~2 mV, and high paracellular permeability leads to considerable paracellular absorption in the proximal tubule. The tight junction protein claudin-2 plays a key role in paracellular cation transport by the proximal tubule; targeted deletion in knockout mice generates a "tight" epithelium in the proximal tubule, with a reduction in Na^+, Cl^-, and fluid absorption.[28] The loss of claudin-2 expression does not affect the ultrastructure of tight junctions, but does lead to a reduction in paracellular cation permeability.[28]

The absorption of K^+ across the paracellular pathway is thought to occur via convective transport—solvent drag due to frictional interactions between water and K^+—rather than diffusional transport.[29] Notably, however, the primary pathway for water movement in the proximal tubule is quite conclusively transcellular, via water channels in the apical and basolateral membrane. Therefore, the apparent convective transport of K^+ would have to constitute pseudosolvent drag, with uncharacterized, coordinating interactions between water traversing the transcellular route and the diffusion of K^+ along the claudin-dependent paracellular pathway.[29]

The Loop of Henle and Medullary Potassium Recycling

Transport by the loop of Henle functions in medullary K^+ recycling (Fig. 6.2). A considerable fraction of K^+ secreted by the CCD is reabsorbed by the medullary collecting ducts and is then secreted into the late proximal tubule and/or the descending thin limbs of long-looped nephrons.[30] In potassium-loaded rats there is thus a doubling of luminal K^+ in terminal thin descending limbs, with a sharp drop after the inhibition of CCD K^+ secretion by amiloride.[31] Enhancement of CCD K^+ secretion with the treatment of 1-desamino-8-D-arginine vasopressin (dDAVP) also results in an increase in luminal K^+ in descending thin limbs.[32] This recycling pathway (i.e., secretion in CCD, absorption in the OMCD and the inner medullary collecting duct (IMCD), secretion in the descending thin limb)

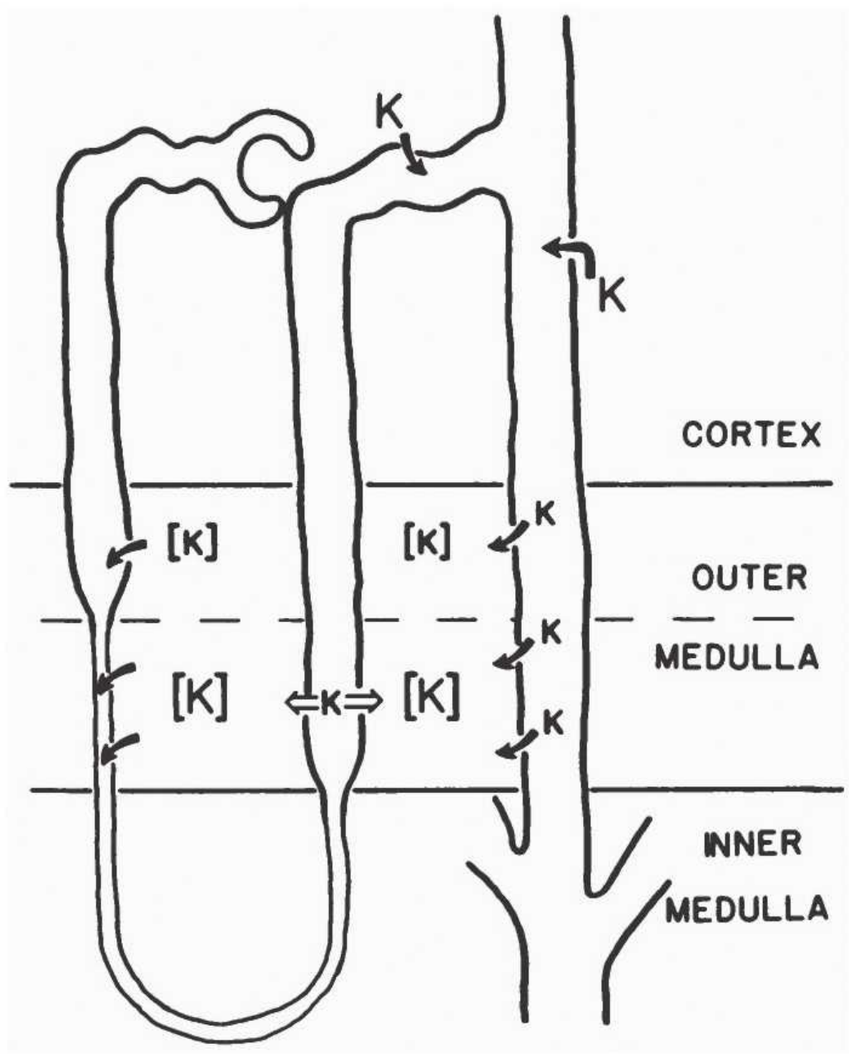

FIGURE 6.2 A schematic representation of medullary K^+ recycling. Medullary interstitial K^+ increases considerably after dietary K^+ loading due to the combined effects of secretion in the cortical collecting duct (CCD), absorption in the outer medullary collecting duct (OMCD), the thick ascending limb (TAL), and the inner medullary collecting duct (IMCD), and secretion in the descending thin limb (see text for details). (From Stokes JB. Consequences of potassium recycling in the renal medulla. Effects of ion transport by the medullary thick ascending limb of Henle's loop. *J Clin Invest*. 1982;70:219–229.)

is associated with a marked increase in medullary interstitial K^+ concentration. Passive transepithelial K^+ absorption by the thin ascending limb and active absorption by the thick ascending limb (TAL)[33] also contribute to this increase in interstitial K^+ (Fig. 6.2). Specifically, the absorption of K^+ by the ascending thin limb, TAL, and OMCD exceeds the secretion by descending thin limbs, thus concentrating K^+ within the interstitium.

K^+ is secreted into descending thin limbs by passive diffusion, driven by the high medullary interstitial K^+ concentration. Descending thin limbs thus have a very high K^+ permeability, without evidence for active transepithelial K^+ transport.[34] Transepithelial K^+ transport by ascending thin limbs has not been measured; however, as is the case for Na^+-Cl^- transport,[35] the absorption of K^+ by thin ascending limbs is presumably passive, via the paracellular pathway.

The physiologic significance of medullary K^+ recycling is not completely clear. However, an increase in interstitial K^+ from 5 to 25 mM dramatically inhibits Cl^- transport by perfused thick ascending limbs.[33] By inhibiting Na^+-Cl^- absorption by the TAL, increases in interstitial K^+ would increase Na^+ delivery to the CNT and CCD, thus enhancing the lumen-negative potential difference (PD) in these tubules and increasing K^+ secretion.[33] A high K^+ diet also reduces the absorption of K^+ by the TAL[36] (i.e., there are direct effects on K^+ secretion by the TAL). The marked increase in medullary interstitial K^+ after dietary K^+ loading is also postulated to limit the difference between luminal and peritubular K^+ in the collecting duct, thus minimizing passive K^+ loss from the collecting duct.

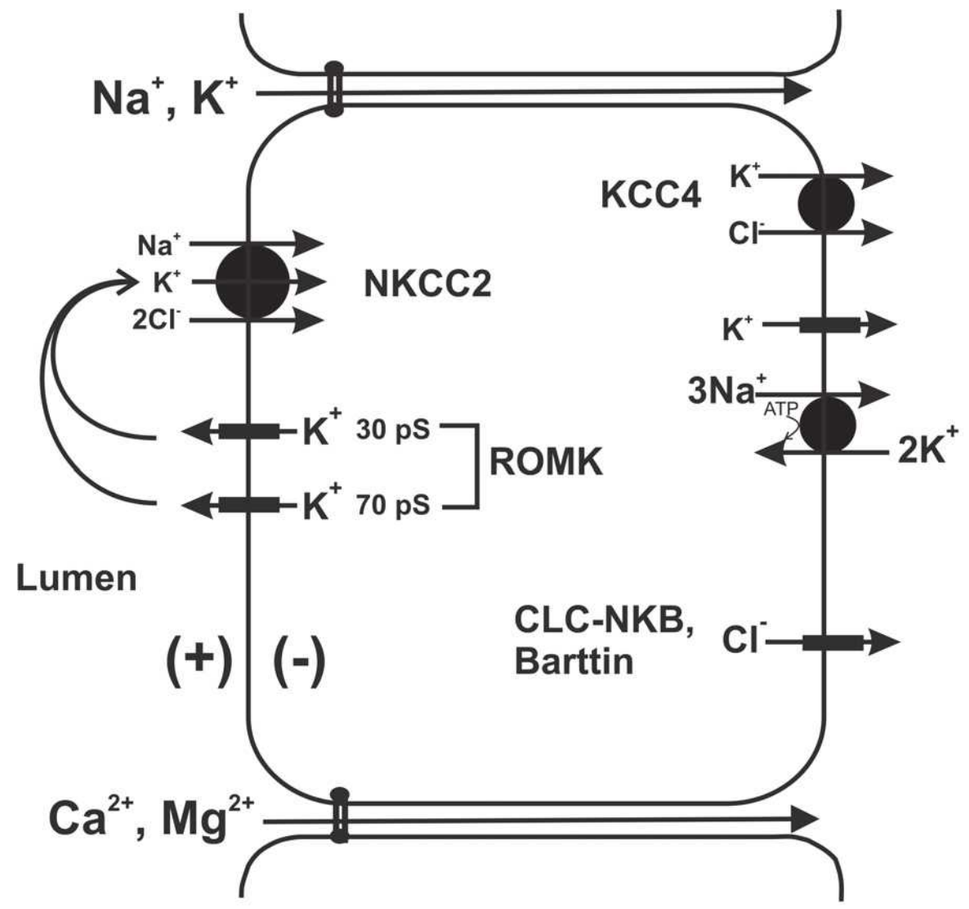

FIGURE 6.3 The potassium transport pathways in the thick ascending limb (TAL); see text for details. *NKCC2*, Na^+-K^+-2Cl– cotransporter 2; *ROMK*, renal outer medullary K^+ channel; *CLC-NKB*, human Cl– channel; *Barttin*, Cl– channel subunit; *KCC4*, K^+-Cl– cotransporter 4.

Potassium Transport in the Thick Ascending Limb

Active transepithelial K^+ transport across the TAL includes both a transcellular component, via the apical Na^+-K^+-$2Cl^-$ cotransporter NKCC2, and a paracellular pathway (Fig. 6.3). Potassium transport appears to differ in the major morphologic subtypes of TAL cells (i.e., rough- and smooth-surfaced cells). Morphologically, the TAL begins abruptly after the thin ascending limb of long-looped nephrons and after the aquaporin-negative segment of short-limbed nephrons. The TAL then extends into the renal cortex, where it meets its parent glomerulus at the vascular pole; the plaque of cells at this junction forms the macula densa, which functions as the tubular sensor for both tubuloglomerular feedback and the tubular regulation of renin release by the juxtaglomerular apparatus. Cells in the medullary TAL are 7 to 8 μm in height, with extensive invaginations of the basolateral plasma membrane and interdigitations between adjacent cells[37]; cells in the cortical TAL are considerably shorter, about 2 μm in height at the end of the cortical TAL in rabbits, with less mitochondria and a simpler basolateral membrane.[37] Macula densa cells also lack the lateral cell processes and interdigitations that are characteristic of medullary TAL cells.[37]

Scanning electron microscopy has revealed that the TAL of both rats[38] and hamsters[39] contains two morphologic subtypes, a rough-surfaced cell type (R cells) with prominent apical microvilli and a smooth-surfaced cell type (S cells) with an abundance of subapical vesicles.[37,40] In the hamster TAL, cells can also be separated into those with high apical and low basolateral K^+ conductance and a low basolateral Cl^- conductance (LBC cells), versus a second population with low apical and high basolateral K^+ conductance, combined with high basolateral Cl^- conductance (HBC) cells.[39,41] The relative frequency of the morphologic and functional subtypes in the cortical and medullary TAL suggests that HBC cells correspond to S cells and LBC cells correspond to R cells.[39]

Morphologic and functional heterogeneity notwithstanding, the cells of the medullary TAL, the cortical TAL, and the macula densa are presumed to share the same basic or at least composite transport mechanisms (see Fig. 6.3). Na^+-Cl^- reabsorption by the TAL is a secondarily active process, driven by the favorable electrochemical gradient for Na^+ established by the basolateral Na^+/K^+–ATPase.[42] The Na^+, K^+, and Cl^- ions are cotransported across by the apical membrane by an electroneutral Na^+-K^+-$2Cl^-$ cotransporter; this transporter generally requires the simultaneous presence of all three ions, such that the transport of Na^+ and Cl^- across the epithelium is mutually codependent and dependent on the luminal presence of K^+. An apical Na^+-K^+-$2Cl^-$ cotransport is mediated by the cation–chloride cotransporter NKCC2, encoded by the SLC12A1 gene.[6] Functional expression of NKCC2 in Xenopus laevis oocytes yields Cl^--dependent and Na^+-dependent uptake of $^{86}Rb^+$ (a radioactive substitute for K^+) and Cl^--dependent and K^+-dependent uptake of $^{22}Na^+$.[6] NKCC2 is sensitive to

micromolar concentrations of furosemide, bumetanide, and other loop diuretics.[6]

Immunofluorescence indicates the expression of the NKCC2 protein along the entire length of the TAL.[6] In particular, immunoelectron microscopy reveals the expression in both rough (R; see previous) and smooth (S) cells of the TAL (see previous).[40] NKCC2 expression in subapical vesicles is particularly prominent in smooth cells,[40] suggesting a role for vesicular trafficking in the regulation of NKCC2. NKCC2 is also expressed in macula densa cells,[40] which have been shown to possess apical Na^+-K^+-$2Cl^-$ cotransport activity. Both tubuloglomerular feedback (TGF) and renal renin secretion are controlled by NKCC2 in the macula densa; luminal loop diuretics block both TGF and the suppression of renin release by the luminal Cl^-.[6]

Microperfused TALs develop a lumen-positive PD during perfusion with Na^+-Cl^-.[43] This lumen-positive PD plays a critical role in the physiology of the TAL, driving the paracellular transport of Na^+, K^+, Ca^{2+}, and Mg^{2+} (see Fig. 6.3). Originally attributed to electrogenic Cl^- transport,[43] the lumen-positive, transepithelial PD in the TAL is generated by the combination of apical K^+ channels and basolateral Cl^- channels.[42] The conductivity of the apical membrane of TAL cells is predominantly, if not exclusively, K^+ selective. Luminal recycling of K^+ via Na^+-K^+-$2Cl^-$ cotransport and apical K^+ channels, along with basolateral depolarization due to Cl^- exit through Cl^- channels, results in the lumen-positive transepithelial PD.[42]

Several lines of evidence indicate that apical K^+ channels are required for transepithelial Na^+-Cl^- transport by the TAL.[42] First, the removal of K^+ from luminal perfusates results in a marked decrease in Na^+-Cl^- reabsorption by the TAL, as measured by a short circuit current; the residual Na^+-Cl^- transport in the absence of luminal K^+ is sustained by the exit of K^+ via apical K^+ channels, because the combination of K^+ removal and a luminal K^+ channel inhibitor (barium) almost abolishes the short-circuit current.[44] Apical K^+ channels are thus required for the activity of NKCC2, the apical Na^+-K^+-$2Cl^-$ cotransporter; the low luminal concentration of K^+ in this nephron segment would otherwise become limiting for transepithelial Na^+-Cl^- transport. Second, the net transport of K^+ across perfused TAL is $<$10% that of Na^+ and Cl^-[33]; ~90% of the K^+ transported by NKCC2 is recycled across the apical membrane via K^+ channels, resulting in minimal net K^+ absorption by the TAL.[42] Third, the intracellular K^+ activity of perfused TAL cells is ~15 to 20 mV above equilibrium, due to furosemide-sensitive entry of K^+ via NKCC2.[45] Given an estimated apical K^+ conductivity of ~12 m per square centimeter, this intracellular K^+ activity yields a calculated K^+ current of ~200 μA per square centimeter; this corresponds quantitatively to the uptake of K^+ by the apical Na^+-K^+-$2Cl^-$ cotransporter. Finally, the observation that Bartter syndrome can be caused by mutations in ROMK (renal outer medullary K^+ channel),[46] a critical component of apical K^+ channels in the TAL (see the following), provides genetic proof for the importance of K^+ channels in Na^+-Cl^- absorption by the TAL. Clearance studies in ROMK-deficient mice also indicate that ~80% of NKCC2 activity is dependent on expression of the ROMK K^+ channel with the TAL.[47]

Three types of apical K^+ channels have been identified in the TAL, a 30 pS channel, a 70 pS channel, and a high-conductance, calcium-activated maxi K^+ channel.[48–50] The higher open probability and greater density of the 30 pS and 70 pS channels, versus the maxi K^+ channel, suggest that these are the primary routes for K^+ recycling across the apical membrane; the 70 pS channel in turn appears to mediate ~80% of the apical K^+ conductance of TAL cells.[51] The low conductance 30 pS channel shares several electrophysiologic and regulatory characteristics with ROMK, the cardinal inward-rectifying K^+ channel (KIR 1.1) that was initially cloned from the renal outer medulla.[52,53] ROMK protein has been identified at the apical membrane of the medullary TAL, the cortical TAL, and the macula densa.[54] Furthermore, the 30 pS channel is also absent from the apical membrane of knockout mice with a homozygous deletion of the gene encoding ROMK.[55] Notably, not all cells in the TAL are labeled with anti-ROMK antibodies,[54,56] suggesting that ROMK might be absent in the HBC cells with high basolateral Cl^- conductance and low apical/high basolateral K^+ conductance (see also previous).[39,41] HBC cells are thought to correspond to the smooth-surfaced morphologic subtype of TAL cells (S cells)[39]; however, distribution of the ROMK protein by immunoelectron microscopy has not as yet been published, hence correlation cannot be made as of yet between morphology and ROMK expression.

Alternative splicing and alternative promoter use of the KCNJ1 gene encoding ROMK/KIR1.1 generates three different protein isoforms, differing in the extreme aminoterminal amino acid sequence: ROMK1, ROMK2, and ROMK3.[57] Although the functional significance of this variation is not clear, these isoforms are differentially distributed along the nephron. ROMK1 is exclusive to the CNT, CCD, and OMCD; ROMK2 is expressed from the TAL to the CCD; and ROMK3 is expressed in the TAL and the distal convoluted tubule (DCT).[57] Apical ROMK channels in the TAL are thus a mixture of ROMK2 and ROMK3 proteins.

ROMK clearly plays a critical role in Na^+-Cl^- absorption by the TAL, given that loss-of-function mutations in this gene are associated with Bartter syndrome.[46] The role of ROMK in Bartter syndrome was initially discordant with the data indicating that the 70 pS K^+ channel is the dominant conductance at the apical membrane of TAL cells[51]; heterologous expression of the ROMK protein in *Xenopus* oocytes had yielded a channel with a conductance of ~30 pS, suggesting that the 70 pS channel was distinct from ROMK. This paradox was resolved by the observation that the 70 pS channel is absent from the TAL of ROMK knockout mice, indicating that ROMK proteins form a subunit of the 70 pS channel.[58] ROMK activity in the TAL is clearly modulated by association with other proteins. That coassociation with other subunits generates the 70 pS channel and is perfectly compatible with

the known physiology of this channel protein. ROMK thus associates with scaffolding proteins NHERF-1 and NHERF-2 via the C-terminal PDZ-binding motif of ROMK; NHERF-2 is coexpressed with ROMK in the TAL.[59] The association of ROMK with NHERFs brings ROMK into closer proximity to the cystic fibrosis transmembrane regulator (CFTR) protein.[59] This ROMK-CFTR interaction is in turn required for reconstitution of the native ATP and glibenclamide sensitivity of apical K^+ channels in the TAL.[60]

TAL cells are phenotypically defined by the apical expression of uromodulin or Tamm-Horsfall glycoprotein (THP), a TAL-specific, GPI-linked membrane protein that is shed into the tubular lumen as the most abundant protein in normal human urine. THP interacts with ROMK protein in yeast two-hybrid screens and activates the channel in coexpression experiments.[61] THP also exhibits functional interactions with NKCC2, activating the cotransporter in coexpression experiments.[62] Membrane trafficking of both ROMK and NKCC2 proteins is affected in THP knockout mice, with greater accumulation of both transport proteins in subapical vesicles. THP knockout mice also exhibit a blunted natriuretic and kaliuretic response to furosemide, which is consistent with a partial deficiency in TAL function.[62] Mutations of the THP gene cause the allelic disorders familial juvenile hyperuricemic nephropathy, medullary cystic kidney disease type II, and glomerulocystic kidney disease. All three disorders share reduced expression of THP at the apical membrane with retention in the endoplasmic reticulum. The associated defects in NKCC2 and ROMK may explain the renal salt wasting and hyperuricemia that can be associated with these genetic disorders.[61,62]

Potassium Secretion by the Distal Convoluted Tubule, Connecting Tubule, and Cortical Collecting Duct

Approximately 90% of filtered K^+ is reabsorbed by the proximal tubule and the loop of Henle (Fig. 6.1); the final adjustments in renal K^+ excretion occur in the downstream distal nephron. The bulk of regulated secretion occurs in the CNT and CCD, whereas K^+ reabsorption primarily occurs in the OMCD (see the following). K^+ secretion is initially detectable in the early DCT, wherein cells expressing the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) express ROMK, the apical K^+ secretory channel (see the following).[54] More recent studies in rat kidneys have localized the protein to both DCT1 and DCT2 segments.[56] Classically, the CCD is considered the primary site for distal K^+ secretion, largely due to the greater ease with which this segment can be perfused and studied. However, as in Na^+ absorption (see below),[63,64] the bulk of distal K^+ secretion appears to occur prior to the CCD,[22] within the CNT.[65] In addition to a lesser endowment with absorptive apical epithelial Na^+ channels (ENaC) and secretory K^+ channels, water absorption in the vasopressin-responsive CCD limits K^+ secretion by allowing the concentration of luminal K^+ to rise toward equilibrium.[66] Water permeability of the CNT is considerably lower than that of the CCD,[66] with a significant number of early (CNT1) CNT cells that do not express aquaporin-2 in the absence of vasopressin. Aquaporin-2 expression is also low or undetectable in CNT1 cells on a high K^+ diet,[56] further optimizing these early CNT segments for K^+ secretion.

The apical membrane of CNT cells and principal cells contain prominent Na^+ and K^+ conductances,[63,65,67] without a measurable apical conductance for Cl^-.[68] The entry of Na^+ occurs via the highly selective ENaC, which is sensitive to micromolar concentrations of amiloride (Fig. 6.4). This selective absorption of a positive charge generates a lumen-negative PD, the magnitude of which varies considerably as a function of mineralocorticoid status and other factors. This lumen-negative PD serves to drive the following critical processes: (1) K^+ secretion via apical K^+ channels, (2) paracellular Cl^- transport through the adjacent tight junctions, and (3) electrogenic H^+ secretion via adjacent type A intercalated cells.

The three ENaC subunits are detectable at the apical membrane of CNT cells and principal cells within the CCD, OMCD, and IMCD. Notably, however, several lines of evidence support the hypothesis that the CNT makes the

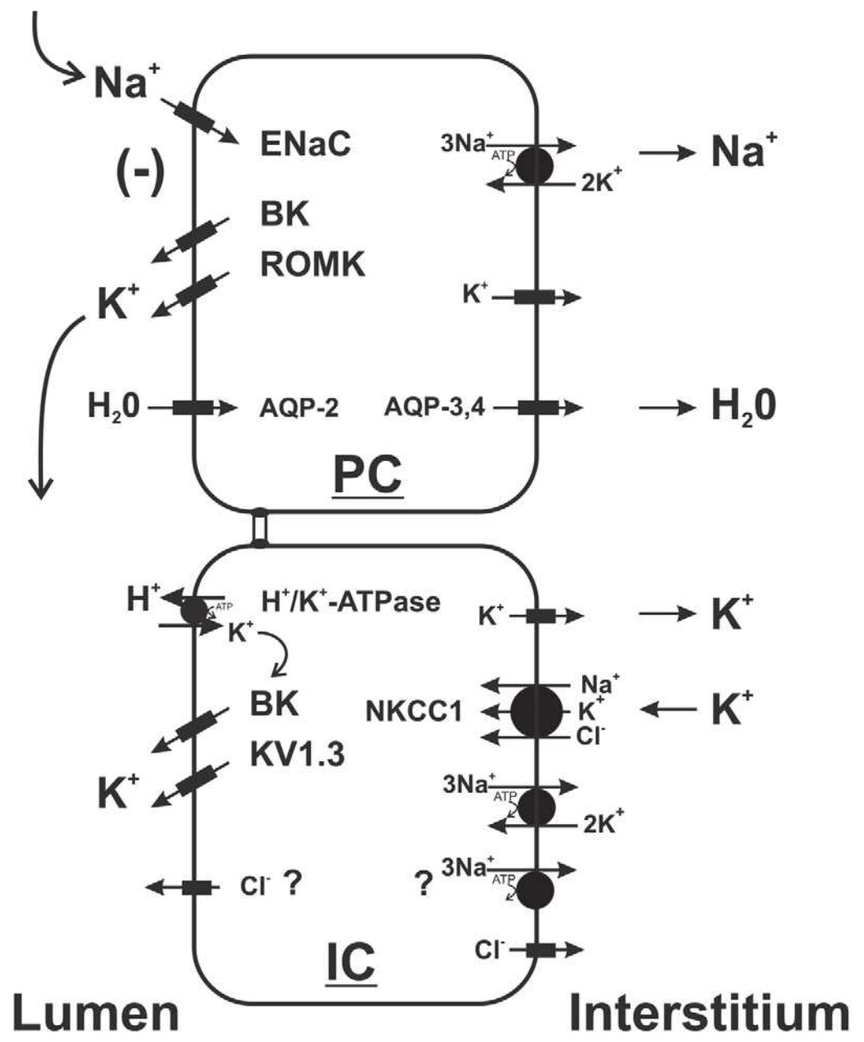

FIGURE 6.4 The transport pathways for potassium in principal/connecting tubule (CNT) cells and intercalated cells. Potassium secretion occurs in CNT and principal cells, driven by the lumen-negative potential difference generated by the epithelial Na^+ channel (ENaC). In intercalated cells, potassium reabsorbed by apical H^+/K^+-ATPase recycles across the apical membrane via K^+ channels; alternatively, in the setting of hypokalemia or potassium deprivation, it exits the cell via basolateral K^+ channels. Potassium that is secreted across intercalated cells enters at the basolateral membrane via NKCC1 and exits via apical BK and Kv1.3 K^+ channels. *BK*, BK K^+ channel; *ROMK*, renal outer medullary K^+ channel; *Kv1.3*, Kv1.3 K^+ channel; *NKCC1*, Na^+-K^+-$2Cl^-$ cotransporter 1.

dominant contribution to amiloride-sensitive Na^+ reabsorption by the distal nephron. First, amiloride-sensitive Na^+ currents in the CNT are two- to fourfold higher than in the CCD; the maximal capacity of the CNT for Na^+ reabsorption is estimated to be ~10 times higher than that of the CCD.[63] Second, targeted deletion of α-ENaC in the collecting duct abolishes amiloride-sensitive currents in CCD principal cells, but does not affect Na^+ or K^+ homeostasis. Thus, the residual ENaC expression in the late DCT and CNT of these knockout mice easily compensates for the loss of the channel in CCD.[69] In contrast, the more extensive deletion of α-ENaC in all aquaporin-2–positive cells in both CNT and CCD causes a profound impairment in Na^+ and K^+ homeostasis.[64] Third, Na^+/K^+–ATPase activity in the CCD is considerably less than that of the DCT[70]; this speaks to a greater capacity for transepithelial Na^+-Cl^- absorption by the DCT and CNT. Fourth, the apical recruitment of ENaC subunits in response to dietary Na^+ restriction begins in the CNT, with progressive recruitment of subunits in the downstream CCD at lower levels of dietary Na^+[71]; under conditions of high Na^+-Cl^- and low K^+ intake, the bulk of aldosterone-stimulated Na^+ transport likely occurs prior to the entry of tubular fluid into the CCD.

In principal cells and CNT cells, the lumen-negative potential difference generated by Na^+ entry via ENaC drives passive K^+ exit through apical K^+ channels. Although there is evolving evidence for ENaC− and Na^+-independent secretion (see also the following),[72] distal K^+ secretion is critically dependent on delivery of adequate luminal Na^+ to the CNT and CCD,[73] essentially ceasing when luminal Na^+ drops below 8 mmol/L.[74] Dietary Na^+ intake also influences K^+ excretion, such that excretion is enhanced by excess Na^+ intake and reduced by Na^+ restriction.[73] Secreted K^+ enters principal cells via the basolateral Na^+/K^+–ATPase, which also generates the gradient that drives apical Na^+ entry via ENaC (Fig. 6.4).

Two major subtypes of apical K^+ channels function in secretion by the CNT and CCD, +/– DCT; a small-conductance (SK) 30 pS channel[55,65] and a large-conductance, Ca^{2+}-activated 150 pS ("maxi-K" or BK) channel.[65,75] The density and high open probability of the SK channel indicates that this pathway alone is sufficient to mediate the bulk of K^+ secretion in the CCD under baseline conditions,[76] hence its designation as the "secretory" K^+ channel. Notably, SK channel density is considerably higher in the CNT than in the CCD,[65] consistent with the greater capacity for Na^+ absorption[63] and K^+ secretion in the CNT. The characteristics of the SK channel are similar to those of the ROMK K^+ channel,[53] and ROMK protein has been localized at the apical membrane of principal cells.[54] SK channel activity is absent from apical membranes of the CCD in homozygous ROMK knockout mice, definitive proof that ROMK is the SK channel.[55] The observation that these knockout mice are normokalemic with an increased excretion of K^+ illustrates the considerable redundancy in distal K^+ secretory pathways[55]; distal K^+ secretion in these mice is mediated by apical BK channels (see the following).[77] Loss-of-function mutations in human KCNJ1 are associated with Bartter syndrome; ROMK expression is critical for the 30 pS and 70 pS channels that generate the lumen-positive PD in the TAL (see Fig. 6.3).[55,58] These patients typically have slightly higher serum K^+ than the other genetic forms of Bartter syndrome,[46] and affected patients with severe neonatal hyperkalemia have also been described; this neonatal hyperkalemia is presumably the result of a transient developmental deficit in apical BK channel activity (see the following).

The apical Ca^{2+}-activated BK channel plays a critical role in *flow*-dependent K^+ secretion by the CNT and CCD.[75] BK channels have a heteromeric structure, with α-subunits that form the ion channel pore and modulatory β-subunits that affect the biophysical, regulatory, and pharmacologic characteristics of the channel complex.[75] BK α-subunit transcripts are expressed in multiple nephron segments, and channel protein is detectable at the apical membrane of principal and intercalated cells in the CCD and CNT.[75] The β subunits are differentially expressed within the distal nephron. Thus β1 subunits are restricted to the CNT,[75] with no expression in intercalated cells,[78] whereas β4 subunits are detectable at the apical membranes of TAL, DCT, and intercalated cells.[78] Increased distal flow has a well-established stimulatory effect on K^+ secretion, due in part to both the enhanced delivery and absorption of Na^+ and to the increased removal of secreted K^+.[73] The pharmacology of flow-dependent K^+ secretion in the CCD is consistent with BK channels,[79] and flow-dependent K^+ secretion is reduced in mice with targeted deletion of the α1 and β1 subunits.[75,80,81] Both mice strains develop hyperaldosteronism that is exacerbated by high-K^+ diet,[81] leading to hypertension in the α1 subunit knockout.[81]

One enigma has been the greater density of BK channels in intercalated cells, in both the CCD[82] and the CNT.[83] This has suggested a major role for intercalated cells in K^+ secretion; however, the much lower density of Na^+/K^+–ATPase activity in intercalated cells has been considered inadequate to support K^+ secretion across the apical membrane.[84] More recent evidence reveals a major role for the basolateral Na^+-K^+-$2Cl^-$ cotransporter NKCC1 in K^+ secretion mediated by apical BK channels (see also Fig. 6.4)[85]; NKCC1 is expressed almost exclusively at the basolateral membrane of intercalated cells,[86] providing an alternative entry pathway for basolateral K^+ secreted at the apical membrane. This still begs the question of how basolateral Na^+ recycles across the basolateral membrane, in the absence of significant Na^+/K^+–ATPase activity; one possibility is an alternative basolateral Na^+ pump, the ouabain-insensitive furosemide-sensitive Na^+–ATPase, a transport activity that has been detected in cell culture models of intercalated cells.[85] At the apical membrane, BK-mediated K^+ secretion is only partially dependent on luminal Na^+[87]; K^+ secretion would eventually hyperpolarize the membrane in the absence of apical Na^+ entry, which is mediated by ENaC in principal cells. An intriguing possibility is that apical Cl^- channels[88] allow for a parallel secretion of K^+ and Cl^- in intercalated cells (see Fig. 6.4).

BK channels also play a critical role in cell volume regulation by intercalated cells, with indirect, flow-mediated influences on distal K^+ secretion. MDCK-C11 cells have an intercalated cell phenotype and express BK α and β4 subunits, as do intercalated cells[78]; shear stress activates BK channels in these cells, leading to a loss of K^+ and cell shrinkage.[89] Mice with a targeted deletion of the β4 subunit exhibit normal K^+ excretion on a normal diet.[84] However, when fed a high K^+ diet, which increases urinary and tubular flow rates and tubular shear stress, the β4-knockout mice develop hyperkalemia with a blunted increase in both K^+ excretion and urinary flow rates. Intercalated cells from β4 knockout may fail to significantly decrease cell volume in response to a high K^+ diet. Intercalated cells thus function as "speed bumps" that protrude into the lumen of distal tubules; flow-activated BK channels reduce the cell volume of intercalated cells after K^+ loading, reducing tubular resistance, increasing tubular flow rates, and increasing distal K^+ secretion.[84]

The physiologic rationale for the presence of two apical secretory K^+ channels—ROMK/SK and BK channels—is not completely clear. However, the high density and higher open probability of SK/ROMK channels is perhaps better suited for a role in basal K^+ secretion, with additional recruitment of the higher capacity, flow-activated BK channels when additional K^+ secretion is required.[75] The evolving evidence also indicates that BK channels function in partially Na^+-independent K^+ secretion by intercalated cells, with ROMK functioning in ENaC- and Na^+-dependent K^+ excretion by DCT, CNT, and CCD cells. Regardless, at the whole organ level, the two K^+ channels can substitute for one another, with BK-dependent K^+ secretion in ROMK knockout mice[77] and an upregulation of ROMK in the distal nephron of α1 subunit BK knockout mice.[80]

Other K^+ channels reportedly expressed at the luminal membranes of the CNT and CCD include voltage-sensitive channels such as Kv1.3,[90] double-pore K^+ channels such as TWIK-1,[91] and KCNQ1.[92] KCNQ1 mediates K^+ secretion in the inner ear and is expressed at the apical membrane of principal cells in the CCD,[92] whereas TWIK-1 is expressed at the apical membrane of intercalated cells.[91] The roles of these channels in renal K^+ secretion or absorption are not fully characterized. However, Kv1.3 may play a role in distal K^+ secretion by intercalated cells (see Fig. 6.4) in that luminal margatoxin, a specific blocker of this channel, reduces K^+ secretion in CCDs of rat kidneys from animals on a high K^+ diet (see also previous).[90] Other apical K^+ channels in the distal nephron subserve other physiologic functions. For example, the apical Kv1.1 channel is critically involved in Mg^{2+} transport by the DCT, likely by hyperpolarizing the apical membrane and increasing the driving force for Mg^{2+} influx via transient receptor potential cation channel 6 (TRPM6); missense mutations in Kv1.1 are a cause of genetic hypomagnesemia.[93]

K^+ channels present at the basolateral membrane of principal cells set the resting potential of the basolateral membrane and function in K^+ secretion and Na^+ absorption at the apical membrane, the latter via K^+ recycling at the basolateral membrane to maintain activity of the Na^+/K^+–ATPase. A variety of different K^+ channels have been described in the electrophysiologic characterization of the basolateral membrane of principal cells, which has a number of technical barriers (reviewed by Gray et al.[94]). However, a single predominant activity can be identified in principal cells from the rat CCD, using whole-cell recording techniques under conditions in which ROMK is inhibited (low intracellular pH or the presence of the ROMK inhibitor tertiapin-Q).[94] This basolateral current is tetraethylammonium (TEA) insensitive, barium sensitive, and acid sensitive (pKa ~6.5), with a conductance of ~17 pS and weak inward rectification. These properties do not correspond exactly to specific characterized K^+ channels, or combinations thereof. However, candidate inward-rectifying K^+ channel subunits that have been localized at the basolateral membrane of the CCD include KIR4.1, KIR5.1, KIR7.1, and KIR2.3.[94] A more recent report suggests that KIR4.1/KIR5.1 channels generate a predominant 40 pS basolateral K^+ channel in murine principal cells.[95] Notably, basolateral K^+ channel activity increases on a high K^+ diet, suggesting a role in transepithelial K^+ secretion.[94]

Similar K^+ channels are found at the basolateral membrane of DCT cells. Cell-attached patches in the basolateral membranes of microdissected DCTs detect an inward rectifying K^+ channel with characteristics similar to heteromeric KIR4.1/KIR5.1 and KIR4.2/KIR5.1 channels.[96] Basolateral membranes of the DCT express immunoreactive KIR4.1[97] and KIR5.1[98] proteins, and DCT cells express KIR4.2 mRNA.[96] Patients with loss-of-function mutations in the KCNJ10 gene that encodes KIR4.1 develop a syndrome of epilepsy, ataxia, sensorineural deafness, and renal tubulopathy.[97,99] The tubulopathy phenotype encompasses hypokalemia, metabolic alkalosis, hypocalciuria, and hypomagnesemia[97,99]; KIR4.1 knockout mice demonstrate a greater natriuresis than littermate controls, in addition to hypocalciuria.[97] As in principal cells, KIR4.1/KIR5.1 and KIR4.2/KIR5.1 channels at the basolateral membrane of DCT cells are hypothesized to function in basolateral K^+ recycling, maintaining adequate Na^+/K^+–ATPase activity for Na^+-Cl^- absorption and other aspects of DCT function.

In addition to apical K^+ channels, considerable evidence implicates apical K^+-Cl^- cotransport (or functionally equivalent pathways)[100] in distal K^+ secretion.[101] In perfused rat distal tubules, a reduction in luminal Cl^- markedly increases K^+ secretion[102]; the replacement of luminal Cl^- with SO_4^- or gluconate has an equivalent stimulatory effect on K^+ secretion. This anion-dependent component of K^+ secretion is not influenced by luminal Ba^{2+},[102] suggesting that it does not involve apical K^+ channel activity. Perfused surface distal tubules are a mixture of the DCT, the connecting segment, and the initial collecting duct; however, Cl^--coupled K^+ secretion is detectable in both the DCT and in the early CNT.[103] In addition, similar pathways are detectable in rabbit CCD, where a decrease in luminal Cl^- from 112 mmol per liter to 5 mmol per liter increases

K^+ secretion by 48%.[104] A reduction in basolateral Cl^- also decreases K^+ secretion without an effect on transepithelial voltage or Na^+ transport, and the direction of K^+ flux can be reversed by a lumen-to-bath Cl^- gradient, resulting in K^+ absorption.[104] In perfused CCDs from rats treated with a mineralocorticoid, vasopressin increases K^+ secretion.[105] Because this increase in K^+ secretion is resistant to luminal Ba^{2+} (2 mmol per liter), vasopressin may stimulate Cl^--dependent K^+ secretion.[106] Recent pharmacologic studies of perfused tubules are consistent with K^+-Cl^- cotransport mediated by the KCCs[101]; however, of the three renal KCCs, only KCC1 is apically expressed in the distal nephron. Other functional possibilities for Cl^--dependent K^+ secretion include the parallel operation of apical H^+-K^+-exchange and Cl^--HCO_3^- exchange in type B intercalated cells[100] and parallel K^+ and Cl^- channels in type A intercalated cells (see Fig. 6.4).[88]

A provocative study by Frindt and Palmer[72] underscores the importance of ENaC-independent K^+ excretion, be it mediated by apical K^+-Cl^- cotransport and/or by other mechanisms (see also Integrated Regulation of Distal Sodium and Potassium Transport). Rats were infused with amiloride via osmotic minipumps, generating urinary concentrations considered sufficient to inhibit >98% of ENaC activity. Whereas amiloride almost abolished K^+ excretion in rats on a normal K^+ intake, acute and long-term high K^+ diets led to an increasing fraction of K^+ excretion that was independent of ENaC activity (~50% after 7 to 9 days on a high K^+ diet).

Potassium Reabsorption by the Collecting Duct

In addition to K^+ secretion, the distal nephron is capable of considerable K^+ reabsorption, primarily during the restriction of dietary K^+.[22,107,108] This reabsorption is accomplished in large part by intercalated cells in the OMCD, via the activity of apical H^+/K^+–ATPase pumps. Under K^+-replete conditions, apical H^+/K^+–ATPase activity recycles K^+ with an apical K^+ channel, without effect on transepithelial K^+ absorption (see Figure 6.4). Under K^+-restricted conditions, K^+ absorbed via apical H^+/K^+–ATPase appears to exit intercalated cells via a basolateral K^+ channel, thus achieving the transepithelial transport of K^+.[109]

H^+-K^+–ATPase holoenzymes are members of the P-type family of ion transport ATPases, which also includes subunits of the basolateral Na^+-K^+–ATPase.[110] HKα-1 and HKα-2 are also referred to as the gastric and colonic subunits, respectively. A specific HKβ subunit interacts with the HKα subunits to ensure delivery to the cell surface and a complete expression of H^+-K^+–ATPase activity; HKα-2 subunits are also capable of interaction with Na^+-K^+–ATPase β subunits. The pharmacology of H^+-K^+–ATPase holoenzymes differs considerably, such that the gastric HKα-1 is classically sensitive to the H^+-K^+–ATPase inhibitors SCH-28080 and omeprazole and is resistant to ouabain; the colonic HKα-2 subunit is usually sensitive to ouabain and resistant to SCH-28080.[110] Within the kidney, the HKα-1 subunit is expressed at the apical membrane of at least a subset of type A intercalated cells in the distal nephron.[111] HKα-2 distribution in the distal nephron is more diffuse, with robust expression at the apical membrane of type A and B intercalated cells and connecting segment cells, and a lesser expression in principal cells.[112]

HKα-1 and HKα-2 are both constitutively expressed in the distal nephron. However, tubule perfusion of K^+-replete animals suggests a functional dominance of omeprazole/SCH-28080-sensitive, ouabain-resistant H^+-K^+–ATPase activity that is consistent with holoenzymes containing HKα-1.[113] K^+ deprivation increases the overall activity of H^+-K^+–ATPase in the collecting duct, with the emergence of a ouabain-sensitive H^+-K^+–ATPase activity.[114] This is consistent with a relative dominance of HKα-2 during K^+-restricted conditions. Consistent with this pharmacology, K^+-restriction also induces a dramatic upregulation of HKα-2 transcript and protein in the outer and inner medulla during K^+ depletion; HKα-1 expression is unaffected.[115,116] Mice with a targeted deletion of HKα-2 exhibit lower plasma and muscle K^+ than wild-type littermates when maintained on a K^+-deficient diet. However, this appears to be due to a marked loss of K^+ in the colon rather than the kidney, because renal K^+ excretion is appropriately reduced in the K^+-depleted knockout mice.[110] Presumably, the lack of an obvious renal phenotype in either HKα-1[110] or HKα-2[110] knockout mice reflects the marked redundancy in the expression of HKα subunits in the distal nephron. Indeed, collecting ducts from the HKα-1 knockout mice have significant residual ouabain-resistant and SCH-28080-sensitive H^+-K^+–ATPase activities, consistent with the expression of other HKα subunits that confer characteristics similar to the "gastric" H^+-K^+–ATPase.[110] However, data from HKα-1 and HKα-2 knockout mice suggest that compensatory mechanisms in these mice are not accounted for by ATPase-type mechanisms.[117]

The importance of K^+ reabsorption mediated by the collecting duct is dramatically illustrated by the phenotype of transgenic mice with generalized overexpression of a gain-of-function mutation in H^+-K^+–ATPase; this effectively bypasses the redundancy and complexity of this reabsorptive pathway. This mutant HKβ subunit has a tyrosine-to-alanine mutation within the C-terminal tail that abrogates regulated endocytosis from the plasma membrane; these mice have higher plasma K^+ than their wild-type littermates, with approximately half the fractional excretion of K^+.[118]

Finally, it should be noted that considerable evidence implicates the H^+-K^+–ATPases in renal acid secretion.[110] In particular, acid extrusion from intercalated cells is markedly reduced in doubly deficient HKα-1/HKα-2 knockout mice.[110] In addition, the H^+-K^+–ATPases may function directly in Na^+ balance, collaborating, for example, with apical Cl^-:HCO_3^- exchangers in the CCD in apical Na^+-Cl^- uptake.[110]

The Regulation of Distal Tubular Potassium Transport

Modulation of ROMK Activity

ROMK and other KIR channels are inward rectifying (i.e., K^+ flows inward more readily than outward). Even though outward conductance is usually less than inward conductance, K^+ efflux through ROMK predominates in the CNT and CCD because the membrane potential is more positive than the equilibrium potential for K^+. Intracellular magnesium (Mg^{2+})[119] and polyamines[120] play key roles in inward rectification, binding and blocking the pore of the channel from the cytoplasmic side.[121] A single transmembrane residue, asparagine-171 in ROMK1, controls the affinity and the blocking effect of both Mg^{2+} and polyamines.[119,120] Intracellular Mg^{2+} in TAL, DCT, CNT, and principal cells is thought to have a significant effect on ROMK activity, because it inhibits outward ROMK-dependent currents in principal cells.[122] The blocking affinity of Mg^{2+} is enhanced at lower extracellular K^+ concentrations,[122] which should aid in reducing K^+ secretion during hypokalemia and K^+ deficiency. A reduction of this intracellular Mg^{2+} block may also explain the hypokalemia associated with hypomagnesemia, wherein distal K^+ secretion is enhanced.[121]

In addition to inward rectification, the endogenous ROMK channels in the TAL and principal cells exhibit a very high channel open probability (P_o). The high P_o of ROMK is maintained by the combined effects of the binding of phosphatidylinositol-4,5-bisphosphate (PIP_2) to the channel protein, direct channel phosphorylation by protein kinase A (PKA), ATP binding to the ROMK-CFTR complex, and cytoplasmic pH. PIP2 binding to ROMK is thus required to maintain the channel in an open state,[123] whereas cytoplasmic acidification inhibits the channel. PKA phosphorylates ROMK protein at one N-terminal serine and two C-terminal serines[124]: S25, S200, and S294 in the ROMK2 isoform. Phosphorylation of all three sites is required for full channel function. The phosphorylation of the N-terminal site overrides the effect of a carboxy-terminal endoplasmic reticulum retention signal, thus increasing the expression of the channel protein at the cell membrane.[125] The phosphorylation of S200 and S294 maintains the channel in a high P_o state, in part by modulating the effects of PIP_2,[126] ATP,[60] and pH.[127]

Because ROMK channels exhibit such a high P_o, the physiologic regulation of the channel is primarily achieved by regulated changes in the number of active channels on the plasma membrane. The associated mechanisms are discussed in the context of the adaptation to K^+ loading/hyperkalemia and K^+ deprivation/hypokalemia.

Aldosterone and Potassium Secretion

Aldosterone has a potent kaliuretic effect,[128] with important interrelationships between circulating K^+ and aldosterone. Aldosterone release by the adrenal is thus induced by hyperkalemia and/or a high K^+ diet,[129] suggesting an important feedback effect of aldosterone on K^+ homeostasis.[130] Aldosterone also has clinically relevant effects on K^+ homeostasis, with a clear relationship at all levels of serum K^+between circulating levels of the hormone and the ability to excrete K^+.

Renin released from the kidney stimulates aldosterone release from the adrenal via angiotensin-II (AT-II). Renin secretion by juxtaglomerular cells within the afferent arteriole is initiated in response to a signal from the macula densa, specifically a decrease in luminal chloride transported through the Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) at the apical membrane of macula densa cells.[6] In addition to this macula densa signal, decreased renal perfusion pressure and renal sympathetic tone stimulate renal renin secretion.

Hyperkalemia is also an independent and synergistic stimulus for aldosterone release from the adrenal gland,[129] although dietary K^+ loading is less potent than dietary Na^+-Cl^- restriction in increasing circulating aldosterone.[130] The resting membrane potential of adrenal glomerulosa cells is hyperpolarized due to the activity of the "leak" K^+ channels TASK-1 and TASK-3; the combined deletion of genes encoding these channels leads to baseline depolarization of adrenal glomerulosa cells and an increase in plasma aldosterone that is resistant to dietary sodium loading.[20] The KCNJ5 K^+ channel also plays a role, in that mutations that produce a depolarizing acquisition of KCNJ5-mediated Na^+ conductance are associated with adrenal adenomas.[21] AT-II and K^+ both activate Ca^{2+} entry in glomerulosa cells via voltage-sensitive T-type Ca^{2+} channels, primarily Cav3.2.[131] Elevations in extracellular K^+ thus depolarize glomerulosa cells and activate these Ca^{2+} channels, which are independently and synergistically activated by AT-II. The calcium-dependent activation of calcium-calmodulin (CaM)-dependent protein kinase, in turn, activates the synthesis and release of aldosterone via the induction of aldosterone synthase. K^+ and AT-II also enhance the transcription of the Cav3.2 Ca^{2+} channel by abrogating repression of this gene by the neuron restrictive silencing factor (NRS); this ultimately amplifies the induction of aldosterone synthase.[131]

The adrenal release of aldosterone due to increased K^+ is dependent on an intact adrenal renin–angiotensin system, particularly during Na^+-Cl^- restriction. Angiotensin converting enzyme (ACE) inhibitors and angiotensin–receptor blockers (ARBs) completely abrogate the effect of high K^+ on salt-restricted adrenals.[132] Direct, G protein–dependent activation of the TASK-1 and/or TASK-3 K^+ channels by AT_{1A} or AT_{1B} receptors is thought to underlie the effect of AT-II on adrenal aldosterone release,[20] with abrogation of this effect by ARBs or ACE inhibitors. Other clinically relevant activators of adrenal aldosterone release include prostaglandins and catecholamines via increases in cAMP.[133] Finally, atrial natriuretic peptide (ANP) exerts a potent negative effect on aldosterone release induced by K^+ and other stimuli,[134] at least in part by inhibiting early events in aldosterone synthesis.[135] ANP inhibits both renal renin release and adrenal aldosterone release, functions that may be central to the roles of this hormone in the pathophysiology of hyporeninemic hypoaldosteronism.[134]

Within the kidney, aldosterone has no effect on the density of apical SK channels in the CCD[136]; it does however induce a marked increase in the density of apical Na^+ channels in the CNT and CCD.[136] Aldosterone activates ENaC via interrelated effects on the transcription, synthesis, trafficking, and membrane-associated activity of the subunits encoding the channel. Aldosterone is thus induced by a high K^+ diet and strongly stimulates apical ENaC activity, which generates the lumen-negative PD that stimulates K^+ secretion by principal cells. The hormone also has significant effects on the basolateral membrane of principal cells, with dramatic changes in cellular morphology and the length of basolateral membranes.[137] This is accompanied by an increase in basolateral Na^+-K^+–ATPase activity, although it has been difficult to determine how much of these cellular and functional changes are due to enhanced Na^+ entry via apical ENaC. It is, however, known that aldosterone increases the expression of the Na^+-K^+–ATPase α1 and β1 subunits in the CCD[138]; these effects are evidently independent of ENaC activity.[139]

Transcriptional effects of aldosterone also include the induction of the α-ENaC subunit via a glucocorticoid-response element in the promoter of the SCNN1A gene. Aldosterone also relieves a tonic inhibition of the SCNN1A gene by a complex that includes the Dot1a (disruptor of telomere silencing splicing variant a) and the AF9 and AF17 transcription factors.[140] This transcriptional activation results in an increased abundance of α-ENaC protein in response to either exogenous aldosterone or dietary Na^+-Cl^- restriction[141]; the response to Na^+-Cl^- restriction is blunted by spironolactone, indicating involvement of the mineralocorticoid receptor. At the baseline, α-ENaC transcripts in the kidney are less abundant than those encoding β- and γ-ENaC. All three subunits are required for the efficient processing of heteromeric channels in the endoplasmic reticulum and trafficking to the plasma membrane, such that the induction of α-ENaC is thought to relieve a major bottleneck in the processing and trafficking of active ENaC complexes.[142]

Aldosterone also plays an indirect role in the regulated trafficking of ENaC subunits to the plasma membrane via the regulation of accessory proteins that interact with preexisting ENaC subunits. Aldosterone rapidly induces the expression of a serine-threonine kinase denoted SGK1.[143] The coexpression of SGK1 with ENaC subunits in *Xenopus* oocytes results in a dramatic activation of the channel due to increased expression at the plasma membrane.[141] Analogous in vivo redistribution of ENaC subunits occurs in the CNT and early CCD from a largely cytoplasmic location during dietary Na^+-Cl^- excess to a purely apical distribution after aldosterone or Na^+-Cl^- restriction.[71,141] Furthermore, there is a temporal correlation between the appearance of induced SGK1 protein in the CNT and the redistribution of ENaC protein to the plasma membrane.[141] The amiloride-sensitive, lumen-negative potential difference generated by ENaC is reduced in SGK1 knockout mice,[144] resulting in a decreased driving force for distal K^+ secretion and marked susceptibility to hyperkalemia.

SGK1 modulates membrane expression of ENaC by interfering with regulated endocytosis of its channel subunits. Specifically, the kinase interferes with interactions between ENaC subunits and the ubiquitin-ligase Nedd4-2.[142] PPxY domains in the C-termini of all three ENaC subunits bind to WW domains of Nedd4-2. Coexpression of Nedd4-2 with a wild-type ENaC channel results in a marked inhibition of channel activity due to retrieval from the cell membrane; Nedd4-2 is thought to ubiquitinate ENaC subunits, resulting in the removal of channel subunits from the cell membrane and degradation in lysosomes and the proteosome.[142] A PPxY domain in SGK1 also binds to Nedd4-2, which is a phosphorylation substrate for the kinase; the phosphorylation of Nedd4-2 by SGK1 abrogates its inhibitory effect on ENaC subunits.[145,146] Aldosterone also stimulates Nedd4-2 phosphorylation in vivo[147] and Nedd4-2 phosphorylation, in turn, results in the ubiquitin-mediated degradation of SGK1.[148]

Finally, aldosterone indirectly activates ENaC channels through the induction of channel activating proteases, which increase open channel probability by the cleavage of the extracellular domain of α- and γ-ENaC. Proteases that have been implicated in the processing of ENaC include furin, elastase, plasmin, kallikrein, and three novel, membrane-associated proteases called channel activating proteases 1, 2, and 3(CAP1, 2, and 3).[149–151] Proteolytic cleavage of ENaC appears to activate the channel by removing the self-inhibitory effect of external Na^+[150]; in furin-mediated proteolysis of αENaC, this appears to involve the removal of an inhibitory domain from within the extracellular loop.[152] The structures of the extracellular domains of ENaC and the related channels resemble an outstretched hand holding a ball, with defined subdomains termed the wrist, finger, thumb, palm, β-ball, and knuckle; functionally relevant proteolytic events target the finger domains of ENaC subunits.[151] Unprocessed channels at the plasma membrane are thought to function as a reserve pool, capable of rapid activation by membrane-associated luminal proteases.[150]

Adaptation to High Potassium Intake

Much of the renal adaptation to high K^+ intake is aldosterone independent. For example, a high K^+ diet increases apical Na^+ reabsorption and K^+ secretion in the CCD in adrenalectomized animals that lack aldosterone.[153] At the tubular level, when basolateral K^+ is increased, there is a significant activation of the Na^+/K^+–ATPase, accompanied by a secondary activation of apical Na^+ and K^+ channels.[154] Increased dietary K^+ also markedly increases the density of SK channels in the CCD (Fig. 6.5), along with a modest increase in ENaC density.[136] This is associated with changes in the subcellular distribution of the ROMK protein, with an increase in apical expression.[155] Notably, this increase in ENaC and SK density in the CCD occurs within hours of assuming a high K^+ diet, with a minimal associated increase in circulating aldosterone.[156] In contrast, a week of low Na^+-Cl^- intake, with almost a 1000-fold increase in aldosterone, has

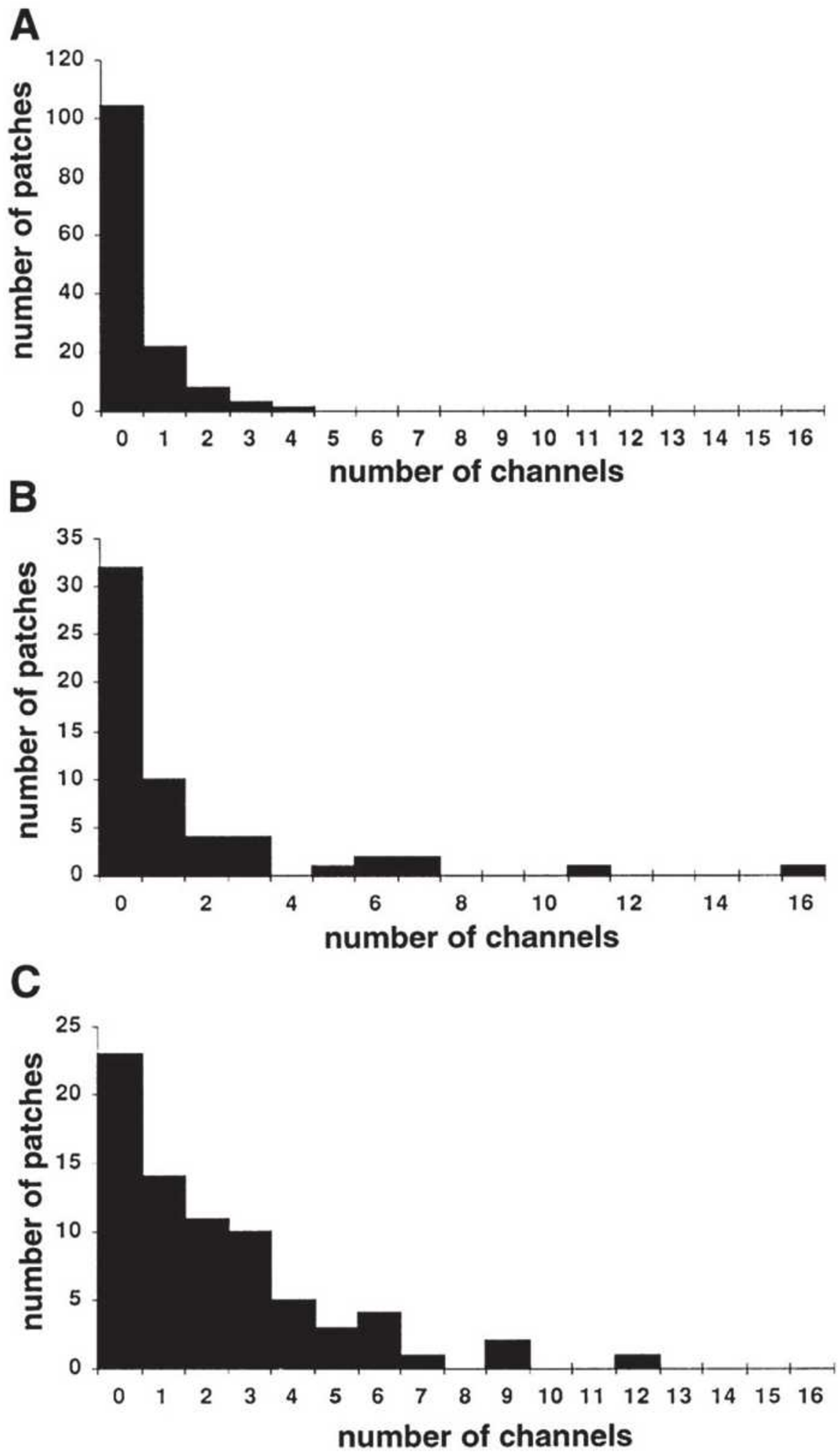

FIGURE 6.5 A high K^+ diet rapidly activates small conductance (SK) channels in the cortical collecting duct (CCD), which is mediated by the renal outer medullary K^+ channel (ROMK) (Kir 1.1) K^+ channel. Histograms of *N* (channels/patch) are shown for rats on a control diet (**A**), on a high K diet for 6 hours (**B**), and on a high K diet for 48 hours (**C**). Each determination of *N* represents a single cell-attached patch. The high K^+ diet results in a progressive recruitment of SK channels at the apical membrane. (From Palmer LG, Frindt G. Regulation of apical K channels in rat cortical collecting tubule during changes in dietary K intake. *Am J Physiol.* 1999;277:F805–812.)

no effect on SK channel density, nor for that matter does 2 days of aldosterone infusion, despite the development of hypokalemia (Table 6.1).[156] Of note, unlike the marked increase seen in the CCD,[136,156] the density of SK channels in the CNT is not increased by high dietary K^+.[65] This appears to be due to difficulties in estimating channel densities in small membrane patches, because the measurement of whole cell currents indicates an upregulation of ROMK activity in the CNT of animals maintained on a high K^+ diet.[157]

BK channels in the CNT and CCD play an important role in the flow-activated component of distal K^+ excretion[75]; these channels are also activated by dietary K^+ loading. Flow-stimulated K^+ secretion by the CCD of both mice[77] and rats[158] is enhanced on a high-K^+ diet, with an absence of flow-dependent K^+ secretion in rats on a low K^+ diet.[158] This is accompanied by commensurate changes in transcript levels for α- and β_{2-4}-subunits of the BK channel proteins in microdissected CCDs (β_1 subunits are restricted to the CNT[75]). Trafficking of BK subunits is also affected by dietary K^+, with largely intracellular distribution of α-subunits in K^+-restricted rats and prominent apical expression in K^+-loaded rats.[158] Aldosterone does not contribute to the regulation of BK channel activity or expression in response to a high K^+ diet.[159]

Regulatory information is notably more extensive for ROMK/SK channels than for BK channels. ROMK channels exhibit a high P_o; physiologic regulation of SK channels is primarily achieved by regulated changes in the number of active channels on the plasma membrane. The changes in trafficking and/or activity of the ROMK channel that are induced by dietary K^+ restriction appear to involve tyrosine phosphorylation of the ROMK protein (see the following); K^+ loading reverses this tyrosine phosphorylation. The forward trafficking of ROMK channels to the apical membrane of CNT and principal cells after K^+ loading is also phosphorylation dependent. PKA phosphorylates ROMK protein at one N-terminal serine and two C-terminal serines.[124] Phosphorylation of the N-terminal serine overrides the effect of a carboxy-terminal endoplasmic reticulum retention signal, thus inducing exit from the Golgi apparatus and expression of the channel protein at the cell membrane.[125] This explains in large part the induction of SK channel density by vasopressin[160] and cAMP.[76] Notably, this N-terminal serine is also a substrate for SGK1, the aldosterone-induced kinase that activates ENaC, which then activates ROMK by increasing membrane expression.[161] Thus, although aldosterone does not appear to have significant effects on SK density,[156] SGK1 is conceivably involved; alternatively, other antagonistic effects of SGK1 on ROMK[162] trafficking abrogate the effect mediated by N-terminal phosphorylation.

A recent series of reports have linked changes in the expression of WNK1 kinase subunits in the response to high K^+ diet. WNK1 and WNK4 were initially identified as causative genes for familial hypertension with hyperkalemia (FHHt), also known as Gordon syndrome or pseudohypoaldosteronism type II.[163] The regulation of NCC, the thiazide-sensitive Na^+-Cl^- cotransporter in the DCT, is a major role of the WNK kinases.[164–166] NCC activity is a major determinant of Na^+ delivery to the downstream CNT, with potent effects on the ability to secrete K^+ (see also Integrated Regulation of Distal Sodium and Potassium Transport). However, the WNK kinases also directly regulate trafficking and activity of both ROMK and BK channels.

ROMK expression at the membrane of Xenopus oocytes is dramatically reduced by the coexpression of WNK4; FHHt-associated mutations dramatically increase this effect, suggesting a direct inhibition of SK channels in FHHt.[167]

6.1 The Effect of a High K^+ Diet, Aldosterone, and/or Na^+-Cl^- Restriction on Small Conductance Channel Density in the Rat Cortical Collecting Duct

Condition	K Channel Density/μm^2	Plasma Aldo (ng/dL)	Plasma K (mM)
Control	0.41	15	3.68
High K diet, 6 h	1.51	36	NM
High K diet, 48 h	2.13	98	4.37
Low Na diet, 7 d	0.48	1260	NM
Aldo infusion, 48 h	0.44	550	2.44
Aldo + high K diet	0.32	521	3.80

K, potassium; h, hour; Na, sodium; d, day; Aldo, aldosterone; NM, not measured.
Modified from Palmer LG, Frindt G. Regulation of apical K channels in rat cortical collecting tubule during changes in dietary K intake. Am J Physiol. 1999;277:F805–F812.

The coexpression of WNK4 also inhibits BK channels in a heterologous expression system, apparently by inducing lysosomal degradation.[168] In the case of WNK1, the analysis is complicated dramatically by the transcriptional complexity of its gene, which has at least three separate promoters and a number of alternative isoforms. In particular, the predominant intrarenal WNK1 isoform is generated by a distal nephron transcriptional site that bypasses the N-terminal exons that encode the kinase domain, thus yielding a kinase-deficient short form of the protein ("WNK1-S").[169] Full-length WNK1 (WNK1-L) inhibits ROMK activity by inducing endocytosis of the channel protein[170–172]; kinase activity and/or the N-terminal kinase domain of WNK1 appear to be required for this effect,[171,172] although Cope et al.[170] have reported that a kinase-dead mutant of WNK1 is unimpaired. WNK1 and WNK4 induce endocytosis of ROMK via an interaction with intersectin, a multimodular endocytic scaffold protein.[173] The additional binding of ROMK to the clathrin adaptor protein autosomal recessive hypercholesterolemia (ARH) is required for basal and WNK1-stimulated endocytosis of the channel protein.[174] The ubiquitination of the ROMK protein is also involved in clathrin-dependent endocytosis, requiring an interaction between the channel and the U3 ubiquitin ligase POSH (plenty of SH domains).[175]

The shorter WNK1-S isoform, which lacks the kinase domain, appears to inhibit the effect of WNK1-L.[171,172] The ratio of WNK1-S to WNK1-L transcripts is reduced by K^+ restriction (greater endocytosis of ROMK)[172,176] and is increased by K^+ loading (reduced endocytosis of ROMK),[171,176] thus suggesting that this ratio between WNK1-S and WNK1-L functions as a "switch" to regulate distal K^+ secretion (Fig. 6.6). The inhibitory effect of WNK1-S tracks to the first 253 amino acids of the protein, encompassing the initial 30

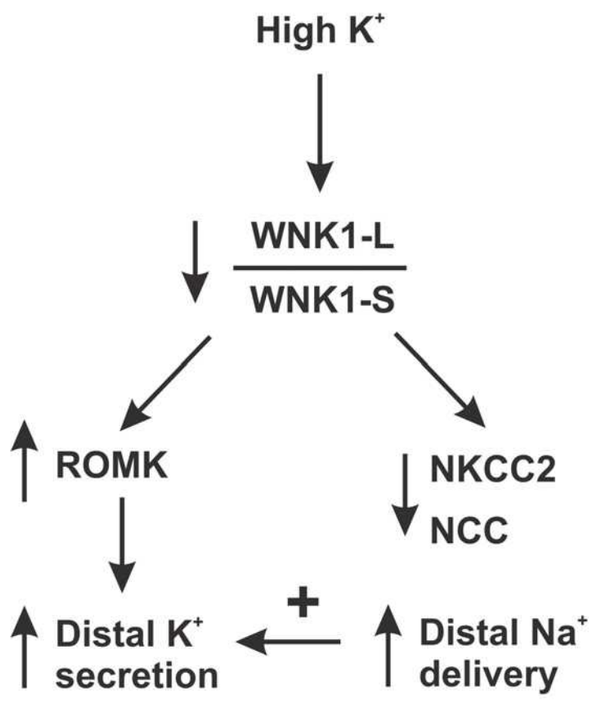

FIGURE 6.6 The balance between the major short (WNK1-S) and long (WNK1-L) isoforms of the WNK1 kinases affects K^+ secretion. The WNK1-S isoform lacks a kinase domain and inhibits the effects of the long isoform. Potassium loading causes a reduction in the ratio of WNK1-L to WNK1-S, resulting in an increased expression of the renal outer medullary K^+ channel (ROMK) at the plasma membrane of connecting tubule (CNT) cells and principal cells, with a concomitant reduction in the apical expression of the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) in the distal convoluted tubule (DCT) and the furosemide-sensitive Na^+-K^+-$2Cl^-$ cotransporter NKCC2 in the thick ascending limb (TAL). The net result is increased Na^+ delivery to the CNT and principal cells with enhanced apical secretory K^+ channel activity, resulting in an enhanced K^+ secretion.

amino acids unique to this isoform and an adjacent autoinhibitory domain.[177] Transgenic mice that overexpress this inhibitory domain of WNK1-S have lower serum K^+ concentrations, higher fractional excretion of K^+, and increased expression of the ROMK protein at the apical membrane of CNT and CCD cells, all of which are consistent with an important inhibitory effect of WNK1-S.[177]

Potassium Deprivation

A reduction in dietary K^+ leads, within 24 hours, to a dramatic drop in urinary K^+ excretion.[176,178] This drop in excretion is due to both an induction of reabsorption by intercalated cells in the OMCD[107,108] and to a reduction in SK channel activity in principal cells.[179] The mechanisms involved in K^+ reabsorption by intercalated cells were previously discussed. Notably, H^+/K^+–ATPase activity in the collecting duct does not appear to be regulated by aldosterone[180]; stimulatory effects of mineralocorticoid on H^+/K^+–ATPase activity are abrogated by K^+ loading, indicating a primary role for hypokalemia.[181]

Dietary K^+ intake modulates trafficking of the ROMK channel protein to the plasma membrane of principal cells, with a marked increase in the relative proportion of intracellular channel protein in K^+-depleted animals[155] and clearly defined expression at the plasma membrane of CCD cells from animals on a high K^+ diet.[155] The membrane insertion and activity of ROMK is modulated by tyrosine phosphorylation of the channel protein, such that the phosphorylation of tyrosine residue 337 stimulates endocytosis and dephosphorylation induces exocytosis.[182,183] This tyrosine phosphorylation appears to play a key role in the regulation of ROMK by dietary K^+.[184] Whereas the levels of protein tyrosine phosphatase-1D do not vary with K^+ intake, intrarenal activity of the cytoplasmic tyrosine kinases c-src and c-yes are inversely related to dietary K^+ intake, with a decrease under high K^+ conditions and a marked increase after several days of K^+ restriction.[179,185] Immunolocalization indicates coexpression of c-src with ROMK in the TAL and the principal cells of the CCD.[155] The inhibition of protein tyrosine phosphatase activity, leading to a dominance of tyrosine phosphorylation, dramatically increases the proportion of intracellular ROMK in the CCD of animals on a high K^+ diet.[155]

The neurohumoral factors that induce the K^+-dependent trafficking and expression of apical ROMK[155] and BK channels[158] have only recently been identified. Several studies have implicated the intrarenal generation of superoxide anions in the activation of cytoplasmic tyrosine kinases and in the downstream phosphorylation of the ROMK channel protein by K^+ depletion.[186,187] Candidates for the upstream kaliuretic factor include AT-II and growth factors such as insulin-like growth factor-1 (IGF-1).[162] AT-II inhibits ROMK activity in K^+-restricted rats, but not in rats on a normal K^+ diet.[188] This inhibition involves the downstream activation of superoxide production and c-src activity, such that the well-known induction of AT-II by a low K^+ diet appears to play a major role in reducing the distal tubular K^+ secretion.[189] IGF-1 is produced in the kidney and upregulated by dietary K^+ restriction[190]. The downstream activation of PI3-kinase by insulin and IGF-1 results in the Akt1- or SGK1-dependent phosphorylation of WNK1, leading to endocytosis of ROMK.[162]

Reports of transient postprandial kaliuresis in sheep, independent of changes in plasma K^+ or aldosterone, suggest that an enteric or hepatoportal K^+ sensor controls kaliuresis via a sympathetic reflex[191]; tissue kallikrein has recently emerged as a candidate mediator for this postprandial kaliuresis (see the following). Regardless of the signaling involved, changes in dietary K^+ absorption have a direct "anticipatory" effect on K^+ homeostasis in the absence of changes in plasma K^+. Such a feed-forward control has the theoretical advantage of greater stability, because it operates prior to changes in plasma K^+.[192] Notably, changes in ROMK phosphorylation status and insulin-sensitive muscle uptake can be seen in K^+-deficient animals in the absence of a change in plasma K^+,[13] suggesting that the upstream activation of the major mechanisms that serve to reduce K^+ excretion (reduced K^+ secretion in the CNT/CCD, decreased peripheral uptake, and increased K^+ reabsorption in the OMCD) does not require changes in the plasma K^+. Consistent with this hypothesis, moderate K^+ restriction—without an associated drop in plasma K^+—is sufficient to induce AT-II-dependent superoxide generation and c-src activation, leading to the inhibition of ROMK channel activity.[189]

Vasopressin

Vasopressin has a stimulatory effect on K^+ secretion by the distal nephron.[32] This serves to preserve K^+ secretion during dehydration and extracellular volume depletion, when circulating levels of vasopressin are high and tubular delivery of Na^+ and fluid is reduced. The stimulation of basolateral V2 receptors results in the activation of ENaC, which increases the driving force for K^+ secretion by CNT cells and principal cells.[193] In addition, vasopressin activates SK channels directly in the CCD,[160] as does cAMP.[76] The ROMK protein is directly phosphorylated by PKA on three serine residues (S25, S200, and S294 in the ROMK2 isoform), with phosphorylation of all three sites required for full activity (see also the previous discussion). Finally, the stimulation of luminal V1 receptors also increases K^+ secretion in the CCD, apparently via the activation of BK channels.[194]

Tissue Kallikrein

The serine protease tissue kallikrein (TK) is involved in the generation of kinins, which ultimately stimulates the formation of bradykinin.[195] Within the kidney, TK is synthesized within CNT cells and is released into the tubular lumen and the peritubular interstitium. Although TK-induced bradykinin has a number of effects on distal tubular physiology,[195] more recent data have revealed a provocative role in postprandial kaliuresis. Thus, oral K^+-Cl^- loading leads to a spike in urinary K^+ and TK excretion in rats, mice, and

humans.[195] The increase in urinary TK after K^+ loading is not accompanied by changes in urinary aldosterone and can be detected in aldosterone synthase knockout mice.[149] Mice deficient in TK demonstrate postprandial hyperkalemia, indicating a role for the protease in postprandial kaliuresis. This transient hyperkalemia is accompanied by a marked increase in K^+ reabsorption by perfused CCDs due to an upregulation of H^+/K^+–ATPase activity and an increase in HKα-2 transcript. The addition of luminal but not basolateral TK inhibits the activated CCD H^+/K^+–ATPase activity in TK knockout mice, which is consistent with direct proteolytic activation. There is also a marked increase in Na^+ reabsorption by perfused CCDs from TK knockout mice, without the development of a lumen-negative PD. This is consistent with an increased activity of the electroneutral Na^+-Cl^- cotransport mediated by the Na^+-driven SLC4A8 Cl^--HCO_3^- exchanger and the SLC26A4 Cl^--HCO_3^- exchanger (see also the following text).[196] This electroneutral transport pathway had previously been shown to be inhibited by bradykinin[197]; hence, the activation by TK deletion presumably reflected a loss of tonic inhibition by TK-generated bradykinin. Prior data had indicated that TK mediates proteolytic cleavage of the γ subunit of ENaC, with reduced ENaC activity in TK-deficient mice[198]; net Na^+ balance is thus neutral in these mice.

In summary, TK secretion from CNT cells is induced by oral K^+-Cl^- loading, causing proteolytic activation of ENaC[198] and thus an increase in ENaC-driven K^+ secretion and the bradykinin-dependent inhibition of electroneutral Na^+-Cl^- cotransport in the CCD.[196,197] Thus, a further augmentation of electrogenic Na^+ transport (favoring K^+ secretion) and a direct luminal inhibition of H^+/K^+–ATPase activity causes a decrease or tonic inhibition of K^+ reabsorption. TK may very well be the postprandial factor[191] that functions in the feed-forward control of plasma K^+.[192]

The Integrated Regulation of Distal Sodium and Potassium Transport

In CNT and principal cells, the lumen-negative potential difference generated by Na^+ entry via ENaC induces the exit of K^+ via apical K^+-selective channels. This arrangement explains much of the known physiology and pathophysiology of renal K^+ secretion, yet has several key consequences that bear emphasis. First, enhanced Na^+-Cl^- reabsorption upstream of the CNT and CCD will reduce the delivery of luminal Na^+ to the CNT and CCD,[73] decrease the lumen-negative potential difference, and thus decrease K^+ secretion; K^+ secretion by the CCD essentially stops when luminal Na^+ drops below 8 mmol per liter.[74] In this respect, the increasingly refined phenotypic understanding of FHHt, caused by kinase-induced gain-of-function of the DCT, has served to underline that a variation in NCC-dependent Na^+-Cl^- absorption, just upstream of the CNT, has major effects on the ability to excrete dietary K^+.[166] Second, aldosterone is a kaliuretic hormone, induced by hyperkalemia. However, in certain circumstances associated with a marked induction of aldosterone, such as dietary sodium restriction, sodium balance is maintained without affecting K^+ homeostasis. This aldosterone paradox—the independent regulation of Na^+-Cl^- and K^+ handling by the aldosterone-sensitive distal nephron—is only recently beginning to yield to investigative efforts. The major factors in the integrated control of Na^+-Cl^- and K^+ transport appear to include electroneutral, NCC-independent, and thiazide-sensitive Na^+-Cl^- transport within the CCD[196,197,199]; K^+-dependent changes in NCC activity within the DCT; ENaC-independent K^+ excretion within the distal nephron[72]; and the differential regulation of various transport and signaling pathways by aldosterone, AT-II, TK, and dietary K^+.[195,200,201]

Thiazide-sensitive electroneutral Na^+-Cl^- transport within the CCD is mediated by parallel activity of the Na^+-driven SLC4A8 Cl^--HCO_3^- exchanger and the SLC26A4 Cl^--HCO_3^- exchanger,[196] expressed in intercalated cells. The molecular identity of this transport mechanism has only recently emerged[196]; hence, regulatory influences are not fully characterized. However, electroneutral Na^+-Cl^- transport within the CCD is evidently induced by both volume depletion and mineralocorticoid treatment.[196,197,199] This mechanism mediates ~50% of Na^+ reabsorption in the CCD under these conditions, all without affecting the lumen potential difference and thus without influencing K^+ excretion. A high K^+ diet also increases the fraction of ENaC-independent, amiloride-resistant K^+ excretion to ~50%; again, this electroneutral, aldosterone-independent pathway for K^+ excretion uncouples distal tubular Na^+ and K^+ excretion.[72]

In a landmark study, Kahle et al.[167] established in 2003 that the WNK4 kinase, encoded by a disease gene for FHHt, inhibits ROMK activity in Xenopus oocytes. This identified WNK-dependent signaling as a major pathway for integrating Na^+-Cl^- and K^+ transport within the distal nephron. Details of the relevant effects of WNK kinases on NCC and ROMK are discussed earlier and elsewhere.[202–204] However, key findings include the differential influence of K^+ intake on circulating AT-II, ROMK activity (i.e., K^+ secretory capacity), the ratio of WNK1 isoforms, and the activity of NCC in the DCT. AT-II activates NCC via WNK4 and the downstream SPAK kinase,[202–204] thus reducing delivery of Na^+ to the CNT and limiting K^+ secretion. AT-II also inhibits ROMK activity via several mechanisms, including the downstream activation of c-src tyrosine kinases.[187–189] Whereas K^+ restriction induces renin and circulating AT-II, increases in dietary K^+ suppress AT-II levels.[189,205] A decrease in circulating and local AT-II explains why NCC phosphorylation and activity is downregulated by a high K^+ diet (Fig. 6.7).[56,206] Teleologically, this serves to increase the delivery of Na^+ to the CNT, thus increasing K^+ secretion. Finally, within CNT cells and principal cells, increases in aldosterone induce the SGK1 kinase, which phosphorylates WNK4 and attenuates the effect of WNK4 on ROMK,[207] while activating ENaC via Nedd4-2-dependent effects. However, when dietary K^+ intake is reduced, c-src tyrosine kinase activity increases under

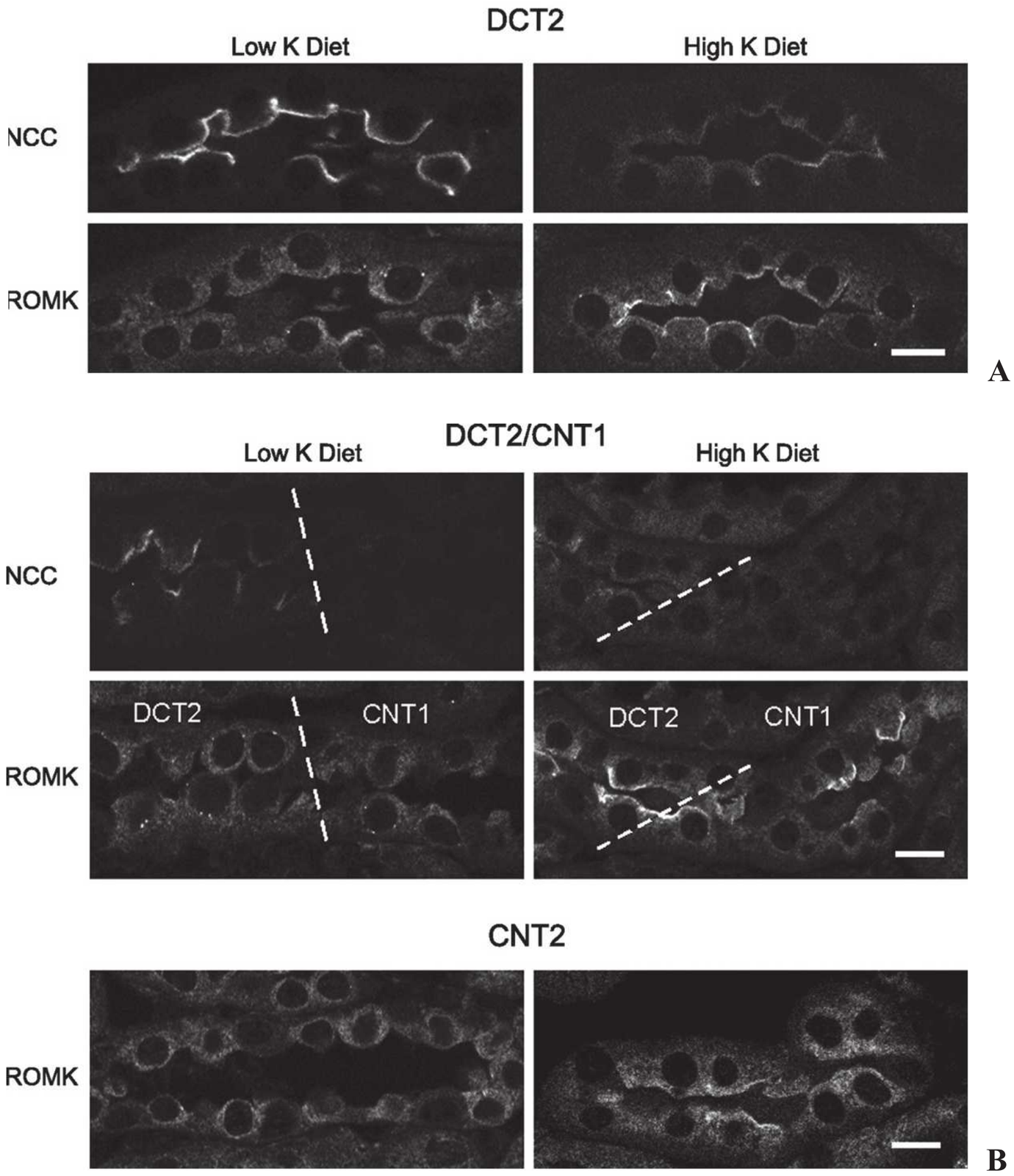

FIGURE 6.7 The differential effects of a high and low K^+ diet on the membrane expression of Na^+-Cl^- cotransporter (NCC) and renal outer medullary K^+ channel (ROMK) in late distal convoluted tubule (DCT) and the connecting tubule (CNT). **A:** Whereas NCC expression at the plasma membrane of DCT2 cells is enhanced in rats treated with a low K^+ diet and is reduced by a high K^+ diet, the opposite occurs for ROMK protein. **B:** This dichotomy persists at the junction between DCT2 and the early CNT (CNT1: the juncture is denoted by a *dashed white line*). However, within CNT2 segments, which lack NCC, apical labeling of ROMK under high K^+ conditions is less striking. (From Wade JB, et al. Differential regulation of ROMK (Kir1.1) in distal nephron segments by dietary potassium. *Am J Physiol.* 2011;300(6):F1385–1393.)

the influence of increased AT-II, causing direction inhibition of ROMK activity via tyrosine phosphorylation of the channel.[182–184] The increase in c-src tyrosine kinase activity also abrogates the effect of SGK1 on WNK4[201]; this latter mechanism can also be induced by AT-II.[208]

The ratio of WNK1 isoforms is also a critical determinant in the balance between distal Na^+ and K^+ transport. The shorter WNK1-S isoform, which lacks the kinase domain, appears to inhibit the effect of WNK1-L.[171,172] The ratio of WNK1-S to WNK1-L transcripts is reduced by K^+ restriction (greater endocytosis of ROMK)[172,176] and is increased by K^+ loading (reduced endocytosis of ROMK).[171,176] This suggests that the ratio between WNK1-S and WNK1-L functions as a switch to regulate distal K^+ secretion (see also Fig. 6.6). Transgenic mice that overexpress this inhibitory domain of WNK1-S have lower serum K^+ concentrations, a higher fractional excretion of K^+, and an increased expression of ROMK protein at the apical membrane of CNT and CCD cells, all of which are consistent with an important inhibitory effect of WNK1-S.[177] In contrast, WNK1-L activates NCC by enhancing expression at the cell membrane; this activation is blocked by WNK1-S. Again, transgenic WNK1-S mice have the expected phenotype, with renal salt wasting and hypotension due to marked suppression of membrane-associated total and phosphorylated NCC in the DCT and NKCC2 in the TAL.[209] In contrast, selective knockout of the WNK1-S exons leads to a major gain in function for NCC and NKCC2.[209] Therefore, K^+ loading will lead to an increase in the ratio of WNK1-S to WNK1-L, reduced endocytosis of ROMK, and enhanced endocytosis of NCC; this will have the effect of reducing Na^+ absorption in the DCT and thus increasing Na^+ delivery and tubular flow rate in the CNT, where ROMK +/– BK channel activity is maximized (see also Fig. 6.6). The converse cascade occurs in K^+ restriction.

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CHAPTER

7 Renal Acid–Base Transport

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ACID–BASE HOMEOSTASIS

The body maintains systemic acid–base homeostasis (a pH in the range of 7.35 to 7.45) in two ways: (1) through chemical buffering in extracellular fluid (ECF) and intracellular fluid (ICF), and (2) through physiologic regulation controlled by the metabolic, renal, and respiratory systems. The central nervous and respiratory systems control CO_2 tension (PCO_2), and the kidneys regulate the plasma HCO_3^- concentration. Buffers in the ECF and ICF guard against acid and base retention. These processes serve to dispose of carbonic and nonvolatile acids on a daily basis and pathologic quantities of acid and alkali as needed. This chapter briefly reviews the role of chemical buffers, but focuses primarily on the renal transport and regulatory processes that maintain acid–base balance.

Buffer Systems

The body's buffer systems keep the blood from becoming too basic or too acidic by combining with or releasing H^+, and thus are comprised of a base (i.e., an H^+ acceptor) and an acid (i.e., an H^+ donor). The most prodigious base is bicarbonate (HCO_3^-) and the most common acid is carbonic acid (H_2CO_3). Because buffer systems attenuate system changes, acid or base loads in the presence of a buffer cause smaller changes in the pH than if the buffer were absent. During homeostasis, the extracellular H^+ concentration ($[H^+]_e$) is constant. H^+ concentration can be expressed either directly as $[H^+]$ or indirectly as pH.

When CO_2 is dissolved in water, H_2CO_3 is formed according to the reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad (1)$$

The rate of this reaction, in the absence of the enzyme carbonic anhydrase, is slow, with a half-life of about 8 seconds at 37°C. The importance of carbonic anhydrase in bicarbonate equilibrium in the kidneys and lungs are discussed in the following paragraphs. The major portion of CO_2 remains as dissolved CO_2; only about 1 part in 1,000 forms H_2CO_3, a nonvolatile acid. Because H_2CO_3 is a weak acid, it dissociates rapidly to yield H^+ and HCO_3^-.

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad (2)$$

Because the concentration of H_2CO_3 remains low and proportional to the concentration of dissolved CO_2, Eqs 1 and 2 can be combined and treated as a single reaction:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (3)$$

The equilibrium constant for this reaction is given by

$$K = \frac{[H^+]\,[HCO_3^-]}{[CO_2]\,[H_2O]} \quad (4)$$

Defining $K' = K[H_2O]$ as the apparent equilibrium constant and using Eq. 4,

$$K' = \frac{[H^+]\,[HCO_3^-]}{\alpha_{co2}\,PCO_2} \quad (5)$$

Taking logarithms of both sides of Eq. 5 and recognizing that $pK' = \log_{10}(K')$, the familiar Henderson-Hasselbalch equation is derived:

$$pH = \frac{pK' + \log_{10}[HCO_3^-]}{(\alpha_{co2}Pco_2)} \quad (6)$$

Using $pK' = 6.1$ in Eq. 6, the Henderson equation is derived, which may be used in the clinical interpretation of acid–base data:

$$[H^+](nmol/L) = \frac{24\ PCO_2\ (mm\ Hg)}{[HCO_3^-]\ (mM)} \quad (7)$$

Physiologic Processes that Protect the Plasma Bicarbonate

Three physiologic processes mitigate changes in the HCO_3^-/CO_2 ratio: (1) metabolic regulation, (2) respiratory regulation, and (3) renal regulation. Metabolic regulation is

of minor importance in terms of the overall physiologic regulation of acid–base balance. Some enzymes are regulated by changes in blood pH. For example, the activity of phosphofructokinase, the pivotal enzyme in the glycolytic pathway, is inhibited by low pH and enhanced by high pH.

Because, under most circumstances, respiratory CO_2 excretion and CO_2 production are matched, the usual steady state arterial PCO_2 ($PaCO_2$) is maintained at 40 mm Hg. Underexcretion of CO_2 produces hypercapnia, and overexcretion produces hypocapnia. Production and excretion are again matched but at a new steady state PCO_2. Therefore, $PaCO_2$ is regulated primarily by neurorespiratory factors and is not subject to regulation by the rate of metabolic CO_2 production. Hypercapnia is primarily the result of hypoventilation, not increased CO_2 production. Increases or decreases in PCO_2 represent derangements of control of neurorespiratory regulation or can result from compensatory changes in response to a primary alteration in the plasma HCO_3^- concentration.

Sources of Endogenous Acids

Pathologically, acid loads may be derived from endogenous acid production (e.g., generation of ketoacids and lactic acids) or loss of base (e.g., through diarrhea) or from exogenous sources (e.g., ammonium chloride or toxin ingestion). Under normal physiologic circumstances, a daily input of acid derived from the diet and metabolism confronts the body. The net result of these processes amounts to about 1 mEq of new H^+ per kilogram per day entering the ECF.[1–4]

Sulfuric acid is formed when organic sulfur from methionine and cysteine residues of proteins are oxidized to SO_4^{2-}. The metabolism of sulfur-containing amino acids is the primary source of acid in the usual Western diet, accounting for approximately 50%. The quantity of sulfuric acid generated is equal to the SO_4^{2-} excreted in the urine.

Organic acids are derived from intermediary metabolites formed by the partial combustion of dietary carbohydrates, fats, and proteins as well as from nucleic acids (uric acid). Organic acid generation contributes to net endogenous acid production when the conjugate bases are excreted in the urine as organic anions. If full oxidation of these acids can occur, however, H^+ is reclaimed and is eliminated as CO_2 and water. The net amount of H^+ added to the body from this source can be estimated by the amount of organic anions excreted in the urine.

Phosphoric acid can be derived from hydrolysis of PO_4^{3-} esters in proteins and nucleic acids if it is not neutralized by mineral cations (e.g., Na^+, K^+, Mg^{2+}). The contribution of dietary phosphate to acid production is dependent on the kind of protein ingested. Some proteins generate phosphoric acid, whereas others generate only neutral phosphate salts.[1–4] Hydrochloric acid is generated by the metabolism of cationic amino acids (lysine, arginine, and some histidine residues) into neutral products. Other potential acid or base sources in the diet can be estimated from the amount of unidentified cations and anions ingested.

Potential sources of bases are also found in the diet (e.g., acetate, lactate, citrate) and can be absorbed to neutralize the acid loads from the three sources just mentioned. The net base absorbed by the gastrointestinal tract is derived from the anion gap (AG) of the diet minus that of the stool. Acid production is partially offset by HCO_3^-, which is produced when organic anions combine with H^+ and are oxidized to CO_2 and H_2O or when dibasic phosphoesters combine with H^+ during hydrolysis. The gastrointestinal tract may modify the amount of these potential bases reabsorbed under particular circumstances of acidosis or growth. It has been confirmed in patients ingesting an artificial diet that urinary net acid excretion is equal to urinary [(SO_4^{2-}) + organic A^- + dietary phosphoester-derived H^+].[1,2,5] Therefore, in summary, the metabolism of certain proteins, nucleic acids, and small fractions of lipids and certain carbohydrates generate specific organic acids that cannot be burned to CO_2 (e.g., uric, oxalic, glucuronic, hippuric acids). In addition, the inorganic acids H_2SO_4 and H_3PO_4, derived respectively from sulfur-containing amino acids and organophosphates, are excreted by the kidneys or the gastrointestinal tract.

Impact of Daily Metabolism on Acid–Base Balance

Human subjects ingesting a typical Western diet are confronted, under most physiologic circumstances, with an acid challenge. Metabolism generates a daily load of relatively strong acids (lactate, citrate, acetate, and pyruvate), but under physiologic circumstances, in the steady state, metabolic production and consumption are matched. However, if production and consumption rates become mismatched, organic acids can accumulate (e.g., lactic acid accumulation with anoxia or ischemia). These acids are buffered by HCO_3^- in the ECF, causing a decline in plasma HCO_3^- concentration as the organic acid concentration increases. During recovery, these organic acids reenter the metabolic pathways to CO_2 production, the removal of H^+, and the generation of HCO_3^- unless the organic anions are excreted (e.g., ketonuria), and thereby are no longer available for regeneration of HCO_3^-.

Both metabolic and renal regulatory mechanisms protect a normal HCO_3^- concentration in blood (25 mEq per liter) despite the daily addition of acid (or alkali) to the ECF. Although the buffering capacity of the body is magnified severalfold by respiratory adjustments in $PaCO_2$, primary changes in $PaCO_2$ may result in acidosis or alkalosis, depending on whether CO_2 is elevated above or depressed below the normal value: 40 mm Hg (*respiratory acidosis* and *respiratory alkalosis*), respectively. A primary change in the plasma HCO_3^- concentration as a result of metabolic production or the retention or excretion of an acid or base by the kidney evokes a ventilator response because a decrease or increase in pH is sensed by chemoreceptors in the circulation and signals the respiratory center to increase or decrease minute ventilation. The respiratory response to acidemia (increase ventilation

and decrease PCO_2) or alkalemia (decrease in ventilation and increase in PCO_2) thereby blunts the change in blood pH that would occur otherwise. Such respiratory alterations that adjust blood pH toward normal are referred to as *secondary* or *compensatory* alterations, because they occur in response to primary metabolic changes. While helpful, respiratory compensation to metabolic acidosis or alkalosis is never sufficient to return blood pH to normal. Therefore, the kidneys must play a very significant role in adjustments to metabolic disturbances by altering bicarbonate reabsorption and net acid excretion.

RENAL PARTICIPATION IN ACID–BASE HOMEOSTASIS

Although temporary relief from changes in the pH of body fluids may be derived from chemical buffering or respiratory compensation, the ultimate defense against the addition of nonvolatile acid or of alkali resides in the kidneys. The addition of a strong acid (e.g., HA) to the ECF titrates plasma HCO_3^-:

$$HA + NaHCO_3 \rightarrow NaA + H_2O + CO_2 \quad (8)$$

The CO_2 is expired by the lungs, and body HCO_3^- declines. This process occurs constantly as endogenous metabolic acids are generated. To maintain a normal plasma HC O_3^- in the face of the constant accession of metabolic acids, the kidneys must (1) conserve the HCO_3^- present in the filtered load (through reclamation), and (2) regenerate the HC O_3^- decomposed by reaction with metabolic acids (Eq. 8).

The first function of the kidneys in maintaining acid–base homeostasis is to reclaim or reabsorb filtered HCO_3^-. The kidneys filter approximately 4,000 mEq of bicarbonate daily (defined as the product of the glomerular filtration rate [GFR] and the plasma HCO_3^- concentration), and the excretion of any significant portion of this leads to metabolic acidosis. The renal tubules essentially reabsorb all of the filtered HCO_3^-, or may excrete excessive HCO_3^- when needed, to maintain the normal plasma HCO_3^- concentration of 25 mEq per liter.

HCO_3^- reclamation is accomplished principally in the proximal tubule. There is a subsequent contribution by the loop of Henle and a minor contribution by more distal nephron segments. Under most circumstances, the filtered load of HCO_3^- is absorbed almost completely, especially during an acid load. Nevertheless, when less acid is generated or if the plasma HCO_3^- concentration increases above the normal value of 25 mEq per liter, HCO_3^- will be excreted efficiently into the urine.

The generation of acid in the body by metabolism is referred to as *net acid production*. Because a typical Western diet generates fixed acids at 50 to 70 mEq per day, net acid excretion must be affected to maintain acid–base balance. Therefore, net acid excretion approximates 50 to 70 mEq per day. If acid production remained stable and unabated by net acid excretion, metabolic acidosis would ensue. Conversely, an increase in net acid excretion above the level of net acid production results in metabolic alkalosis. Each milliequivalent of net acid excreted corresponds to 1 mEq of HCO_3^- returned to the ECF. This process is called HCO_3^- regeneration and is necessary for replacing the HCO_3^- lost by the entry of fixed acids into the ECF or, less commonly, replacing the HCO_3^- excreted in stool or urine.

To prevent metabolic acidosis, this excreted acid must be recovered. The kidney recovers this acid through the second process, HCO_3^- regeneration, which is represented by the renal output of acid or **net acid excretion (NAE)**, or the sum of ammonia (NH_3)/ammonium (NH_4^+) plus excreted titratable acids (TA) minus any bicarbonate excreted.

$$\text{Net acid excretion (NAE)} = (U_{NH_4^+}V) + (U_{TA}V) - (U_{HCO_3^-}V) \quad (9)$$

where V is urinary volume per 24 hours and U refers to the concentration of the moiety in the urine.

TA excretion in the urine is represented by buffers that are filtered at the glomerulus and then titrated to acid moieties in the tubule lumen. Because renal H^+-secretory mechanisms cannot generate such steep pH gradients between the cell (pH ~7.3) and lumen, excretion of 70 mEq of acid per day requires that most of this acid be buffered in the luminal fluid.

Buffers with a *pK* between 5.0 and 7.4, the pH range of the luminal fluid, are most effective chemically. In this regard, the most abundant and effective buffer in the urine is the phosphate buffer pair, $H_2PO_4^- - HPO_4^{2-}$, with a p*K* of 6.9. The excretion of phosphate, the major titratable buffer, is usually regulated in accordance with phosphate homeostasis. Phosphate excretion increases modestly in metabolic acidosis, but the magnitude of the increase is limited by the filtered load of phosphate and, therefore, is significantly regulatable. In general, the buffers responsible are the $NH_3 - NH_4^+$ and titratable buffer, such as $HPO_4^{2-} - H_2PO_4^-$, systems. Physiologically, the most significant of these is the NH_3–NH_4^+ system.

Ammoniagenesis

Ammoniagenesis by protein catabolism is balanced by the generation of new HCO_3^- through renal NH_4^+ and TA excretion (Fig. 7.1A). The products of these neutral reactions are HCO_3^- and NH_4^+. Most ammoniagenesis occurs in the proximal tubule. NH_4^+ produced by the metabolism of amino acids reacts with HCO_3^- or forms urea and thus has no impact on acid–base balance. A portion of this NH_4^+ is diverted to glutamine synthesis, the amount of which is regulated by pH. Acidemia promotes and alkalemia inhibits glutamine synthesis. Each glutamine molecule metabolized produces two NH_4^+ and two HCO_3^- molecules (Fig. 7.1A) (water, CO_2, and glucose are also formed). This breakdown occurs as follows: glutamine is transported into the proximal

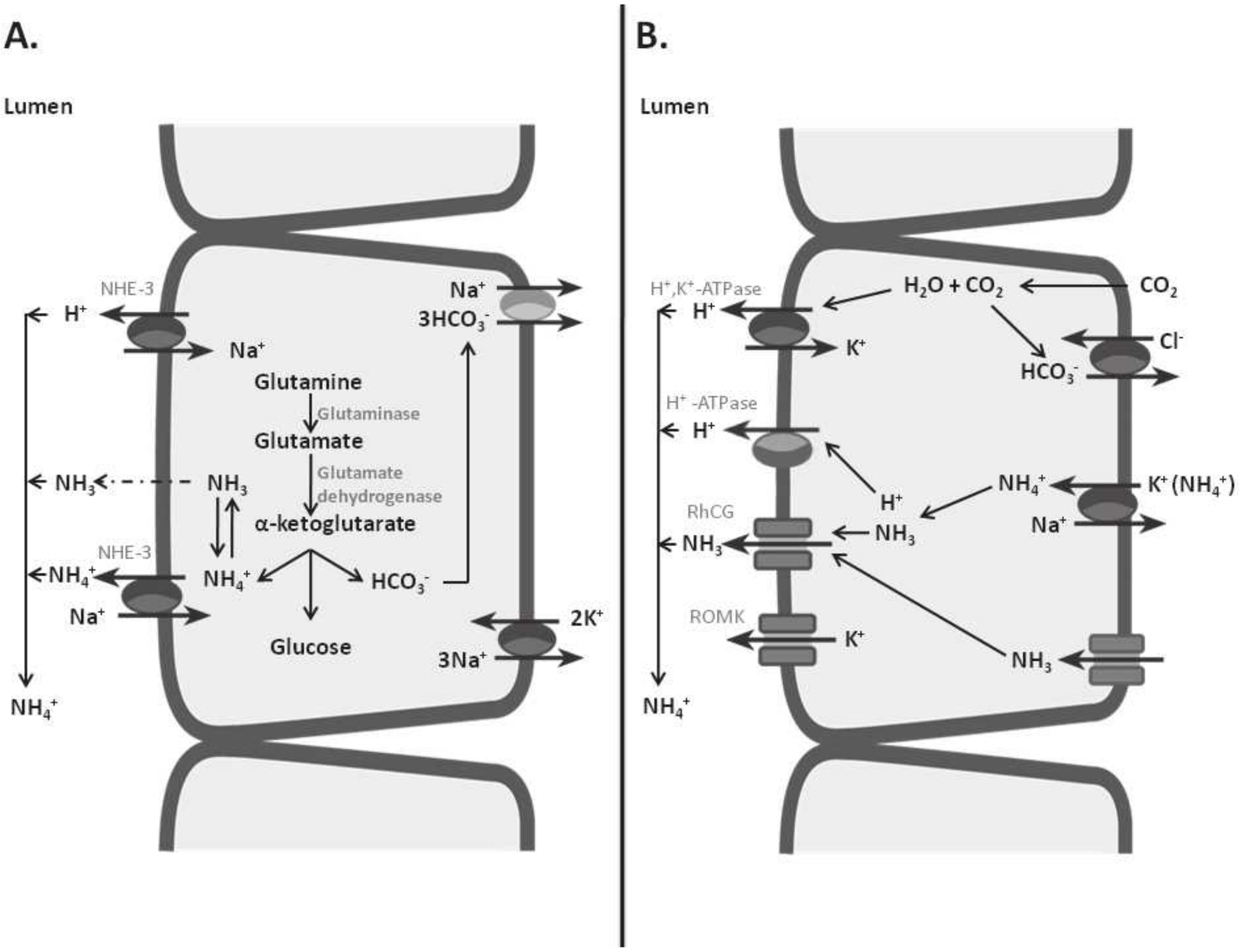

FIGURE 7.1 Cell models of ammonia synthesis and excretion pathways. **A:** A proximal convoluted tubule. Ammonia is derived from glutamine precursors to produce 2 NH_4^+ and 2 HCO_3^- molecules through an enzymatic pathway activated by acidemia and hypokalemia and inhibited by alkalemia and hyperkalemia. **B:** A type A intercalated cell in the collecting tubule. Ammonium enters across the basolateral membrane through the substitution of K^+ for NH_4^+ in K^+ conductance and is secreted across the apical membrane via renal outer medullary potassium (ROMK) or RhCG (see text). In both **A** and **B**, NH_3 diffusion coupled with H^+ secretion traps NH_4^+ in the tubule lumen.

tubule across the apical and basolateral membranes and is then transported into the mitochondria. In the mitochondria, an NH_4^+ molecule is formed when glutamine is deaminated by glutaminase to glutamate. When glutamate is then deaminated to α-ketoglutarate, a second NH_4^+ molecule is formed. Finally, the α-ketoglutarate molecule is metabolized in the Krebs cycle to CO_2, H_2O, glucose, and the two HCO_3^- molecules. Glutamine deamination in the kidney is highly regulated by systemic pH, so that acidemia augments and alkalemia inhibits NH_4^+ and HCO_3^- production. Hepatic regulation of NH_4^+ metabolic pathways appears to facilitate glutamine production when NH_4^+ excretion is stimulated by acidemia or, conversely, blunts glutamine production when excretion is inhibited by alkalemia.[5]

Renal Excretion of Ammonia/Ammonium

The ultimate control, however, resides in the renal excretion of NH_4^+, because the NH_4^+ must be excreted to escape entry into the hepatic urea synthetic pool. Hepatic urea synthesis would negate the new HCO_3^- realized from α-ketoglutarate in the kidney. Thus, ammoniagenesis does not contribute to acid–base homeostasis until the urine is acidified and the HCO_3^- produced by glutamine metabolism is added to the ECF. In order for this to occur, the ammonia synthesized in the proximal tubule has to be transported to the terminal nephron segments and is then excreted by the kidney (the pathway is described in detail in the following paragraphs). This ammonia transport occurs through both nonionic diffusion (for NH_3) and ionic transport (for NH_4^+). NH_3 quickly and easily diffuses across the plasma membrane to compartments of lower pH, where it is rapidly converted to NH_4^+, thus leading to accumulated NH_4^+ in acidic compartments. NH_4^+ can diffuse across tight junctions or can substitute on transporters such as the apical membrane Na^+–H^+ (NHE-3) antiporter (as Na^+–NH_4^+ exchange).[6] In general, NH_3 has a higher membrane permeability than NH_4^+, but the concentration of NH_4^+ exceeds that of NH_3 by approximately 100-fold at a pH 7.4 (pK 9.3). Thus, the relative

permeability of the two moieties defines whether either crosses membranes by nonionic NH_3 diffusion or ionic NH_4^+ transport; for example, if the NH_4^+ moiety is transported in a specific nephron segment as in the thick ascending limb of the loop of Henle by substitution for potassium on the Na^+–K^+–$2Cl^-$ cotransporter.

The first step of ammonia transport and urinary excretion following ammoniagenesis in the proximal tubule is for the ammonia to enter the proximal tubule lumen, and it can do so in two ways. Because the luminal pH is lower than cell pH, much of the NH_3 is diffused into the tubule fluid and is converted into NH_4^+. The apical membrane Na^+-H^+ antiporter also plays an important role in NH_3–NH_4^+ transport (Fig. 7.1A).[7,8] Transport mechanisms for NH_3–NH_4^+ in more distal segments function to ensure that the NH_3– NH_4^+ issuing out of the proximal tubule fluid is excreted into the urine in a regulated fashion.

From the lumen, ammonia enters the medullary interstitium (Fig. 7.2) via the thick ascending limb of the loop of Henle. If ammonia did not enter the interstitium, it would return to the distal nephron and diffuse back into the blood, thereby failing to be excreted in the urine. The thick ascending limb of the loop of Henle also creates a "corticomedullary concentration gradient" for NH_3–NH_4^+. The NH_3–NH_4^+ transported into the medullary interstitium accumulates at a higher concentration by countercurrent multiplication and can reenter the collecting tubule for a final excretion in a more regulated way. Nonionic diffusion of NH_3 into the medullary interstitium is not possible because the thick ascending limb's apical membrane is highly impermeable to NH_3.[9] NaCl transport enables the ionic transport of NH_4^+ into the interstitium. NH_4^+ can substitute for K^+ on the Na^+–K^+–$2Cl^-$ cotransporter, or it can be transported via the renal outer medullary potassium (ROMK) channel for transport across the apical membrane (Fig. 7.1B). Alternatively, lumen-positive voltage can drive NH_4^+ diffusion across the tight junction. NH_4^+ that enters the thick ascending limb cell can exit the basolateral membrane by nonionic diffusion driven by cell-to-interstitial NH_3 concentration gradient. Once in the interstitium, the NH_3

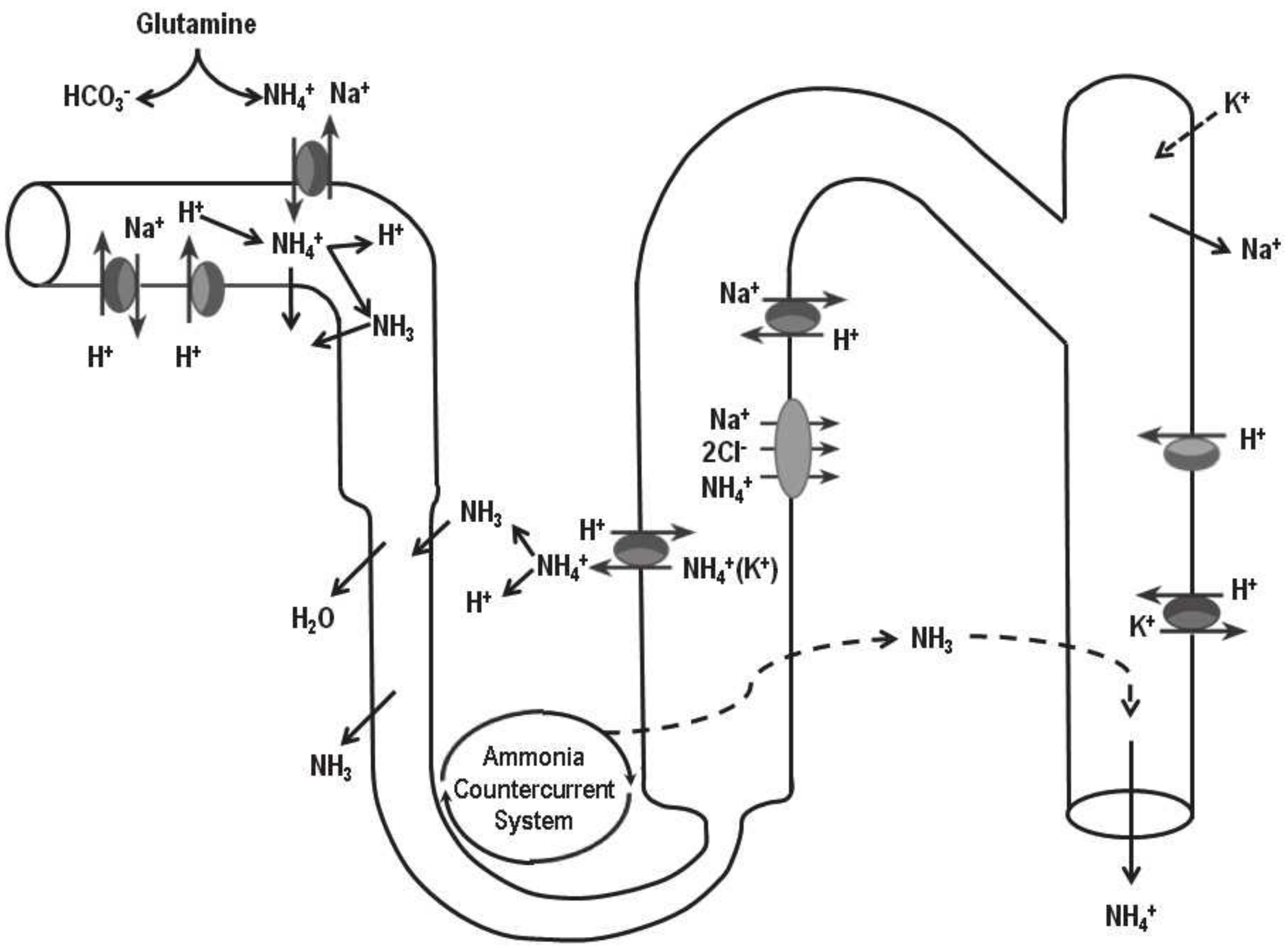

FIGURE 7.2 Nephron sites of ammonia production, secretion, transport, and ultimately, excretion. Note that approximately 75% of ammonia/ammonium delivered to the loop of Henle is transported into the medullary interstitium and is accumulated through countercurrent multiplication, thus resulting in higher concentrations from the cortex to the medulla. In acidosis, both the production of ammonia/ammonium and the transport, multiplication, and excretion of NH_4^+ is augmented. Therefore, the physiologic response of the kidney to an acid load is to increase bicarbonate reabsorption and ammonia production and excretion. The resulting urine should demonstrate a pH $<$5.5 and a higher concentration of ammonium. If these conditions are met, a normal gap metabolic acidosis would be nonrenal in origin.

will again accept a H^+, forming NH_4^+. Furosemide, which inhibits the Na^+–K^+–$2Cl^-$ cotransporter and secondarily inhibits the lumen-positive voltage, significantly inhibits all three mechanisms of thick ascending limb NH_3–NH_4^+ absorption.[6,10,11]

Through these processes, NH_3–NH_4^+ accumulates in the renal medulla (Fig. 7.2). The countercurrent system concentrates the solute toward the tip of the medulla and preserves the cortical-to-medullary gradient. Blood in the descending vasa recta has lower NH_3–NH_4^+ concentrations than the surrounding interstitium, allowing net entry. Conversely, blood in the ascending vasa recta has a higher NH_3–NH_4^+ concentration than the surrounding interstitium, causing net efflux. In patients with an intact urinary concentrating system and medullary architecture, the net result is a steep gradient, with the highest concentrations of NH_3–NH_4^+ in the inner medullary interstitium.

Ultimately, NH_3–NH_4^+ is transported from the medullary interstitium into the lumen of the collecting tubule for final urinary excretion (Figs. 7.1B and 7.2). The apical and basolateral membranes of the medullary collecting tubule are permeable to NH_3, allowing nonionic diffusion to be driven by the low pH of the collecting tubule fluid. In addition, NH_4^+ can be transported from the medullary interstitium into the collecting tubule cell on the Na^+, K^+-ATPase.[12] Once inside the cell, the dissociation of NH_4^+ into NH_3 and H^+ provides a source of H^+ for transport into the luminal fluid by the vacuolar H^+-ATPase. H^+ secreted into the luminal fluid will combine with secreted NH_3 to form NH_4^+, maintaining the gradient for nonionic NH_3 diffusion into the luminal compartment, which has a much lower pH, and this way NH_4^+ is "trapped" in tubule fluid and is swept into the collecting system. Medullary interstitial NH_3–NH_4^+ concentrations are determined by the rate of ammonia synthesis in the proximal tubule, the efficiency of ammonia transport into the lumen of the proximal tubule and subsequently into the medullary interstitium in the thick ascending limb, and the efficiency of countercurrent trapping of ammonia in the medullary interstitium. Both ammonia synthesis and each step in the highly integrated response by the nephron to excrete ammonia are upregulated by metabolic acidosis and chronic hypokalemia, and are inhibited by alkalosis and hyperkalemia (see Chapter 18).

The Rh glycoprotein (RhCG/Rhcg), which is expressed not only in both principal and intercalated cells but also in the distal convoluted tubule, connecting tubule, initial collecting tubule, cortical collecting duct, and the outer and inner collecting ducts, is now thought to be critical for the excretion of ammonia by the kidney (Fig. 7.1B).[13,14] RhCG expression is greater in A-type intercalated cells than in principal cells but is not detectable in B-type intercalated cells.[15] RhCG is thought to play a role in renal ammonia excretion because of some recent genetic studies. Biver and colleagues[16] found that global RhCG deletion impaired ammonia excretion in the urine and decreased basal ammonia excretion in response to metabolic acidosis. Similar findings were observed when RhCG was deleted only from the collect ducts.[17] In summary, that both intercalated and principal cells express RhCG and that metabolic acidosis increases RhCG in both cell types suggests that RhCG contributes to transepithelial ammonia secretion.[16]

Therefore, the sum of TA and ammonia/ammonium excretion is represented, as noted previously, as net acid excretion (Eq. 9). All three components of net acid excretion are dependent on the operation of an H^+ secretory mechanism into the tubule fluid. Therefore, increases in the rate of H^+ secretion will increase urinary ammonium and TA excretion and will lower urinary HCO_3^- excretion. A protein-rich diet leads to acid production, so the kidneys must both reclaim the filtered bicarbonate and also excrete an acid equivalent to that produced in the diet. In this way, in a steady state, net acid production equals net acid excretion, and acid–base balance is maintained. In the presence of nonprotein-rich diets, the tubules do not reclaim all of the filtered bicarbonate and they decrease production of new bicarbonate in order to maintain acid–base homeostasis.

SPECIFIC MECHANISMS OF H^+–HCO_3^- TRANSPORT ALONG THE NEPHRON

How the transport of H^+ and HCO_3^- in the nephron occurs is generally defined by the specific characteristics of each epithelium and cell type within the nephron segments. Bicarbonate reabsorption is largely the responsibility of the proximal tubule and net acid secretion is largely of the distal tubule. A number of transport mechanisms across either the apical (for H^+) or basolateral (for alkali) membranes have been identified and are detailed in the following paragraphs.

Apical Membrane H^+-Transport Mechanisms

Renal tubule cells use both primary and secondary transport mechanisms for active H^+ secretion. A primary transport couples the metabolism to a transport mechanism, whereas a secondary transport couples the transporter to the metabolism, which generates a concentration gradient for a solute.

There are two mechanisms for primary active H^+ secretion, both of which are directly related to the metabolism of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (P_i). The first and quantitatively most important mechanism involves the vacuolar H^+-ATPase, or V-type ATPase, a multisubunit protein complex that secretes H^+,[18] and is electrogenic, thereby generating a positive charge in the tubule lumen. The ability of the H^+-ATPase to affect H^+ secretion depends on where it is located; when it is in the proximal tubule, it minimally mediates H^+ secretion into the lumen, but in type A (acid secreting) intercalated cells of the collecting tubule, it significantly mediates H^+ secretion into the lumen. This

mechanism is able to adapt as needed by increasing mRNA and protein abundance to protect against metabolic and respiratory acidosis.

The second mechanism for primary active H^+ secretion involves P-type ATPases. The P-type ATPases, which are isoforms of the H^+–K^+–ATPase ($HK\alpha_1$, the gastric isoform, and $HK\alpha_2$, the colonic isoform), participate importantly in H^+ transport by the collecting tubule. These P-type ATPases are coupled to ATP metabolism, have a phosphorylated high-energy intermediate, and possess α and β subunits. Both the gastric and colonic isoforms are expressed on the apical membranes of type A intercalated cells (IC) in the cortical collecting tubule, and the outer and inner medullary collecting tubules.[19–24] These transport processes are electroneutral, mediating H^+ secretion across the apical membrane into the tubule lumen in exchange for K^+ entry (into the cell). Both isoforms react to metabolic or respiratory acidosis by increasing in abundance and activity. Alternatively, the two types of H^+-transporters are also located on the basolateral membranes of type B (bicarbonate secreting) ICs in the cortical and outer collecting tubule.[25–29] The H^+-ATPase or H^+, K^+-ATPase in this location mediates H^+ transport across the basolateral membrane into the interstitium.

The secondary active H^+ transporter is an isoform of the Na^+-H^+ antiporter, NHE-3. Although there are eight known isoforms of the Na^+-H^+ antiporter, NHE-3 (*SLC9A3*) is the most important for bicarbonate reabsorption. It is located in the S_1 and S_2 proximal tubule and thick ascending limb of the loop of Henle on the apical membrane and mediates H^+ secretion into the tubule fluid.[30–33]

Basolateral Bicarbonate Transport

Two transporters mediate passive base efflux along the nephron. One transporter is the electroneutral Cl^-/HCO_3^- exchanger, which carries HCO_3^- out and Cl^- into the cell across the basolateral membrane in the S3 segment of the proximal tubule, the thick ascending limb of the loop of Henle, and the cortical and medullary collecting tubules. This Cl^-/HCO_3^- exchanger is a member of the anion exchanger 1 (AE1) family and is a truncated form of the red cell AE1 Cl^-/HCO_3^- exchanger,[34–37] or kAE1 (*SLC4A1*). The mRNA lacks the first three exons of red cell mRNA,[38] but the net result of this truncation is a protein with similar transport characteristics but altered cytoskeletal interactions. Cl^- enters the cell in exchange for HCO_3^- and exits the basolateral membrane through a Cl^- conductance.[39,40]

The second is the sodium bicarbonate cotransporter (NBC), of which there are four isoforms. The NBCe1 (*SLC4A4*) isoform is the most important for base efflux, mediating the majority of base efflux (~80%) out of the cell and into the interstitium.[41–44] It is present on the basolateral membrane of the proximal tubule and is electrogenic because it transports three base molecules with one Na^+ ion ($Na^+/n\ HCO_3^-$ or *SLC4A4*).[41–44]

SEGMENTAL CONTRIBUTION TO BICARBONATE ABSORPTION AND ACIDIFICATION OF THE URINE

Proximal Tubule

The proximal tubule reabsorbs approximately 80% of the filtered HCO_3^- load; the HCO_3^- concentration at the end of the proximal tubule is approximately 8 mEq per liter and the pH is 6.7.[45,46] The reabsorption rate is a function of proximal tubule segmentation, so the rate of HCO_3^- reabsorption is highest in the early segment (S_1), decreases in the midsegment (S_2), and is lowest in the terminal segment (S_3).

The specific mechanisms responsible for H^+–HCO_3^- transport in the proximal tubule are shown in Figure 7.3. Approximately two-thirds of H^+ secretion is mediated by the apical membrane Na^+–H^+ antiporter, and the remaining one-third is mediated by the vacuolar H^+–ATPase.[47–49]

The active secretion of H^+ into HCO_3^--rich glomerular ultrafiltrate by the NHE-3 results in the formation of H_2CO_3. Luminal carbonic anhydrase (type IV) facilitates the conversion of H_2CO_3 to CO_2 and H_2O and prevents the generation of limiting pH gradients across the proximal convoluted tubule.[50] CO_2 diffuses readily into the cell where, under the influence of cytoplasmic carbonic anhydrase (type II), it forms H_2CO_3 that dissociates rapidly to H^+ and HCO_3^-, which are transported, respectively, across the apical and basolateral membranes. Studies from Weinman's group[51] have clearly established that NHERF-1 (sodium-hydrogen exchange factor 1) is required for a protein kinase A (PKA)–associated downregulation of NHE-3 activity. NHE-3 has a specific requirement for NHERF-1 for cAMP-mediated phosphorylation and inhibition. In addition to H^+ secretion via NHE-3, an apical H^+-ATPase is also responsible for a small but significant fraction of bicarbonate reclamation in the proximal tubule.

HCO_3^- generated in the proximal tubule cell exits passively across the basolateral membrane Na^+/n-HCO_3^- cotransporter, or NBCe1, encoded by *SLC4A4* which, as mentioned previously, transports the equivalent of three HCO_3^- ions out in parallel with one Na^+ out (Fig. 7.3).[41–44,52,53] This is a passive process because this transporter carries a net of two negative charges, and the negative cell voltage provides a strong, favorable driving force for base efflux.

As noted previously, in addition to bicarbonate reabsorption, a second function of the proximal tubule in acid–base balance is to synthesize the NH_3 needed for HCO_3^- regeneration (see Fig. 7.1A) (see previous discussion on ammoniagenesis and ammonia/ammonium transport).

The Loop of Henle

Approximately 50% to 70% of the HCO_3^- delivered out of the proximal tubule is reabsorbed in the thick ascending limb of the loop of Henle.[54] The secretion of H^+ across the apical membrane is mediated by NHE-3 as in the proximal tubule (Fig. 7.4B).[30,54] A low cellular Na^+ concentration, as in the proximal tubule, is maintained by the

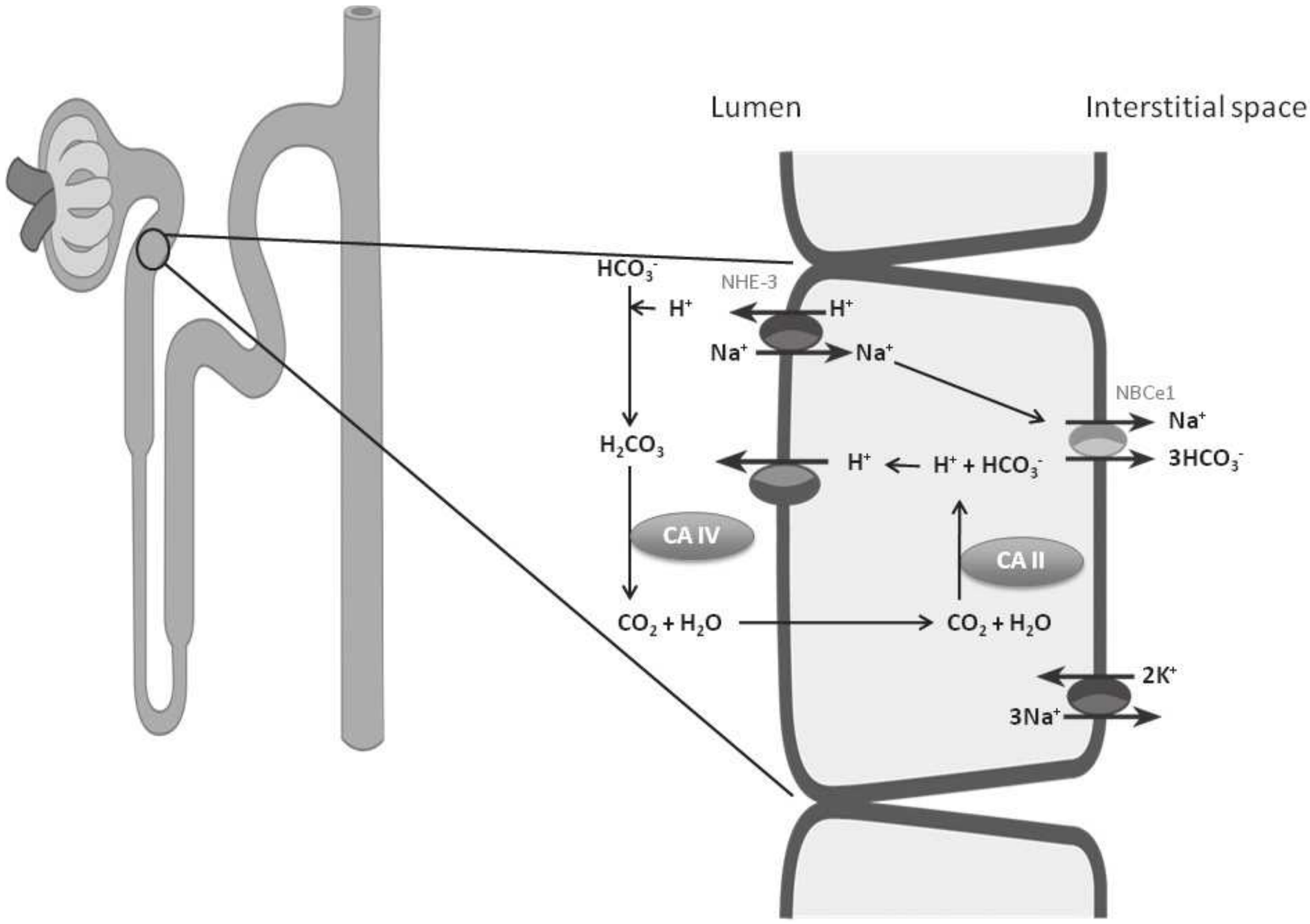

FIGURE 7.3 Aproximal convoluted tubule: the coupled apical and basolateral transporters responsible for bicarbonate reabsorption.

basolateral membrane Na^+, K^+-ATPase, to provide the driving force for operation of the Na^+/H^+ exchanger. Both AE1 (chloride–bicarbonate exchange) and NBC-2 or -3 (sodium–bicarbonate cotransport) are expressed on the basolateral membrane, where they mediate bicarbonate exit across the basolateral membrane.[55–57]

The Distal Tubule and the Collecting Duct

The distal tubule and the collecting duct are responsible for the final acidification of the urine. This process involves three steps: (1) the residual HCO_3^- in tubule fluid is reabsorbed; (2) the final titration of buffers is accomplished (TA excretion); and (3) the H^+ secreted traps ammonium in the lumen so that the majority of ammonia produced is excreted as NH_4^+ (Fig. 7.2). Stoichiometrically, excretion of NH_4^+ is necessary to regenerate the HCO_3^- lost in the buffering of acid products of metabolism. Therefore, each NH_4^+ excreted replenishes one HCO_3^- that is returned to the ECF via the renal vein. If the NH_4^+ is not excreted by the kidney, NH_3 is returned to the liver where each NH_3 molecule generates $2H^+$ in ureagenesis. Conversely, when the kidney is required to excrete alkali, the distal nephron is able to secrete HCO_3^-.[58]

The distal nephron, which begins at the macula densa, is a short segment that exhibits a small amount of amiloride-sensitive HCO_3^- reabsorption mediated by an apical membrane Na^+-H^+ antiporter isoform NHE2 (Fig. 7.4C).[29,59–61] Subsequent segments, including the connecting tubule, the initial cortical collecting tubule, and the cortical collecting tubule all possess ICs that mediate H^+–HCO_3^- transport. Type A ICs secrete H^+ into the tubule fluid and type B ICs secrete HCO_3^- across the apical membrane (Fig. 7.4D).

Type A ICs accomplish H^+ ion secretion through a process mediated by two ATP-dependent H^+ pumps, a V-type H^+-ATPase, and an H^+, K^+-ATPase (which is a P-type ATPase) (Fig. 7.5). There is abundant evidence supporting a role for both transporters. The H^+-ATPase has been localized by (1) the labeling of cells with antibodies against subunits of the vacuolar H^+-ATPase,[25,26] (2) the inhibition of H^+ transport by bafilomycin A_1, a specific inhibitor of vacuolar H^+-ATPases,[27,29] and (3) the electrogenicity of acidification in this nephron segment.[62] With regard to the latter, luminal acidification is associated with a lumen-positive voltage. Generation of a transepithelial voltage requires electrogenic or conductive processes on both apical and basolateral membranes. Because the IC does not possess significant apical membrane conductance,[39,40] electrogenic acidification requires an electrogenic H^+ pump.

Immunohistochemical studies show localization of the electroneutral H^+, K^+-ATPase to the apical membrane of a type A IC using antibodies against both the gastric ($HK\alpha_1$) and the colonic ($HK\alpha_2$) H^+, K^+-ATPase isoforms (Fig. 7.5).[63–65] Bicarbonate transport and potassium absorption are both

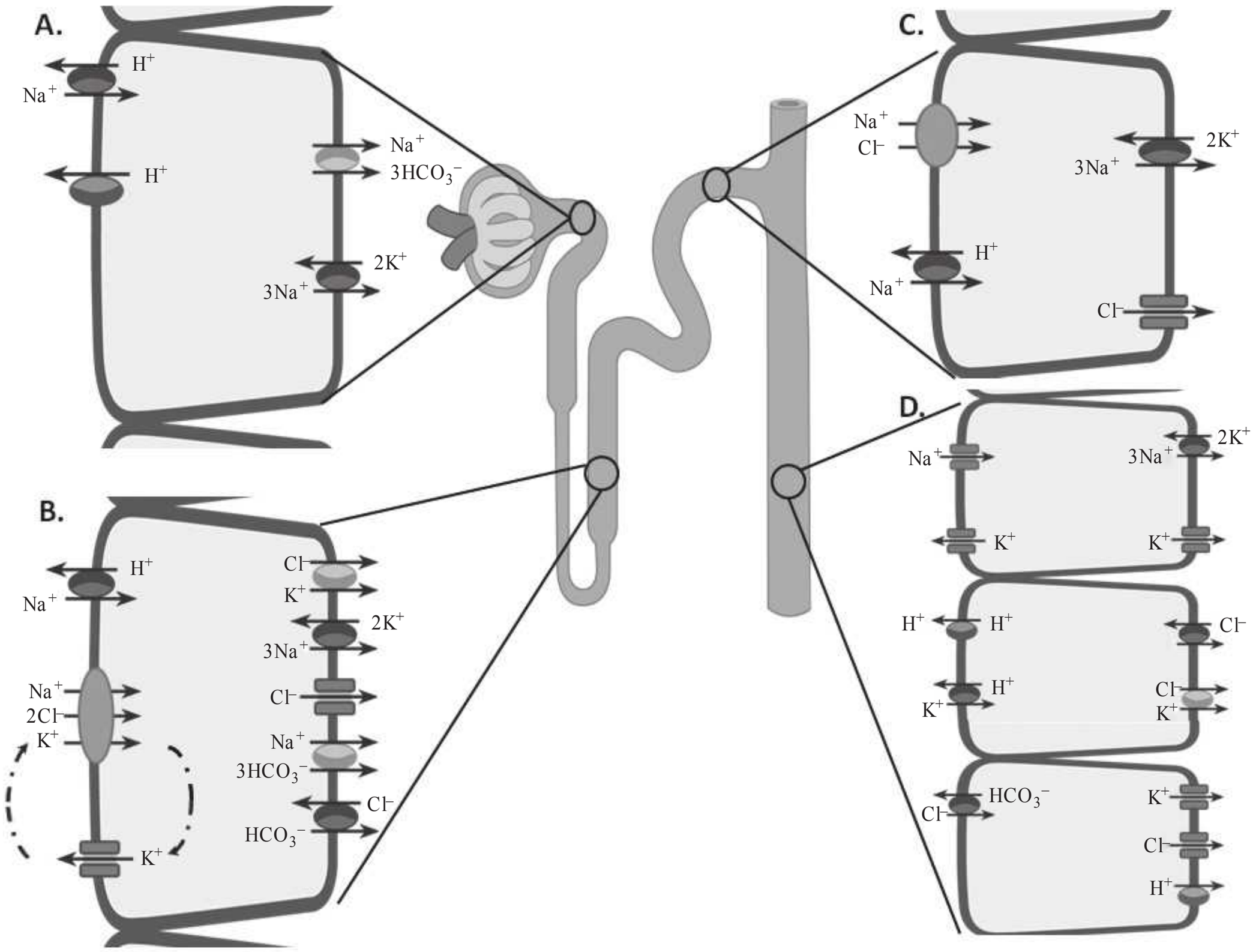

FIGURE 7.4 The H^+–HCO_3^- transport in the proximal tubule (*A*), the thick ascending limb of the loop of Henle (*B*), the distal convoluted tubule (*C*), and the collecting duct (*D*). Note that three cell types are displayed for the collecting tubule (*D*): the principal cell that does not participate directly in H^+ secretion, the type A intercalated or acid-secreting cell, and the type B intercalated or base-secreting cell.

inhibited by SCH 28080, a specific inhibitor of the gastric H^+, K^+-ATPase.[29,66–69]

The colonic isoform of H^+, K^+-ATPase, $HK\alpha_2$, is highly responsive to and upregulated by chronic potassium depletion. The site of regulation is most robust in the outer and inner medulla. The H^+, K^+-ATPase may play a highly significant role in potassium depletion, when its expression is significantly increased to minimize K^+ loss in the urine.[24,70] This regulatory response may explain the role of persistent chronic hypokalemia in the maintenance of metabolic alkalosis by maintaining H^+ secretion mediated by the H^+, K^+-ATPase, to prevent dangerous hypokalemia. In addition, both $HK\alpha_1$ and $HK\alpha_2$ increase abundance and activity due to either metabolic or respiratory acidosis. There is no specific inhibitor for $HK\alpha_2$ but Li and associates[71] in the DuBose laboratory have shown inhibition by intermediate concentrations of ouabain in vitro. Therefore, in summary, both the V-type H^+-ATPase and the H^+, K^+-ATPases contribute to H^+ secretion, although their relative contributions appear to be nephron segment–specific.

Type BICs secrete HCO_3^- into tubule fluid through the coupling of active secretion of H^+ across the basolateral membrane into the interstitium (Fig. 7.5) by both a vacuolar H^+-ATPase and an H^+, K^+-ATPase.[66] Therefore, these cells are mirror images of type A ICs and appear to be involved in protecting against metabolic alkalosis through a more efficient excretion of chronic bicarbonate loads. Type B ICs secrete HCO_3^- across the apical membrane into the collecting duct via a Cl^-/HCO_3^- exchanger. However, this Cl^-/HCO_3^- exchanger is not the same AE1 Cl^-/HCO_3^- exchanger found on the basolateral membrane of the proximal tubule. Rather, the anion exchanger pendrin, which is encoded by *SLC26A4*, is localized to the apical membrane of nonacid-secreting ICs in the kidney cortical collecting duct (CCD) (Fig. 7.5). Cl^- that enters the cell in exchange for HCO_3^- exits the cell through a basolateral membrane Cl^- conductance. This process does not generate a transepithelial voltage, because there is no net transcellular current.

The ability to secrete HCO_3^- disappears in the outer medullary collecting tubule. Here, the H^+ ion secretion is mediated by cells that are functionally similar to type A ICs.

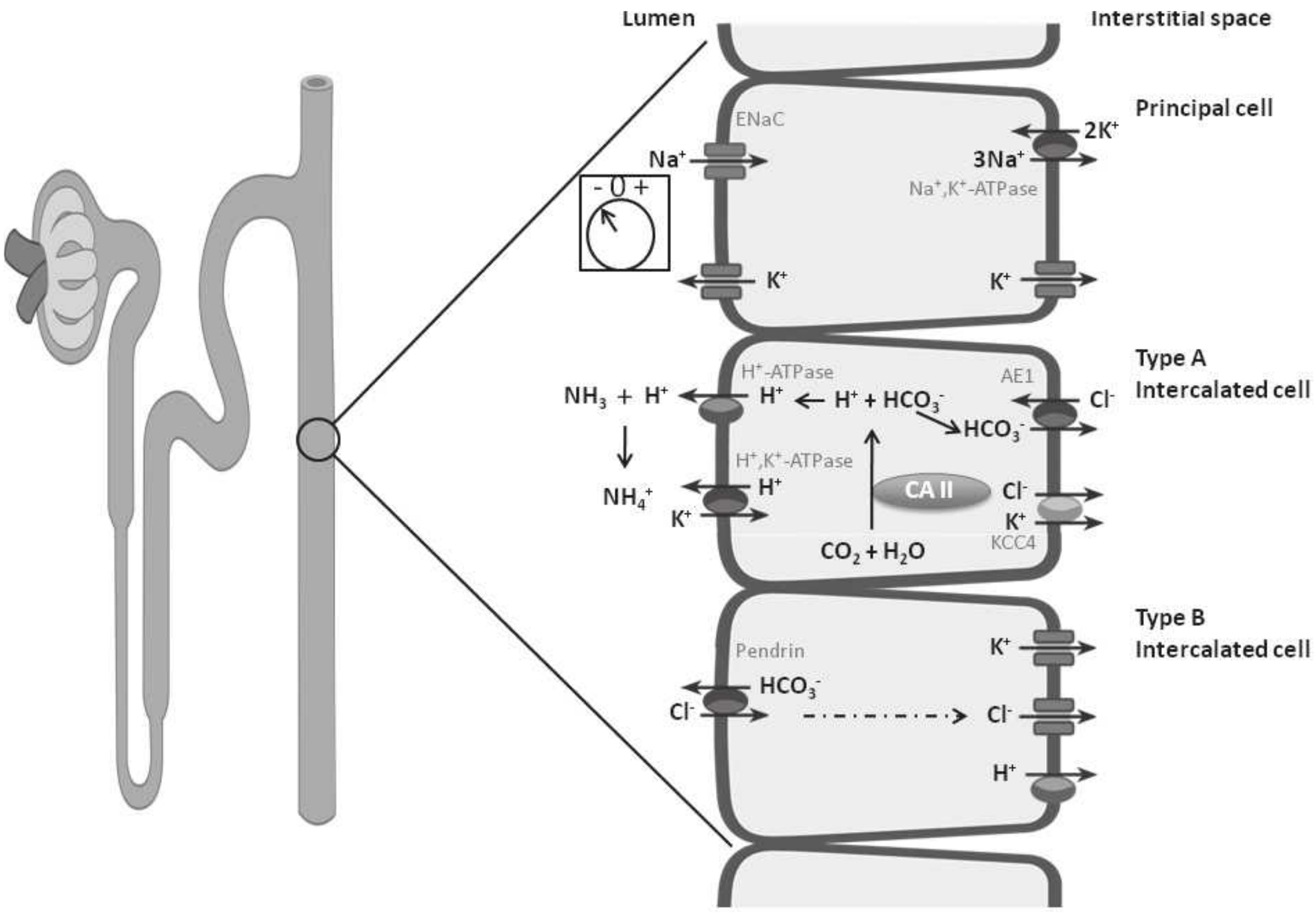

FIGURE 7.5 The H^+–HCO_3^- transport in the collecting tubule, which occurs in type A and type B intercalated cells.

In some cases, these cells are designated as non-A and non-B intercalated cells. The inner stripe of the outer medullary collecting tubule is a high-capacity segment for H^+-secretion. The inner medullary collecting tubule has been difficult to study because of the difficulty in isolating this nephron segment for tubular perfusion. Nevertheless, studies in our laboratory provide evidence for a mechanism of H^+ secretion similar to that described for the type A ICs.[24,70] Moreover, it should be emphasized that this key segment is responsible for the final acidification of the tubule fluid and generates the low urinary pH typical of high acid excretion states.

INTEGRATED KIDNEY REGULATION OF URINARY ACIDIFICATION AND EXCESS BASE EXCRETION

The integrated response of heterogeneous nephron segments to regulate acid–base balance requires the regulation of transport in each of the nephron segment in concert (Fig. 7.4). In the following sections, the most important regulatory responses of H^+–HCO_3^- transport are emphasized.

Luminal HCO_3^- Delivery

In order to avoid metabolic acidosis or alkalosis, H^+ ion secretion and HCO_3^- reabsorption must work in concert. GFR increases lead to luminal flow rate increases, which in turn signal the tubules to secrete H^+ more rapidly. If the rate of acid excretion did not change, too little bicarbonate would be reabsorbed and the luminal pH would decrease too slowly. Alkaline tubule fluid stimulates apical membrane H^+ transporters.[72] The tubule flow rate can also directly regulate the rate of proximal tubule H^+ secretion, regardless of HCO_3^- concentration or luminal composition.[73]

Systemic pH

As noted previously, the kidneys respond to changes in systemic arterial pH (PCO_2) by either increasing urinary acidification (for acidosis) or by inhibiting acidification and increasing bicarbonate excretion (for alkalosis). Because changes in plasma pH cause changes in renal interstitial pH, the mechanisms influencing bicarbonate efflux on the basolateral membrane are also affected. Acidosis enhances HCO_3^- efflux and alkalosis inhibits basolateral membrane HCO_3^- efflux. Thus, in acidosis, cell pH decreases; whereas in alkalosis, it increases.[74] It is the change in intracellular pH that determines apical membrane H^+ ion secretion; relative acidity stimulates H^+ secretion, whereas a more alkaline pH reduces or inhibits H^+ secretion.

The Na^+-H^+ antiporter is activated by decreases in intracellular pH,[75] which also stimulates the insertion of H^+ transporters into the apical membrane.[76,77] These "reserved" H^+ ions in the apical membrane can enhance the rate of H^+

transport when needed. Long-term adaptations in tubular function result from chronic systemic pH changes. In the proximal tubule, chronic metabolic and respiratory acidosis enhances the proximal tubule capacity for H^+ secretion and ammoniagenesis.[78,79] These changes are mediated by parallel increases in apical membrane Na^+-H^+ antiporter and electrogenic basolateral membrane Na^+/n-HCO_3^- cotransporter activity.[80–83]

Such transporter adaptations are known to be a direct effect of pH because they can be experimentally induced by incubating cultured proximal tubule cells in acid media.[84] Enhanced activity of the apical Na^+-H^+ antiporter is mediated by the trafficking of NHE-3 to the apical membrane, and increasing whole cell NHE-3 abundance.[85] Several groups have demonstrated that endothelin-1 (ET-1) increases the activity of NHE-3 and that metabolic acidosis stimulates renal proximal tubule ET-1 expression.[86–89] Studies in ET_B receptor knockout mice demonstrate that the ET-1/ET_B signaling pathway mediates acid stimulation of NHE-3 activity.[90,91] In addition, a consensus sequence (KXXXVPKXXXV) in the second intracellular loop of the ET_B receptor appears to be responsible for ET-1/ET_B stimulation of NHE-3 activity.[92]

How the extracellular pH and the systemic acid–base status are sensed by renal epithelial cells and initiate a regulatory response has not been elucidated. Factors such as pH, PCO_2 and bicarbonate, along with possible hormonal stimuli[28] have all been proposed at one time or another. Early studies by Pitts dating to the 1940s indicated that metabolic acidosis induced by the infusion of 0.1 N HCl causes an increase in net acid excretion, predominately as ammonium excretion.[93,94] Schwartz and Al-Awqati[77] demonstrated that an increase in ambient PCO_2 stimulated proton secretion in isolated perfused proximal tubules and in the collecting ducts of rabbit nephrons. It was later shown that respiratory acidosis was associated with the apical insertion of the H^+-ATPase. Recent studies have suggested that the nonreceptor tyrosine kinases Pyk2 and c-Src may represent a possible signaling pathway involved in the increase in NHE-3 function in the proximal tubule in response to metabolic acidosis.[95–97] Although there is preliminary evidence that Pyk2 is expressed and may also function in the collecting tubule as a pH sensor, the signaling pathway has not yet been delineated (K. Fisher, personal communication, February 2012).

Recently, a small number of G protein-coupled receptors (GPCR) have been identified, the activation of which is stimulated by a reduction in extracellular pH, prompting them to be termed "proton-sensing" receptors. One of these putative proton-sensing GPCRs is the orphan receptor GPR4.[98] GPR4 is an upstream activator of cAMP-PKA signaling, and our preliminary studies implicate GPR4 in the upregulation of proton transporters in the collecting duct of the kidney. Together with existing literature on the activation of other proton translocating ATPases, these findings indicate the presence of an underlying signaling network involved in coordinating the renal adaptive response to acidosis.

Petrovic and colleagues[99] recently reported that deletion of GPR4 in vivo was associated with a reduction in net acid excretion and a relatively alkaline urine pH, parallel with a spontaneous nongap metabolic acidosis, thus providing evidence for an acidification defect in this model. Moreover, some of the features of the renal acidification defect in GPR4-/- resemble that which is reported for mice with the deletion of one of the genes that encodes for the rate-limiting bicarbonate transporter, AE1, in the kidney collecting tubule. Therefore, GPR4 appears to function as a pH sensor in the collecting tubule.

Recent studies have revealed that soluble adenylyl cyclase (sAC) is highly expressed in the collecting tubule and may colocalize with the H^+-ATPase in the apical membrane of type A ICs.[100] It has been suggested that sAC-regulated cAMP signaling may constitute a general sensing mechanism for regulating H^+-ATPase-mediated proton transport. Although parallels exist in the male reproductive tract,[101] how sAC functions to sense bicarbonate, primarily, and how it might function to play a regulatory role in the response to an acid intracellular or extracellular pH, which is pivotal for the renal defense against metabolic acidosis, has not been satisfactorily explained to date.

INTEGRATION OF KIDNEY RESPONSE TO DEFEND AGAINST ACID–BASE DISTURBANCES

As noted previously, a high-protein diet, such as that consumed by most Westerners, requires renal reclamation of most of the filtered bicarbonate as well as net acid excretion that is equivalent to net acid production. The ammonia produced in the kidney is derived from the synthesis of glutamine, and the amount produced is regulated by pH. Acidemia promotes and alkalemia inhibits glutamine synthesis.

Long-term changes in dietary acidity can cause chronic changes in H^+ and HCO_3^- secretion. For example, in the cortical collecting tubule, chronic increases in dietary acid result in an increased capacity for H^+ secretion into the lumen, whereas chronic increases in dietary alkali lead to an increased capacity for HCO_3^- secretion.[102] As noted previously, type A ICs mediate H^+ secretion and type B ICs mediate HCO_3^- secretion. The elegant studies of Schwartz and Al-Awqati[103] over many years have demonstrated that these cells can "switch" phenotypes based on chronic metabolic changes. For example, during metabolic acidosis, the number of type B ICs decreases and the number of type A ICs increases, but the total number of intercalated cells does not change.[104–107] This "switch" could be due to an extracellular protein called hensin.[103] Recent results demonstrate that acidosis induces the polymerization of this novel extracellular matrix protein as follows: The deposition of hensin in the matrix leads to the conversion of a HCO_3^--secreting epithelium to an acid-secreting epithelium. Hensin is deposited in the extracellular matrix of H^+-secreting type A ICs following acid incubation.[108] Alternatively, this "switch" could also be due to the exocytotic insertion of H^+-ATPase into the

apical membrane of type A ICs and the endocytic retrieval of the Cl^-/HCO_3^- exchanger from the apical membrane of the type B ICs.

As noted previously, the kidneys eliminate the acid that is produced daily and have the capacity to increase urinary net acid excretion (and, hence, HCO_3^- generation) in response to endogenous or exogenous acid loads. The renal excretion of acid is usually matched to the net production of metabolic and dietary acids, so little disturbance in systemic pH occurs.

As an acid load is incurred, the kidneys respond to restore balance by increasing the ammonium excretion (TA excretion has limited capacity for regulation). With continued acid loading, the renal net acid excretion increases over the course of 3 to 5 days (Fig. 7.2) but does not quite achieve the level of acid production. Progressive positive acid balance ensues, which is presumably buffered by bone carbonate.

K^+ DEFICIENCY AND HYPERKALEMIA

Chronic K^+ deficiency leads to adaptations in H^+ ion secretion that are similar to those evoked by chronic metabolic acidosis. Thus, chronic K^+ deficiency leads to an increased capacity for transepithelial H^+ secretion by the proximal tubule through parallel increases in the activities of NHE-3 and the basolateral membrane Na^+–$3HCO_3^-$ cotransporter[109] and increases in activities of ammoniagenesis that are also stimulated by chronic metabolic acidosis (Fig. 7.1A).[110] The similarity between these two responses may be the result of chronic K^+ deficiency, which decreases intracellular pH and increases the NHE-3 transport rate.[111,112]

In the collecting tubule, chronic K^+ deficiency increases H^+ secretion through the insertion of H^+ pumps into the apical membrane of H^+-secreting cells (A-type ICs) and through a robust increase in the activity of the H^+, K^+-ATPase (Fig. 7.1B).[70,113–115] The result of the latter is to enhance H^+ ion secretion while, at the same time, to enhance K^+ absorption by the distal nephron. The stimulation of renal acidification in chronic K^+ deficiency explains the association of K^+ deficiency with chronic metabolic alkalosis and is primarily an adaptive upregulation of the H^+, K^+-ATPase, thus offering an explanation for the paradoxical increase in bicarbonate absorption in the face of chronic metabolic alkalosis with hypokalemia.[116] Furthermore, the metabolic alkalosis associated with hypokalemia cannot be corrected until the potassium deficiency is repaired.

Hyperkalemia appears to have the opposite effect on acid–base balance because hyperkalemia inhibits proximal tubule ammoniagenesis and ammonium transport. This decrease in ammonium excretion due to hyperkalemia contributes to the acidification defect characteristic of type 4 renal tubular acidosis.[117,118] Hyperkalemia not only suppresses ammoniagenesis, but also inhibits NH_4^+ reabsorption in the thick ascending limb, resulting in low NH_3 levels in the renal medulla, and, thus, a reduced capacity to buffer H^+ secreted in the collecting tubule.[118] Correcting hyperkalemia increases net acid excretion and helps correct the acidosis in disorders associated with hyperkalemia and the metabolic acidosis of renal origin.[20,114]

THE EFFECT OF MINERALOCORTICOIDS ON URINARY ACIDIFICATION

Mineralocorticoids such as aldosterone stimulate urinary acidification through a number of different mechanisms. Aldosterone binds to its intracellular mineralocorticoid receptor in collecting tubule cells and stimulates the rate of H^+ ion transport[119,120] by parallel increases in the activities of the apical membrane V-type H^+-ATPase[121] and the basolateral membrane Cl^-/HCO_3^- exchanger.[122] It also has indirect effects on H^+ secretion by increasing Na^+ reabsorption in the cortical collecting tubule (CCT) through increasing the open probability of the apical sodium channel in principal cells. This secondarily increases the transepithelial negative potential difference, which is an important driving force for H^+ secretion.[123]

Effective Arterial Volume

Effective arterial volume is an important regulator of renal acidification and is reviewed in the following paragraphs.

Extracellular Volume Expansion

The addition of sodium bicarbonate to the body results in prompt cellular buffering and respiratory compensation. However, as with an acid load, the kidneys have the ultimate responsibility for the disposal of base to restore a normal serum bicarbonate concentration. The renal response is more rapid with the addition of HCO_3^- than with acid ingestion. The speed and efficiency by which HCO_3^- can be excreted renders it challenging to cause more than mild metabolic alkalosis in a patient with normal renal function even with as much as 24 mEq per kilogram per day of sodium bicarbonate over several weeks.[124,125] Type B ICs in the collecting tubule are capable of HCO_3^-/Cl^- exchange (bicarbonate secretion). However, the role of HCO_3^- secretion in chronic alkali loading has never been quantitated precisely in whole organ clearance studies. Presumably, HCO_3^- secretion by the type B IC moderates the development of a more severe alkalosis, enhancing the HCO_3^- excretory response (Fig. 7.5).

The proximal tubule is principally responsible for HCO_3^- excretion when the blood HCO_3^- concentration increases. Absolute proximal HCO_3^- reabsorption does not increase in proportion to the load of HCO_3^- because alkalemia suppresses proximal acidification,[126] causing an increase in the HCO_3^- delivery to the distal nephron. The limited capacity of the distal nephron to secrete H^+ can be overwhelmed easily, and bicarbonaturia increases progressively. NH_4^+ and TA excretion are moderated in response to the increasing urine pH.[124,126]

Acute graded HCO_3^- loads that concomitantly increase ECF also function in human subjects to progressively increase urinary HCO_3^- excretion as the plasma HCO_3^- concentration increases.[126] In summary, an acute base load is excreted entirely when the kidney function is normal, and the blood HCO_3^- concentration is returned to normal within 12 to 24 hours because of the depression of fractional proximal HCO_3^- reabsorption. In addition to the suppression of the filtered HCO_3^- load reabsorption, direct HCO_3^- secretion by the CCT is another means for HCO_3^- disposal during metabolic alkalosis.[124]

The increased delivery of HCO_3^- out of the proximal tubule in response to an increased blood HCO_3^- concentration (filtered HCO_3^- load) in the setting of ECF expansion facilitates HCO_3^- excretion and the return of blood pH to normal. However, other factors may independently enhance distal H^+ secretion sufficiently to prevent HCO_3^- excretion and thus counterbalance the suppressed fractional proximal HCO_3^- reabsorptive capacity. Under these circumstances, the alkalosis is maintained. For example, in the setting of primary hyperaldosteronism, despite the expanded ECF, a stable mild alkalotic condition persists in most experimental models owing to augmented collecting duct H^+ secretion.[124] In such cases, concurrent hypokalemia facilitates the generation and maintenance of metabolic alkalosis by enhancing NH_4^+ production and excretion.[124,126] Moreover, chronic hypokalemia dramatically enhances the abundance and functionality of the H^+, K^+-ATPase in the medullary collecting tubule, thus increasing rather than decreasing bicarbonate absorption.[124,127–129] Enhanced nonreabsorbable anion delivery, as with drug anions, also increases the net collecting tubule H^+ secretion by increasing the effective luminal negative potential difference or by suppressing HCO_3^- secretion in the cortical collecting duct (CCD).

Extracellular Volume Contraction

The renal response to an increase in plasma HCO_3^- concentration can be modified significantly in the presence of ECF contraction.[128,130] Because the distribution volume of Cl^- is approximately equal to ECF, the depletion of the ECF is roughly equivalent to the depletion of Cl^-. The critical role of effective ECF and K^+ stores in modifying net HCO_3^- reabsorption has been demonstrated in numerous experimental models.

In most cases, metabolic alkalosis is maintained by decreases in effective arterial volume. This is due, in part, to volume-induced decreases in GFR (leading to a decrease in filtered HCO_3^- load).[131] Moreover, a decrease in effective arterial volume increases renal acidification by (1) decreasing paracellular HCO_3^- permeability in the proximal tubule, which inhibits HCO_3^- back leak[132]; (2) decreasing the distal delivery of Cl^-, thus inhibiting HCO_3^- secretion in the cortical collecting tubule (by limiting the apical membrane Cl^-–HCO_3^- exchange); and (3) secondarily increasing aldosterone and angiotensin II, as well as renal nerve activity. When taken together, these factors enhance HCO_3^- reabsorption and regeneration.

Deficiency of both Cl^- and K^+ is common in metabolic alkalosis because of renal and/or gastrointestinal losses that occur concurrently with the generation of the alkalosis.[129,130] With Cl^- depletion, the normal bicarbonaturic response to an increase in plasma HCO_3^- is prevented, and metabolic alkalosis can develop. Potassium depletion, even without mineralocorticoid administration, can cause metabolic alkalosis in rats and humans. When Cl^- and K^+ depletion coexist, severe metabolic alkalosis may develop in all species studied.

Two general mechanisms exist by which the bicarbonaturic response to hyperbicarbonatemia can be prevented by Cl^- and/or K^+ depletion: (1) As the plasma HCO_3^- concentration increases, there is a reciprocal fall in GFR and, if inversely proportional to the rise in the plasma HCO_3^- concentration, the filtered HCO_3^- load would not exceed the normal level. Accordingly, normal rates of proximal and distal HCO_3^- reabsorption would suffice to prevent bicarbonaturia. (2) Cl^- deficiency or K^+ deficiency increases the overall renal HCO_3^- reabsorption in the setting of a normal GFR and a high filtered HCO_3^- load. In this case, overall renal HCO_3^- reabsorption, and therefore acidification, would be increased. An increase in renal acidification might occur as a result of an increase in H^+ secretion by the proximal or the distal nephron or by both nephron segments.[124,127,129]

The augmented HCO_3^- absorption in distal nephron segments appears to be due to a primary increase in H^+ secretion that is independent of the delivered HCO_3^- load. Chronic hypokalemia dramatically enhances the abundance and function of the colonic isoform of the H^+, K^+-ATPase in the medullary collecting tubule, which may serve as a significant factor in the maintenance of chronic metabolic alkalosis.[70,115,127]

In summary, the physiologic response by the kidney to a base load associated with volume expansion is to excrete the base. Base is retained, however, if there is enhanced distal HCO_3^- reabsorption as a result of K^+ and/or Cl^- deficiency.

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CHAPTER

8 Hormones and the Kidney

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HORMONAL MODULATION OF NEPHRON FUNCTION: AN OVERVIEW

Modulation of Glomerular Filtration Rate

The major physiologic determinants of single nephron glomerular filtration rate (GFR) are glomerular plasma flow (Q_A), glomerular transcapillary hydraulic pressure (P_{GC}), and the ultrafiltration coefficient (K_f).[1] These variables are determined, in part, by the contractile state of the afferent arteriole, efferent arteriole, and mesangial cells. K_f also varies with alterations in the hydraulic permeability of the capillary filtration barrier, which consists of endothelial cells, visceral epithelial cells, and the glomerular basement membrane. By binding to specific receptors on cellular and structural components of the glomerulus, circulating and locally produced hormones influence one or more of the physiologic determinants of GFR. Figure 8.1 summarizes the effects of different hormones on pregomerular, glomerular, and postglomerular contractility. Because the same substance can act at different sites in the glomerular unit, the net effect on GFR will depend on whether its actions are antagonistic or complementary. For example, atrial natriuretic peptide (ANP) decreases afferent arteriolar resistance, whereas increasing efferent arteriolar resistance results in an augmented P_{GC} and a rise in GFR.[2] Under certain conditions, ANP also increases K_f, which, in turn, contributes to the enhancement of GFR.[3] On the other hand, angiotensin (Ang) II-mediated constriction of both afferent and efferent arterioles results in opposite effects on glomerular plasma flow and P_{GC} and, therefore, no change in single nephron GFR.[4] Regulation of glomerular hemodynamics is further complicated by multiple interactions between hormones in the kidney. For example, infusion of Ang II along with a cyclooxygenase inhibitor causes a significant decrease in single nephron GFR, suggesting that endogenous prostaglandin production antagonizes the glomerular effects of Ang II.[5] The net effects of renally relevant hormones on GFR are discussed in more detail later in this chapter.

Modulation of Tubule Water and Electrolyte Transport

Renal tubule cells express receptors for many circulating and locally synthesized hormones. The effects of a particular hormone on water or electrolyte transport are partly determined by the differential distribution of its receptor on functionally specialized segments of the renal tubule. For example, arginine vasopressin (AVP) binds almost exclusively to principal cells in the collecting tubule (CT), where it influences the physiologic functions of this segment, primarily water and urea absorption.[6] Parathyroid hormone (PTH), on the other hand, exerts its biologic actions on renal tubule segments proximal to the collecting duct, where it modulates calcium, phosphate, magnesium, sodium, and bicarbonate transport.[7] Table 8.1 summarizes the net effects of renally relevant hormones on water and solute handling in different tubule segments. Each hormone is discussed in detail in the sections that follow. As in the glomerulus, hormones may modulate their own actions by altering the production of counterregulatory hormones. For example, AVP induces local synthesis of prostaglandin E (PGE), which opposes the effect of AVP on water permeability in the CT.[8]

ARGININE VASOPRESSIN

In 1895, Oliver and Schafer were the first to describe a substance with potent vasopressor effects originating from the posterior pituitary, hence the name, vasopressin.[9] It was not until later in the 20th century that the effects of this hormone on modulation of water excretion by the kidney were discovered. In fact, vasopressin, also called AVP or antidiuretic hormone (ADH), is a key hormone for osmoregulation and maintenance of body homeostasis.[10] AVP was also found to exert effects on body temperature, glucose metabolism, memory, social behavior, and the hypothalamic-pituitary-adrenal axis.

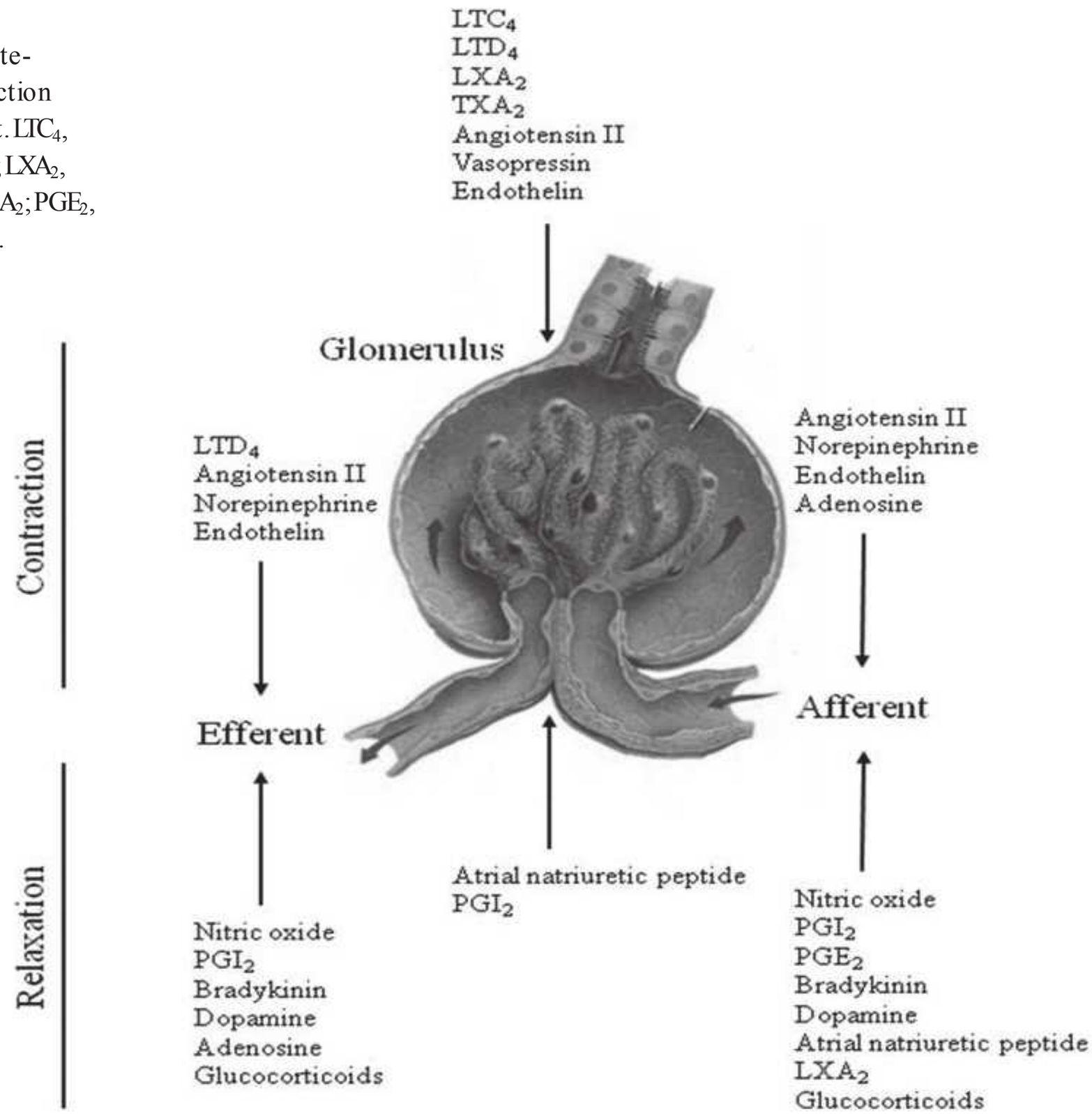

FIGURE 8.1 Hormones regulating glomerular, afferent, and efferent arteriolar contractility. Glucocorticoid action may be pharmacologic and indirect. LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LXA_2, leukotriene A_2; TXA_2, thromboxane A_2; PGE_2, prostaglandin E_2; PGI2, prostacyclin.

Structure and Synthesis of Arginine Vasopressin

AVP is a 9-amino acid neuropeptide synthesized by magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus.[11] The AVP gene, located on chromosome 20, consists of three exons that encode the signal peptide, AVP, neurophysin II (NPII), and a glycopeptide.[10] After cleavage of the signal peptide within the ER, the resulting prohormone is folded and packaged into secretory granules in neuronal bodies. These granules are then transported along the axons to nerve terminals in the posterior pituitary (neurohypophysis).[12] During axonal transport, further processing of the prohormone within secretory granules yields AVP, neurophysin II (NpII), and a glycopeptide. Interestingly, mutations of NpII impair AVP secretion, suggesting that NpII assists in the processing or secretion of AVP.[13] In addition to the posterior pituitary, AVP synthesis has been detected in the pancreas, adrenal gland, ovary, testis, and regions of the brain.[14] Its physiologic function in these sites, however, remains to be clarified.

Physiology of Arginine Vasopressin in the Kidneys

The most sensitive stimulus for AVP secretion into the bloodstream is increased plasma osmolality. Osmosensitive neurons that respond to changes in plasma osmotic pressure by varying their intracellular water content have been identified in the anterior hypothalamus, specifically in the organum vasculosum of the lamina terminalis (OVLT) and the subfornical organ (SFO).[15] When stimulated by osmosensitive neurons, the magnocellular neurons release the stored AVP into the posterior pituitary (an area that lacks a blood–brain barrier) and AVP enters the general circulation. As little as 1% change in plasma osmolality leads to a change in AVP concentration that is sufficient to modify renal water excretion.[16] AVP secretion is almost completely suppressed when plasma osmolality decreases below an average of 280 mOsm per kg of water in humans.[17]

Secretion of AVP is also influenced by alterations in intravascular volume and blood pressure, sensed by baroreceptors located in the heart, aortic arch, and carotid sinus.[18] These signals are transferred through the vagal nerves to the

8.1 Hormonal Modulation of Tubular Transport

	Hormones with Major Effects		
	Reabsorption	**Stimulatory**	**Inhibitory**
Proximal tubule	Na	Ang II, catecholamines, insulin	Dopamine, PTH
	P_i	IGF-1	PTH
	HCO_3	Ang II	PTH
	Ca		PTH
TALH	Na	AVP,[a] catecholamines	PGE
	Ca	PTH, calcitonin, glucagon	
	Mg		AVP, PTH, calcitonin, glucagon
DCT	P_i		PTH
	Ca	PTH	
CCD	H_2O	AVP	PGE, bradykinin, ANP, α-adrenergic agents
	Na	Aldosterone	ANP, PGE, EGF
IMCD	Urea	AVP	
SECRETION CCD	K	AVP, aldosterone	β_1 agonists
	H	Aldosterone	

[a]Unlikely in humans.
TALH, thick ascending limb of the loop of Henle; DCT, distal convoluted tubule; CCD, cortical collecting duct; IMCD, inner medullary collecting duct; Ang II, angiotensin II; IGF, insulinlike growth factor; AVP, arginine vasopressin; PTH, parathyroid hormone; PGE, prostaglandin E; EGF, epidermal growth factor.

nucleus solitarius in the brainstem, from which postsynaptic pathways project to the magnocellular neurons. Whereas a 5% to 8% decrease in blood volume or systemic arterial pressure has little effect, further hemodynamic compromise leads to a steep increase in circulating AVP levels. Significant reductions (10%–30%) in circulatory arterial volume or blood pressure can override osmoregulation and result in markedly increased AVP levels in the face of decreased plasma osmolality.[19] Other less potent stimuli for AVP secretion include fever, emesis,[20] and oropharyngeal osmoreceptors.[21]

AVP circulates in the plasma nearly in an unbound form. Levels of circulating AVP depend on both the rate of AVP release from the posterior pituitary and the rate of AVP degradation. As discussed, the major factor controlling AVP release is plasma osmolality. The liver and the kidney both contribute to the breakdown of AVP and the decline in AVP levels when secretion ceases. In fact, the half-life of AVP in the circulation is 18 minutes due to rapid clearance by hepatic and renal vasopressinases.[22] Under physiologic conditions, plasma vasopressin concentrations vary with serum osmolarity between 0 to 5 pg per mL.[22]

It is noteworthy that AVP levels are difficult to measure in plasma because of the instability of this peptide and the low sensitivity of available AVP antibodies. Copeptin (or C-terminal proarginine vasopressin, CT-proAVP) is the C-terminal part of AVP, which is secreted stoichiometrically with AVP in a manner similar to C-peptide and endogenous insulin.[23] CT-proAVP provides a reliable means for estimating prevailing AVP levels in the circulation, thus facilitating the study of AVP in human diseases. A recent cohort study in renal transplant patients suggests that high CT-proAVP strongly correlates with a negative renal prognosis. In fact, in this study, the plasma concentrations of CT-proAVP predicted renal function loss over a 3.2-year follow-up.[24]

AVP exerts its biologic actions through three specific cell-surface AVP receptors identified as V_1R (also called $V_{1a}R$), V_2R, and V_3R (also $V_{1b}R$).[25] These receptors belong to the G protein coupled receptor superfamily (Table 8.2). In the human kidney, mRNA for V_1Rs predominates in cortical collecting ducts (CCD), gradually decreasing as the collecting duct enters the medulla.[26] In addition, whereas V_1R mRNA is diffusely expressed in the CCD, it is restricted to the intercalated cells in OMCD. V_1Rs are responsible for mediating vascular smooth muscle cell vasoconstriction by activating G protein-dependent phospholipase C (PLC) and the downstream effectors, diacylglycerol (DAG), and inositol 1,4,5-triphosphate (IP_3). In turn, DAG stimulates protein kinase C (PKC), whereas IP_3 increases cytosolic Ca^{2+}, thus

8.2 Vasopressin Receptor Types, Genetics, Location, and Main Physiologic Effects

Receptor	Chromosome location	No. of amino acids	Site of action	Main second messenger	Main effects
V_1 (V_1a)	12 (q14–q15)	418	Vascular smooth muscle, platelets, liver, testes, brainstem, adrenal glands	PLC→IP3 + DAG/calcium and PKC	Vasoconstriction, platelet aggregation, glycogenolysis, stimulation of aldosterone and cortisol synthesis
V_2	X (q28)	371	Collecting duct cells of kidney, inner medulla, heart, pancreas	Adenylate cyclase/cAMP	Water retention, stimulation of atrial natriuretic peptide, stimulation of insulin synthesis, coronary and pulmonary artery vasodilation
V_3 (V_1b)	1 (q32)	553	Hypothalamus, anterior pituitary gland	PLC→IP3 + DAG/calcium and PKC	Modulation of ACTH synthesis; stimulation of ACTH, GH, and procaltin release
Oxytocin	3 (p25)	389	Uterus, breast, vascular endothelium	PLC→IP3 + DAG/calcium and PKC	Myometrial contraction, ductal myoepithelial contraction, vasodilation
P_2 Purinergic	11 (q13.5–14.1)		Cardiac endothelium	ATP	Vasoconstriction, reduced cardiac output

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initiating the second-messenger cascade responsible for the cellular actions of AVP.[25] Stimulation of V_1R, although not directly involved in control of tubular water and electrolyte transport, increases sodium excretion because of the influences on blood pressure, effective arterial circulating volume, glomerular filtration rate, and circulation in the vasa recta system.[27,28] Additional biologic effects of AVP mediated through V_1Rs include platelet aggregation[29] and increased glycogenolysis and gluconeogenesis in the liver.[30]

The V_3R ($V_{1b}R$), also coupled to PLC signaling, is present on neurons in the anterior pituitary (adenohypophysis) and is thought to mediate AVP-induced corticotropin secretion.[25,31,32] They are also found elsewhere in the brain, especially in the pyramidal neurons of the hippocampal CA2 field, in which they mediate fundamental physiologic actions such as memory and body temperature control as well as social behavior.[33] In addition, this receptor has been localized to pancreatic islet cells, modulating insulin secretion.[34] However, its presence and role at the level of the medulla in rat kidney remain unclear.

V_2Rs, on the other hand, are heavily expressed in the medullary TAL, macula densa (MD), connecting tubule, and cortical and medullary collecting duct, as well as weakly expressed in cortical thick ascending limb (TAL) and distal convoluted tubule.[35] These are the best characterized and studied vasopressin receptors. By binding to V_2R, AVP increases water reabsorption through multiple mechanisms.[31] Activation of V_2R results in increased cyclic adenosine monophosphate (cAMP) levels and activation of PKA, which promotes insertion of water channels into the luminal suface of the epithelial tubular cells.[36] This ultimately mediates the antidiuretic effect of AVP by allowing back diffusion of water down its concentration gradient.[37] In addition, V_2Rs modulate sodium reabsorption through the epithelial Na^+ channel (ENaC) across principal cells.[38–41] This facilitates free water reabsorption by supporting the axial corticomedullary hyperosmotic gradient. It was also recently shown in rats that AVP modulates sodium reabsorption even in the distal convoluted tubule by acting on the thiazide-sensitive Na^+-Cl^- cotransporter (NCC).[42] NCC is important in defining sodium delivery to the collecting duct,

which is necessary for ENaC activity. Finally, V_2Rs activate urea transporters, such as UTA1, in the distal nephron.[43–45] This increase in urea reabsorption and recycling maximizes sodium reabsorption in the TAL by supporting the axial hyperosmotic gradient drawing water from the distal nephron.[46] The clinical importance of the V_2R in water balance disorders is underlined by the current use of V_2R antagonists in a clinical setting (see later).

It is noteworthy that AVP also binds to two nonspecific receptors: oxytocin receptors and P2 purinoreceptors. Oxytocin receptors are found in the breast, ovary, uterus, and hypothalamus. Since the affinity of this receptor for vasopressin is relatively low, the clinical effects of this hormone are limited under physiologic conditions.[47] AVP may also act on P2 purinoreceptors in the heart, causing coronary vasoconstriction and contributing to the reduction in cardiac output.[48]

Water Channels

The discovery of the family of aquaporin water channels was crucial to the understanding of the mechanism by which AVP can increase water permeability in the kidney. The group of Peter Agre discovered the first aquaporin in human erythrocytes.[49] To date, seven different aquaporin (AQP) have been shown to be expressed in the human kidney and to be involved in renal water reabsorption.[50]

AQP2 is the vasopressin-sensitive water channel expressed in the principal cells of the collecting duct, where it shuttles between intracellular storage vesicles and the apical membrane.[51] Knocking out the AQP2 gene produces a severe concentration defect in these mice, resulting in postnatal death.[52] It has now been shown to be involved in many clinical disorders (see later). AQP1 is constitutively expressed on the basolateral and apical membrane of epithelial cells lining the proximal tubule and thin descending limb, as well as endothelial cells of the descending vasa recta. It not only plays a role in water reabsorption from urine in these segments but is also critical for a functional countercurrent multiplication system.[53] AQP3 and AQP4 are expressed on the basolateral membrane of the principal cells of the collecting duct, and they represent an exit pathway from these cells for water entering through AQP2.[54,55] Similar to AQP1, AQP7 is expressed at the apical membrane of proximal tubules (S3 segment) and has been shown to mediate glycerol reabsorption in addition to water.[56] AQP6 is found in intracellular vesicles of acid-secreting α-intercalated cells in the collecting duct.[57] It is thought to be involved in urinary acid secretion. AQP11 is localized to the endoplasmic reticulum (ER) in the proximal tubule. Interestingly, knocking out the AQP11 gene in mice is fatal because of the onset of polycystic kidney disease.[58]

Clinical Pathophysiologic Role of Arginine Vasopressin in the Kidneys

AVP is implicated in major clinical syndromes of alterations in water metabolism, namely nephrogenic diabetes insipidus (NDI) and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). In addition to water metabolism, it has been recently suggested that AVP may play a role in the initiation and the progression of chronic kidney disease (CKD) and in the most prevalent form of hereditary renal disease, namely the adult polycystic kidney disease (Fig. 8.2).[59]

NDI is characterized by impaired AVP-induced water reabsorption, resulting in polyuria and polydipsia.[60] If water intake is inappropriate, patients with NDI may fail to thrive, suffer from mental retardation, and die early. NDI can be acquired such as following lithium treatment, hypokalemia, hypercalcemia, or ureteral obstruction, all of which lead to downregulation of AQP2.[61–64] NDI can also be inherited (congenital).[65] In fact, two gene mutations have been linked to congenital NDI: one in the AVPR2 gene encoding V_2R (X-linked NDI) or mutations in the AQP2 gene (autosomal recessive or autosomal dominant NDI). More than 90% of patients with congenital NDI suffer from X-linked NDI. In both cases, patients cannot concentrate their urine despite normal or elevated plasma concentrations of vasopressin, leading to a massive loss of water through the kidney. So far, NDI is managed by salt restriction combined with hydrochlorothiazide diuretics to reduce urine output.[66] However, no cure is available so far.

Abnormal water handling of central origin includes SIADH in which AVP levels are abnormally elevated and not suppressed when plasma osmolality concentration falls below the osmotic threshold for physiologic AVP secretion.[67] SIADH is characterized by impaired water excretion in the absence of renal insufficiency, adrenal insufficiency, or any recognized stimulus for AVP secretion. Renal water retention and extracellular fluid expansion are compensated for by increased urinary Na^+ excretion leading to life-threatening hyponatremia. The most common causes of the syndrome of inappropriate AVP secretion are neoplasia, neurologic disorders, congestive heart failure, liver cirrhosis, preeclampsia, and drugs such as thiazide diuretics or selective serotonin reuptake inhibitor antidepressants.[67] Four patterns of AVP dysregulation in patients with SIADH have been observed.[68] The most common pattern (in ~40% of patients with SIADH) is the excessive and unregulated release of AVP, which is unrelated to plasma osmolality. In the second most common pattern (~30% of patients), referred to as "reset osmostat," AVP release continues to regulate water excretion at a lower plasma osmolality set-point. Although most tumors (e.g., lung carcinoma) manifest the first type of SIADH, some also present with the second type, thus the pattern of abnormal AVP secretion cannot be utilized to predict the cause of SIADH. A third rare pattern is characterized by an inability to stop AVP secretion at low plasma osmolalities, but the osmoregulation of AVP is otherwise normal. This pattern may be due to dysfunction of inhibitory hypothalamic neurons, leading to persistent low-grade basal AVP secretion. In the fourth pattern, a rare clinical picture of SIADH, the normal osmoregulation of AVP secretion is not altered (~10% of patients) but AVP levels are low or undetectable. It is thought that a nephrogenic SIADH

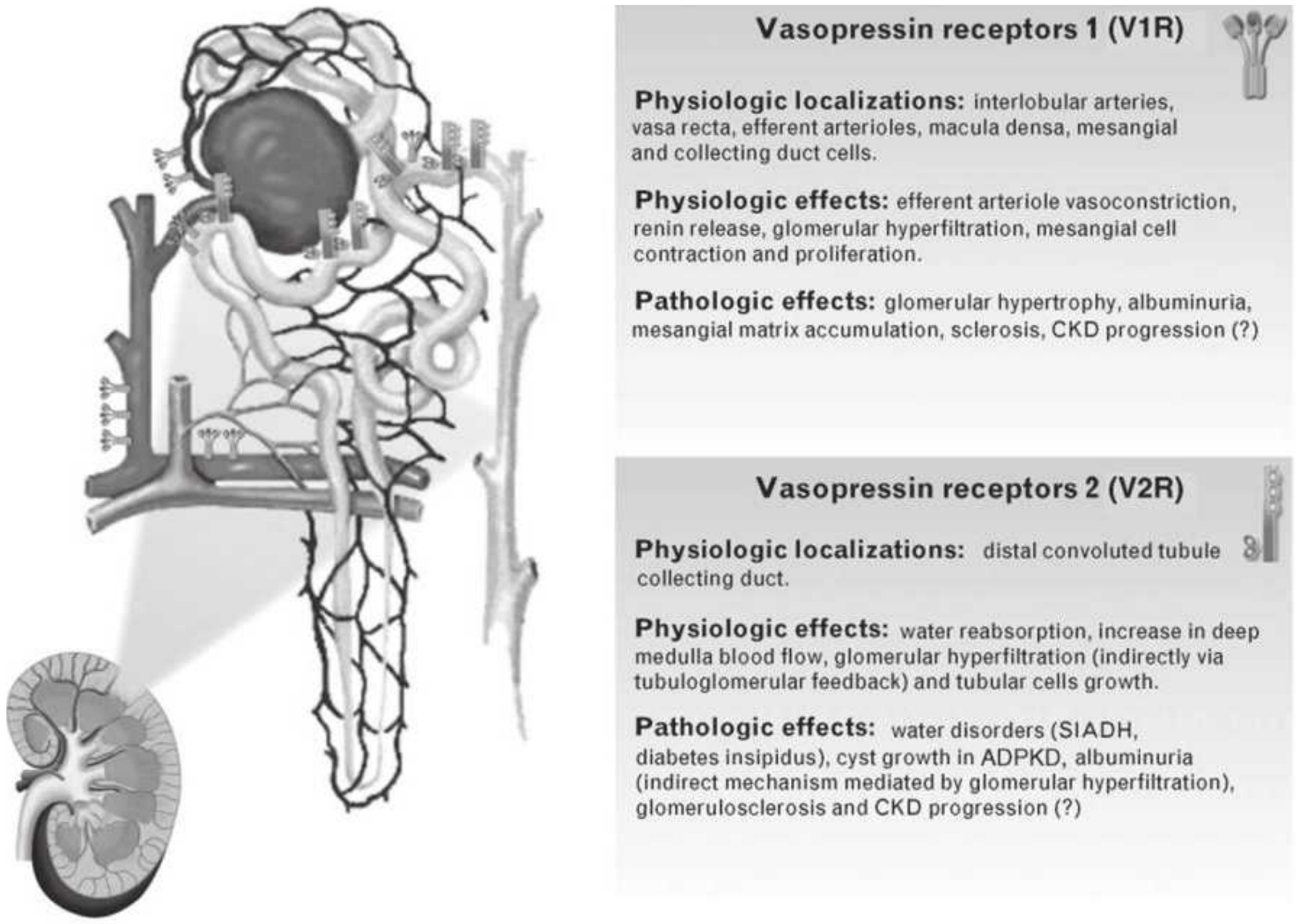

FIGURE 8.2 Vasopressin receptors: physiology and pathologic involvement in renal diseases. (Reproduced from Bolignano D, Zoccali C. Vasopressin beyond water: Implications for renal diseases. *Curr Opin Nephrol Hypertens*. 2010;19(5):499–504.)

(NSIADH) may be responsible for this picture. In fact, in some children, it appears to be due to an activating mutation of V_2R. In other patients, it may be due to abnormal control of aquaporin-2 water channels in renal collecting tubules or production of an antidiuretic principle other than AVP.[69] In a study with SIADH patients, treatment with V_2R antagonists, commonly referred to as vaptans, increased serum Na^+ concentration and decreased its excretion. Tolvaptan has been recently approved in the United States and Europe for the treatment of hyponatremia associated with SIADH, as well as cirrhosis and congestive heart failure. Recently, a dual vasopressin V_1R and V_2R antagonist, conivaptan, improves hyponatremia in rats with SIADH, suggesting a therapeutic potential for conivaptan in the treatment of SIADH.[70]

AVP has also been shown to modify vascular tone in renal microvessels. Short-term infusion of AVP does not alter either renal blood flow or the GFR.[71] Alternatively, chronic AVP administration increases the intraglomerular capillary pressure and GFR through tubuloglomerular feedback.[72] In addition, a sustained increase in water intake and the consequent AVP suppression reduce proteinuria and the severity of glomerular and tubular damage in 5/6 nephrectomized rats.[73,74] Thus, it seems likely that a chronic AVP-induced hyperfiltration may alter the glomerular barrier and start a series of events leading to enhanced protein loss and glomerulosclerosis. An increase in urinary albumin excretion represents an early predictor of glomerular damage in diabetes mellitus and a risk factor for cardiovascular complications in hypertension. Studies show that the Brattleboro rat, a model of central diabetes insipidus with complete lack of AVP, is protected from hyperfiltration, albuminuria, and renal hypertrophy after streptozotocin-induced diabetes mellitus.[75] This suggests that AVP plays a role in hyperfiltration and glomerular damage induced by diabetes. These observations are also of relevance to humans. A marked increase in AVP plasma levels is well documented in diabetes mellitus.[76] AVP through V_1R induces contraction of cortical efferent, but not afferent, arterioles. Administration of a V_1R selective antagonist to noninsulin-dependent diabetic patients modestly reduces albuminuria partly by decreasing intraglomerular capillary pressure.[77] Thus, although V_1Rs (but not V_2Rs) are downregulated in diabetes mellitus, they could mediate part of the increase in albumin excretion. Interestingly, administering desmopressin, a selective V_2R agonist, to healthy humans and patients with central diabetes insipidus significantly increases urinary albumin excretion, but this effect is absent in those with hereditary nephrogenic diabetes insipidus secondary to V_2R mutations.[78] These findings suggest that the AVP-induced rise in albuminuria depends on V_2Rs. This is further confirmed by the observation that plasma copeptin levels correlate with microalbuminuria

in the PREVEND study.[79] However, V_2 receptors have not been found in glomeruli or proximal tubules, suggesting indirect effects. Recent evidence suggests a strong interaction between AVP and the renin-angiotensin system (RAS).[80,81] In fact, chronic RAS blockade by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) prevents the desmopressin-induced albuminuria, indicating that RAS mediates the effects of AVP on glomerular hemodynamics.[82] Recent studies using V_1R knockout mice show that AVP regulates body fluid homeostasis and the GFR by activating RAS through V_1Rs in MD cells, and subsequently the V_2R-aquaporin 2 system.[83] Simultaneous AVP and RAS blockade may represent a good therapeutic approach for delaying renal disease progression.

Another interesting observation is that mesangial cells express V_1Rs, which mediate AVP-induced cell contraction.[84] In addition, prolonged exposure to AVP promotes mitogenesis and proliferation of these cells, which ultimately leads to an increased accumulation of extracellular matrix, a pathologic feature found in various glomerular diseases.[85,86] In fact, addition of AVP to cultured mesangial cells increases in a dose-dependent manner the synthesis and release of matrix proteins, such as type I and IV collagen, fibronectin, and transforming growth factor β.[87] In addition, AVP inhibited the synthesis of matrix metalloproteinase (MMP)-2, which degrades matrix proteins including type IV collagen, and stimulated endothelin (ET)-1 secretion from mesangial cells, another mitogenic factor.[88]

In the remnant kidney model in rat, a model of progressive CKD in humans, V_1R antagonists (but not V_2R) prevent proteinuria and glomerulosclerosis in the initial phases of disease, but have limited effectiveness in established renal damage.[89,90] Similar observations were reported in 5/6 nephrectomized, salt-loaded spontaneously hypertensive rats (SHRs), in which the increase in urinary protein excretion and the progression of nephrosclerosis were attenuated with a V_1R antagonist but not with a V_2R antagonist.[91]

AVP has also been linked to autosomal dominant polycystic disease (ADPKD), an inherited disorder characterized by the development within renal tubules of innumerable cysts that progressively expand to cause renal insufficiency.[92] It has been shown that 3′-5′-cyclic adenosine monophosphate (cAMP) stimulates tubule cell proliferation and transepithelial fluid secretion, both of which contribute to enlarge renal cysts.[93] AVP operates continuously in ADPKD patients to promote cAMP production in the distal nephron and collecting ducts via V_2Rs, thereby contributing to cyst enlargement and renal dysfunction.[94] Studies in animal models of ADPKD provide compelling evidence that blocking AVP's actions dramatically improves disease progression.[95,96] This prompted the initiation of an international clinical trial[97] testing the efficacy of tolvaptan, a V_2R inhibitor, in the treatment of ADPKD.[98] Alternatively, a recent review discusses that the impact of simply increasing the amount of solute-free water drunk evenly throughout the day by patients with ADPKD on decreasing plasma AVP concentrations and mitigating the actions of cAMP on the renal cysts.[99]

In conclusion, in recent years, AVP has been implicated in the initiation and progression of many kidney diseases, playing a role beyond water metabolism. Further studies are required to determine whether AVP antagonists and/or AVP suppression by a high water intake can be useful for the treatment of nephropathies, from ADPKD to diabetic and nondiabetic CKD.

THE RENIN-ANGIOTENSIN SYSTEM

Historical Review

The discovery of renin goes back to 1898, when the Finnish physiologist Robert Tigerstedt and his student Per Gunnar Bergman found that extracts of rabbit renal cortex had a slowly developing and sustained pressor effect. Based on its origin, they named this substance renin.[100] This effect was not observed with extracts of renal medulla and persisted despite removal of sympathetic activation. Subsequently, efforts to verify these experiments were unsuccessful until the 1930s when Harry Goldblatt and his colleagues demonstrated that clamping the renal artery in dogs produced chronic hypertension.[101] This work converged with other subsequent experiments performed by leading scientists such as Juan Fasciolo and Bernardo Houssay to suggest the presence of a vasoactive substance produced by the kidney, other than renin.[102,103] This substance was later isolated from the blood and was named hypertensin. Based on their physiologic properties, renin and hypertensin were clearly two different compounds, but the relationship between them was not established. Renin was later identified by an Argentine group as a proteolytic enzyme that acts on a plasma constituent to produce hypertensin as the final product of the enzymatic reaction.[104] Subsequent research led to the characterization of the components of the renin–angiotensin system and Braun-Menéndez and Page gave the final nomenclature of the whole enzymatic system in 1958: the renin substrate was named angiotensinogen, hypertensin renamed angiotensin, and the enzymes that metabolize angiotensin were named angiotensinases.[105,106]

Components of the Renin-Angiotensin-Aldosterone System

Angiotensinogen

Angiotensinogen is a large molecular weight globulin primarily formed by hepatic cells. It is constitutively secreted into the circulation by the liver, therefore plasma levels are generally stable.[107] Other sources of angiotensinogen have been identified and mRNA expression detected in tissues such as the kidney, heart, brain, vessels, placenta, and adrenal glands.[108] It is currently accepted that intrarenal angiotensinogen is formed and secreted locally for several reasons: first, the molecular size of the molecule makes it unlikely for it to filter through the glomerular capillaries; second, intrarenal angiotensinogen mRNA and protein have been identified in proximal tubule cells[109]; third,

concentrations of angiotensinogen in the proximal tubule of anesthetized rats greatly exceeded the free angiotensin I and II concentrations[110]; and fourth, human angiotensinogen was not detected in urine of normotensive rats infused with the molecule.[111]

Renin and Prorenin

Renin is produced and stored in granular juxtaglomerular cells, which are modified smooth muscle cells found in the media of afferent arterioles.[112–114] Genomic analysis of the renin gene identified a single locus in humans and rats designated Ren-1, whereas mice have two renin genes, designated Ren-1 and Ren-2.[112] This duplicated renin gene in mice leads to production of substantial amounts of renin from submandibular and submaxillary glands.[115] Renin is synthesized in an inactive precursor form, preprorenin. Cleavage of the signal peptide from the carboxyl terminal of preprorenin results in prorenin, which is also biologically inactive. Subsequent glycosylation and proteolytic cleavage leads to formation of renin, a 37 to 40 kDa proteolytic enzyme. Both circulating active renin and prorenin are released mostly from the kidneys; however, other tissues also secrete these substances.[116,117] Because prorenin is the major circulating form, it is postulated that significant conversion of prorenin to renin follows secretion. Prorenin-activating enzymes have been localized to neutrophils, endothelial cells, and the kidney.[112] In addition to juxtaglomerular cells, renin production has also been detected in the submandibular gland, liver, brain, prostate, testis, ovary, spleen, pituitary, thymus, and lung.[112] Circulating renin, however, appears to be derived entirely from the kidney.

Angiotensin I

Angiotensin I is an inactive decapeptide formed upon cleavage of angiotensinogen by active renin in the circulation. Its rate of formation is highly determined by renin activity. Angiotensin I is easily hydrolyzed to angiotensin II given the widespread availability of ACE on endothelial cells of many vascular beds, including the lungs; the octapeptide angiotensin II is therefore formed by cleavage of the C-terminal dipeptide of angiotensin I.[107]

Angiotensin-converting Enzyme

The ACE, also known as kininase II, is a membrane-bound peptidase that catalyzes the conversion of angiotensin I (Ang I) to angiotensin II (Ang II), the primary active product of the renin-angiotensin-aldosterone system (RAAS). This enzyme is localized on the membrane of various cell types, mostly the endothelial cells. Other cell types include the epithelial cells of the kidney (e.g., the brush border of the proximal tubule cells) and the neuroepithelial cells. The ACE exists also in the plasma as a soluble circulating enzyme, but it is thought that the membrane-bound form is the physiologically active one.[107] Other metabolic activities of ACE include the inactivation of the vasodilator peptides bradykinin and kallidin. Therefore, the functional activity of ACE results in enhanced vasoconstriction and reduced vasodilation. Differences between humans and various animal species regarding ACE localization in the kidney have been reported. Normal nonhypertensive human subjects show a widespread expression of ACE on the brush border of tubule epithelial cells and less expression on glomerular vascular endothelial cells, whereas the renal microvasculature of rats show more preponderant ACE expression compared to epithelial cells. These findings imply that the contribution of circulating angiotensin I to the local formation of angiotensin II in the kidney may be minimal.[118]

In the plasma, all conversion of Ang I to Ang II occurs by the activity of ACE with no species variation reported. However, non–ACE-dependent pathways exist at the tissue level and have species variation. In humans, tissue activity of chymase can allow for the local formation of Ang II in the heart, arteries, and kidney. In rats and rabbits, tissue activity of chymase is associated with the local degradation (instead of formation) of Ang II. Therefore, one must carefully evaluate experimental animal data when pharmacologic blockade of the renin-angiotensin system is used.[119]

Angiotensin Peptides

Ang II is the primary active product of the RAAS. It is an octapeptide derived from Ang I after cleavage of the C-terminal dipeptide by the ACE. Most of the physiologic actions of the RAAS on the vasculature and transport functions are mediated by Ang II action on angiotensin receptors, primarily type 1 (AT1) receptors. However, other angiotensin peptides with reported biologic activity have been identified, such as angiotensin III (Ang III) and IV (Ang IV), which are formed from Ang II by the sequential removal of amino acids from the N-terminus by aminopeptidases (Fig. 8.3). They are predominantly seen in the kidney and brain, where aminopeptidases A and N are prevalent.[107,117] Ang III, also known as angiotensin 2-8, is a heptapeptide with suggested role in blood pressure maintenance in the brain. Ang IV (angiotensin 3-8), on the other hand, is a hexapeptide that possibly enhances Ang II signaling. The heptapeptide Ang (1-7) is currently considered one of the biologically active end products of the RAAS. It is formed from Ang I or Ang II in the kidney and heart by the action of tissue peptidases at the C-terminus. Once formed, it is rapidly hydrolyzed by ACE; in conditions of ACE inhibition or AT_1 receptor antagonism, its concentration may increase severalfold. There are two major interactions between Ang (1-7) and bradykinin (BK): potentiation of BK by Ang (1-7) and mediation of vascular actions of Ang (1-7) by kinins. Both mechanisms are involved in the cardioprotective effects of ACE inhibitors. At the kidney level, the proposed role of Ang (1-7) is natriuresis and diuresis as opposed to Ang II, an effect blocked by AT_1 receptor antagonists like losartan. In the vascular bed, it antagonizes the vascular effect of Ang II by acting as a competitive antagonist to AT_1 receptors.[120]

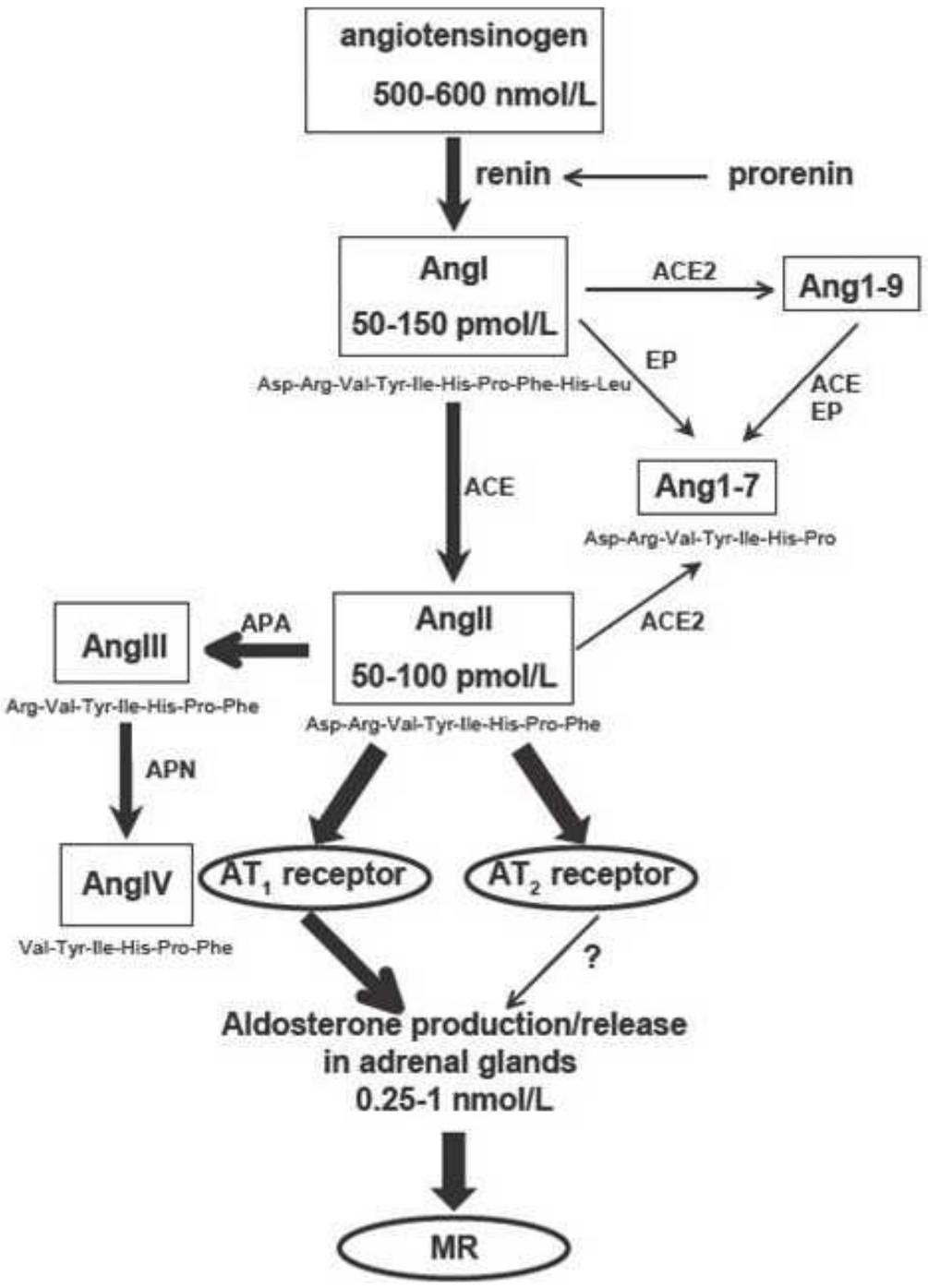

FIGURE 8.3 Schematic representation of the renin-angiotensin system showing plasma concentrations of some components as measured in anesthetized rats. *EP*, endopeptidase; *APA*, aminopeptidase A; *APN*, aminopeptidase N.

Angiotensin II Receptors

Circulating Ang II exerts its biologic effects by binding to specific receptors on the cell surface.[112,121] At least four angiotensin receptor subtypes have been identified. AT_1 receptors bind Ang II with higher affinity than Ang III and are selectively blocked by the biphenylimidazole compound losartan. AT_2 receptors bind Ang II and III with similar affinity and are selectively blocked by tetrahydroimidazopyridines, such as PD123177.[122] The type 3 angiotensin receptor AT_3 has no known function and the type 4 (AT_4) is thought to mediate the release of plasminogen activator inhibitor by Ang II, III, and IV.

Megalin is an abundant membrane protein heavily involved in receptor-mediated endocytosis. Megalin is a receptor for Ang II and Ang II internalization in some tissues is megalin-dependent. Megalin may play a role in regulating proximal tubule Ang II levels.[123] Ang (1-7) exert its vasodilator and natriuretic actions presumably through its binding to a unique receptor, the Mas receptor.[124]

In rodents, two isoforms of AT_1 receptors exist: AT_{1A} (nephron) and AT_{1B} (glomerulus), whereas in humans there is only one AT_1 isoform. The AT_1 receptor has widespread expression in the human adult and is found in the kidney, adrenal gland, heart, and brain. In the kidney, AT_1 receptors are found in the glomeruli, proximal tubule brush border and basolateral membranes, thick ascending loop, proximal convoluted tubule, renal vasculature, the proximal and distal nephron segments, and in both cortical and medullary regions.[125,126]

Aldosterone

Aldosterone was identified by Simpson SA in 1953 and named electrocortin.[127] Later studies characterized more the nature of this hormone and identified it as a mineralocorticoid synthesized and secreted by the zona glomerulosa of the adrenal cortex. The physiology of aldosterone action has been well established after several breakthroughs in research experiences, such as the identification of the mineralocorticoid receptor (MR) as the principal aldosterone receptor, the characterization of sites of aldosterone action in target tissues such as the ENaC, and the demonstration of post-receptor processes involved in physiologic responses to aldosterone.[128–130] Aldosterone secretion is determined by several stimuli, the most important being Ang II and plasma potassium concentration. The MR is the principal receptor for aldosterone, but other target proteins can also bind aldosterone, such as the glucocorticoid receptor. Aldosterone has been implicated in the pathophysiology of several cardiovascular and renal diseases and will be discussed in details in other sections.[129]

Physiologic Actions of Angiotensin II

Systemic Effects of Angiotensin II

AT_1 receptors have been shown to mediate many of the functions of Ang II in the regulation of blood volume, cell contraction, cell proliferation, aldosterone secretion, pressor and tachycardic responses, increased thirst, and hypertension secondary to renal artery stenosis. AT_1 receptors are positively coupled to phospholipase C and mitogen-activated protein kinases (PI3 and MAP) and negatively to adenylate cyclase.[122,125,131] The predominant function of the renin-angiotensin system is regulation of vascular tone and renal salt excretion in response to changes in the volume of extracellular fluid or blood pressure. Ang II represents the effector limb of this hormonal system, acting on several organs, including the vascular system, heart, adrenal glands, central nervous system, and kidneys. Through direct action on smooth muscle cells, Ang II significantly increases arteriolar resistance in renal, mesenteric, dermal, coronary, and cerebral vascular beds.[132] Skeletal muscle and pulmonary vessels, on the other hand, are not affected because of Ang II-stimulated production of vasodilatory prostaglandins by endothelial and smooth muscle cells in these vascular beds.[133,134] Ang II exerts indirect pressor effects via the central and peripheral nervous systems. Its effects on the central nervous system include increased sympathetic discharge and decreased vagal tone.[135] Peripherally, Ang II augments the vasoconstrictive response to renal nerve stimulation in

dogs[136] and its inhibition attenuates the pressor response to norepinephrine in humans.[137] Experimental data suggest the presence of a local renin–angiotensin system in the vasculature that contributes to the regulation of vascular tone.[112]

A more recently recognized function of Ang II is its growth-promoting effects in smooth muscles of the vasculature, heart, and the kidney. Ang II has been shown to induce hypertrophy and mitogenesis in cultured vascular smooth muscles.[138,139] This effect is at least, in part, mediated through autocrine production of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β).[140] Some studies suggest that the renin–angiotensin system contributes to neointimal formation and restenosis after angioplasty.[112] Ang II has been shown to have direct inotropic, chronotropic, mitogenic, and hypertrophic effects on isolated atria and ventricles.[112] Amelioration of hypertensive cardiomyopathy by ACE inhibitors suggests that the renin-angiotensin system plays a role in cardiac hypertrophy.[112]

The predominant physiologic role of the AT_2 receptor is to initiate vasodilation and natriuresis as a counterregulatory response to the vasoconstriction caused by activation of the AT_1 receptor.[141] Other functions include inhibition of growth and hypertrophy, and stimulation of apoptosis.[142] This has been most clearly demonstrated in AT_2 receptor knockout mice that have slightly elevated blood pressure in the basal state, but have an exaggerated increase in blood pressure in response to Ang II infusion compared to wild type mice.[143,144] The intracellular signaling pathways coupled to the AT_2 receptor are unclear. However, there is evidence that the AT_2 receptor may be coupled to the production of a variety of renal vasodilator substances, counterregulating the pressor effects of Ang II via AT_1 receptors.[142] The most likely candidates are bradykinin and nitric oxide (NO)-stimulated cyclic guanosine monophosphate (cGMP),[145,146] but other candidates include products of cyclooxygenase, such as PGE_2 and PGI_2.[141]

Renal Effects of Angiotensin II

Ang II serves at least three important functions in the kidney: regulation of blood flow and GFR, reduction of salt excretion through direct and indirect actions on renal tubule cells, and growth modulation in renal cells expressing AT_1 receptors.

In conditions of decreased renal blood flow, GFR is preserved at nearly constant value over a wide range of perfusion pressures. This phenomenon is known as autoregulation of GFR. At low levels of renal perfusion pressures, Ang II contributes to this phenomenon.[147] Micropuncture studies have shown that Ang II exerts substantial effects on the renal microvasculature and hemodynamics; however, the individual contribution of the systemic and local renin-angiotensin systems to the overall regulation is still controversial. Ang II constricts both the afferent and efferent arterioles, reduces single nephron glomerular filtration rate and plasma flow, increases the filtration fraction, and decreases glomerular filtration coefficient. Other studies have shown that Ang II infusion preferentially vasoconstricts efferent arterioles counteracted by endothelial-derived NO[148] and constricts descending vasa recta (DVR) through Ca^{2+} signaling in pericytes.[149] The disproportionate increase in postglomerular resistance results in a marked increase in glomerular capillary hydrostatic pressure (P_{GC}), ultrafiltration pressure, and filtration fraction, thus preserving GFR in the face of declining renal plasma flow (RPF). The selectivity of the vasoconstrictive action of Ang II for the efferent arteriole results from stimulation of vasodilatory prostacyclin synthesis by the afferent arteriole and not a preferential action of Ang II on the efferent arteriole.[150] In fact, Ang II increases both afferent and efferent resistance in the presence of a cyclooxygenase inhibitor.[150] Under certain pathophysiologic conditions, afferent arteriolar constriction predominates, leading to a decrease in both RPF and GFR.[151] Deep nephrons have higher postglomerular Ang II tone and also higher Ang II sensitivity than superficial nephrons. The better preserved GFR in deep cortex during Ang II action may contribute to maintaining the renal-concentrating ability by providing NaCl for reabsorption by the ascending limb of the loop of Henle.[152]

In addition to its vascular effects, Ang II induces mesangial cell contraction, which leads to decreased K_f in vivo.[153,154] This effect, however, is attenuated by the concomitant production of prostaglandins by mesangial cells.[155]

Ang II is one of the most potent sodium-retaining hormones in the body. Increased tubular sodium reabsorption is enhanced by both direct and indirect tubular effects of Ang II. Physiologic concentrations of Ang II (10^{-12} to 10^{-10} M) stimulate proximal tubule NaCl and $NaHCO_3$ absorption at the proximal tubule.[156] Indirectly, Ang II stimulates ion transport in the proximal tubule by changing the peritubular milieu. Ang II can both decrease peritubular capillary hydrostatic pressure and increase peritubular oncotic pressure, resulting in an increased driving force for ion reabsorption. Directly, Ang II can stimulate transport in the proximal tubule through interaction with the AT_1 receptors found on both the apical and basolateral membranes of the tubule cells.[157,158] It also stimulates calcineurin phosphatase activity in proximal tubule epithelial cells through a mechanism involving AT_1 receptor-mediated tyrosine phosphorylation of the PLC isoform, both linked to sodium transport in the proximal tubule.[159] Specifically, Ang II stimulates the activity of apical membrane Na–H antiporters and basolateral membrane Na–HCO_3–CO_2 cotransporters[160] and the activity of Na–K-ATPase by changing phosphorylation and conformation of Na–K-ATPase.[161] The net effect is increased proximal tubule reclamation of Na and HCO_3. Denervation of the proximal tubule results in attenuation of the Ang II-stimulated NaCl, but not $NaHCO_3$ absorption, suggesting that Ang II enhances proximal Na transport indirectly by increasing presynaptic catecholamine release.[159] Of note is that supraphysiologic concentrations of Ang II (10^{-9} to 10^{-7} M) inhibit NaCl and water reabsorption in the proximal tubule and also inhibit Na–glucose cotransporter translocation by inactivation of PKA and decrease of PI3-kinase activity mediated through the AT_1 receptor.[162,163] Ang II increases proximal tubule phosphate absorption by

direct stimulation of Na/P_i cotransport activity as a result of increase in the expression of brush-border membrane NaPi-IIa protein level and that stimulation is most likely mediated by posttranscriptional mechanisms.[164]

Intraluminal conversion of Ang I to Ang II can occur in the cortical collecting duct, resulting in enhanced apical sodium entry.[165] The AT_2 receptor regulates epithelial sodium channel (ENaC) abundance, consistent with a role for Ang II in regulation of collecting duct function via AT_1 receptors.[166,167]

Ang II increases basolateral K-channel activity via the stimulation of AT_1 receptors and the stimulatory effect of Ang II is mediated by a NO-dependent cGMP pathway.[168]

Other effects of Ang II on proximal tubule cells include enhanced gluconeogenesis and ammonia production.[169] The effects of Ang II on distal tubule transport of Na and K are mediated through aldosterone release.[170] In addition to proximal tubule epithelial cells, vasa recta and outer medullary vascular bundles express high density of AT_1 receptors.[121] ACE inhibitors increase descending vasa recta blood flow, whereas Ang II infusion markedly decreases medullary blood flow in rats.[171] It is postulated that Ang II influences urinary dilution and concentration by modulating blood flow to the medulla.

Ang II induces hypertrophy of proximal tubule epithelial cells in vitro.[172] It also exerts similar growth-promoting effects on mesangial cells.[173] The signaling mechanism by which Ang II exerts this effect is not precisely known, but p27Kip1 is required for Ang II-induced hypertrophy.[174] Downstream potential targets of Ang II are the extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) and Ang II activates ERK1/ERK2 via the AT_1 receptor.[175] Studies have shown that connective tissue growth factor (CTGF) might be an important mediator of Ang II-induced renal hypertrophy, which suggests that inhibiting the production of CTGF might be the new strategy in early prevention of renal fibrosis.[176] Ang II can stimulate human kidney fibroblast (KFB) proliferation and enhance the expression of interleukin-6 in KFB. These findings suggest that Ang II might play a part in the mechanisms for modulating tubulointerstitial changes and inducing renal fibrosis.[177]

Previous in vivo studies in cardiomyopathic hamsters suggested that the expression of vasopressin (AVP) V_2 mRNA is upregulated by Ang II. Ang II caused a significant increase in the AVP V_2 mRNA in a dose-dependent manner mediated by PKA, whereas PKC suppresses the expression of V_2 mRNA in the inner medullary collecting duct (IMCD) of the rat kidney.[178]

The role of Ang II in the pathogenesis of hypertension is complex. Several studies have demonstrated a crucial role of the intrarenal renin-angiotensin system in the development of hypertension and kidney disease.[117] Animal studies have shown that blockade of the AT1 receptor resulted in increased plasma Ang II levels while it limited renal Ang II contents in response to Ang II infusion.[179] This dissociation between systemic and local Ang II regulation has been demonstrated in several animal hypertension models including renovascular hypertension.[180,181] Clinical studies have also shown that local intrarenal Ang II formation was central to the development of hypertension in humans.[182]

In addition to its effects on the maintenance of blood pressure, AT_1 receptors may play a role in embryonic nephrogenesis. Blockage of the renin-angiotensin system with ACE inhibitors or AT_1 inhibitors results in abnormal renal development that is characterized by both papillary and tubular atrophy and by interstitial fibrosis and infiltration. In addition, knockout mice lacking both the AT_{1A} and AT_{1B} receptor have similar renal abnormalities.[183] Ang II also modulates mesangial cell growth, and induces proximal tubular cell hypertrophy in humans, effectively inhibited by irbesartan, an Ang II receptor antagonist.[184]

The AT_2 receptor is expressed predominantly in fetal tissues, but in almost all tissues there is postnatal downregulation. AT_2 receptor mRNA is expressed in the fetal and neonatal rat kidney, but disappears after the neonatal period and is not expressed in the normal adult. Although the AT_2 receptor mRNA is not found in the adult kidney, both immunohistochemistry and Western blot analysis have detected AT_2 receptor protein in the glomeruli, cortical tubules, and interstitial cells of the adult kidney.[121,185] It was thought that AT_2 receptors might play a role in the development of the kidney and urinary tract given the high levels of expression in the fetus. However, although early studies of AT_2 receptor knockout mice showed no gross morphologic abnormalities of the kidney,[143,144] a more recent study has demonstrated the presence of increased numbers of congenital anomalies of the urinary tract.[186] The AT_2 receptor may be implicated in some congenital abnormalities of the urinary tract or may be involved in the pathophysiologic response to ureteral obstruction by protecting against the formation of interstitial fibrosis.[186,187] Ang II modulates the over-expression of AT_2 receptors in renal ablation experiments through its own AT_2 receptor and functional expression of this effect may represent a counterregulatory mechanism to modulate the renal damage induced by renal ablation.[188]

Ang II regulates renal parathyroid hormone-related protein (PTHrP), a vasodilator and mitogenic agent upregulated in kidney injury, and its type-I receptor (PTH1R) system via AT_1 receptors.[189]

Local Effects of Ang II in Nonrenal Organs

Ang II exerts its actions on other organs, such as the adrenals and the brain. At the level of the adrenals, it stimulates aldosterone synthesis and secretion.[170] Acute angiotensin administration stimulates the activity of 11β-hydroxysteroid dehydrogenase (HSD) type 2 in human kidneys and exerts a dual effect on the MR receptor (i.e., an indirect agonistic effect by increasing aldosterone availability and a direct or indirect antagonistic effect by stimulation of renal 11β HSD type 2 activity).[190]

At the central nervous system (CNS) level, it stimulates thirst and salt appetite[191,192] and may increase secretion of

vasopressin and oxytocin from the posterior pituitary, and adrenocorticotropic hormone (ACTH), prolactin, and luteinizing hormone from the anterior pituitary.[193]

Regulation of the Systemic Renin-Angiotensin-Aldosterone System

Ang II in the systemic circulation is produced primarily from circulating angiotensinogen through the proteolytic action of renin. Most of the circulating renin is derived from the kidney, although other organs can secrete prorenin in the circulation. Angiotensinogen is mostly formed and secreted in the circulation by liver cells, allowing for systemic formation of angiotensin peptides, principally Ang II. The circulating concentrations of angiotensinogen are more than 1,000 times greater than those of Ang I and Ang II, therefore changes in plasma renin activity is the major determinant of Ang I formation (Fig. 8.3). Renin secretion is stimulated by various dynamic parameters such as renal perfusion pressure, tubular fluid sodium chloride concentration at the MD, sympathetic discharge to the kidney, and endocrine and paracrine hormones and growth factors.[107]

Stimulation of renin release by juxtaglomerular cells is mediated by increased intracellular cAMP, whereas a rise in cytosolic free calcium is inhibitory.[194] Renin release responds inversely to changes in renal perfusion pressure.[114] Elevation of intrarenal arterial pressure inhibits renin release and induces a "pressure" natriuresis. At least two mechanisms have been postulated. Increased afferent arteriolar wall tension secondary to increased renal perfusion elevates intracellular calcium in juxtaglomerular cells and inhibits renin secretion.[114,194] Increased perfusion pressure also stimulates NO production and release by endothelial cells. NO, in turn, suppresses renin secretion.[195,196] Conversely, decreased renal perfusion results in increased production of prostacyclin (prostaglandin I_2), which enhances renin release.[197] Mechanical strain leads to upregulation of the AT1 receptor and increased Ang II production in conditionally immortalized podocytes. The resulting activation of a local tissue angiotensin system leads to an increase in podocyte apoptosis, mainly in an AT1 receptor-mediated fashion.[198]

Decreased NaCl delivery to MD cells stimulates renin secretion, whereas increased urinary NaCl exerts an opposite effect.[180] Schlatter and coworkers[199] demonstrate that changes in luminal Cl^- concentration alter the rate of Na^+-K^+-$2Cl^-$ transport in MD cells.[199] The precise mechanism by which variation in the activity of this transporter translates to a signal that regulates renin release by adjacent juxtaglomerular granular cells is not entirely clear. Postulated mediators include adenosine, which inhibits renin secretion via activation of A_1 receptors on juxtaglomerular cells, and alterations in interstitial osmolality, which may affect renin secretion directly.[96] Experimental evidence also suggests that NO produced by MD cells and endothelial cells regulates renin secretion.[195,200]

The importance of renal sympathetic innervation in controlling renin secretion is well recognized.[201] Stimulation of postjunctional β-adrenergic receptors increases renin release. The role of α-adrenergic receptors, on the other hand, is controversial.[201] Ample evidence suggests that dopamine stimulates renin secretion by direct activation of dopamine A1 (DA_1) receptors on juxtaglomerular cells.[201,202]

Several endocrine and paracrine hormones regulate renin secretion by the kidney. ANP has been shown to inhibit renin release from isolated juxtaglomerular cells.[203] Other inhibitory hormones include AVP, endothelin, and adenosine (A_1-receptor agonists).[114,195] Regulation of renin secretion by Ang II is probably the most physiologically relevant.[169] Ang II inhibits renin secretion and renin gene expression in a negative feedback loop. Treatment of transgenic mice bearing the human renin gene with an ACE inhibitor increases renin expression in the kidney by five- to tenfold.[204] Similarly, ACE inhibition in rats augments renal renin mRNA expression, an effect that is reversed by infusion of Ang II.[205] The effects of Ang II are believed to be direct and not dependent on changes in renal hemodynamics or tubular transport. Arachidonic acid metabolites produced in the kidney also play an important role in renin secretion.[195] Intrarenal infusion of arachidonic acid increases (and indomethacin decreases) plasma renin activity in rabbits.[206] Several studies have since confirmed that prostaglandins of the I series are potent stimulators of renin secretion.[195,197] On the other hand, lipoxygenase products of arachidonic acid metabolism (12-HPETE, 15-HPETE, and 12-HETE) and cytochrome P450-mediated epoxides (14,15-epoxyeicosatrienoic acid) have been shown to inhibit renin release in renal cortical slices.[207,208]

The Local Renin-Angiotensin System

The renin-angiotensin system has been characterized as an endocrine, paracrine, and autocrine system. Contribution of systemically formed mediators to local control of dynamics within tissues is difficult to delineate. Recent evidence suggests that local formation is a major determinant of Ang levels in organs and tissues. In the brain, for example, Ang peptide levels are regulated in an autonomous manner.[209] Local renin-angiotensin systems have been identified in several organs, including the kidney, heart, vasculature, brain, and adrenals.[210] Although most organs have elements of the renin-angiotensin system, the adult kidney is unique in expressing all the components of the system.[156,169]

Compared with plasma levels, the renal Ang II contents are much higher despite suppression of renin secretion and release.[126] Renin is principally produced by juxtaglomerular cells of the distal afferent arteriole, but has been shown to be expressed in the proximal tubule cells,[126] whereas its substrate, angiotensinogen, is expressed by proximal tubule cells. ACE activity in the kidney has also been localized to the proximal tubule, with the highest concentration present on the brush border. Several studies have provided evidence for production of Ang II by the kidney, mainly concentrated in the proximal tubules,[126] suggesting that the intrarenal renin-angiotensin

system is, indeed, functional.[156] Some investigators have proposed that this local system plays a role in proximal tubule NaCl and HCO_3 absorption, pathogenesis of essential hypertension, and expression of the phenotype of autosomal dominant polycystic kidney disease.[156] Independent regulation of renal Ang II production has not been definitively demonstrated. Circulating Ang II stimulates renal angiotensinogen mRNA production and intact urine angiotensinogen suggests its presence along the whole nephron and that renin and ACE activity are available all through the nephron.[126]

Endogenous Ang II in both peritubular blood and luminal fluid is important for maximal expression of the stimulatory influence of this peptide on proximal tubule fluid uptake.[211] Intraluminal conversion of Ang I to Ang II can occur in the cortical collecting duct, resulting in enhanced apical sodium entry.[165]

Renal degradation of Ang II is constitutively high, unaffected by chronic levels of arterial blood pressure, and is independent of long-term changes in levels.[212]

Low-density lipoproteins (LDLs) increase Ang II production by mesangial cells, which, in turn, results in increased O_2 production, cell proliferation, and hypertrophy—these effects of Ang II are mediated by the AT_1 receptor.[213]

Established Clinical Pathophysiologic Role of the Renin-Angiotensin-Aldosterone System in Humans

The RAAS has been implicated in the pathophysiology of several diseases of the cardiovascular system and the kidney, mostly hypertension and renal injury. The local renal renin-angiotensin system is activated in the renovascular type of hypertension in the stenotic kidney and accounts for most of the Ang II concentration in the renal tissue.[214] In addition, it plays a crucial role in other forms of hypertension. Administration of a renin inhibitor to normal subjects and hypertensive patients showed sustained local renal vascular responses but time-dependent decreased drug activity at the systemic level.[215] Several other studies demonstrated the importance of the intrarenal renin-angiotensin system in the pathophysiology of hypertension.[216,217]

Ang II promotes fibrogenesis and oxidative stress in the kidney by stimulating fibrogenic mediators, altering renal hemodynamics especially facilitating glomerular hypertension, inducing tubulointerstitial hypoxia, and enhancing free radical formation. Studies have shown that the degree of mesangial hypercellularity and expansion in IgA nephropathy correlated closely with glomerular expression of mRNAs for renin, ACE, chymase, and AT1 and AT2 receptors.[217,218] The role of the intrarenal renin angiotensin system has been also established in the pathogenesis of membranous nephropathy.[219] In diabetic nephropathy, the renin-angiotensin system plays a crucial role in disease progression: the intrarenal generation of Ang II is increased, despite suppression of the systemic RAAS. Details of the pathophysiologic mechanisms implicated in diabetic nephropathy are beyond the scope of this chapter.

The role of the RAAS in end-stage renal disease (ESRD) is even more complicated and implicates many parameters related to residual renal function, presence of renal replacement therapy and modality, and drug treatment of associated conditions. In addition, renal transplantation adds a new dimension to the complexity by introducing an immune component through graft rejection and immunosuppressive drugs. Despite the substantial decrease in renal blood flow and function in patients with ESRD on dialysis, studies have shown that those with remaining kidneys have an activated intrarenal renin-angiotensin system.[117] On the other hand, renal transplant diseases, especially rejection and cyclosporine-induced nephropathy, were associated with increased activity of systemic and local RAAS in several studies.[220,221]

In conclusion, the RAAS is one of the most powerful pressure and volume regulatory systems in the body, involved in physiologic responses and pathophysiologic processes at both the systemic and local levels. Autonomous and independent control of local renin-angiotensin systems in various organs and tissues may exist and contribute to disease progression. New areas of interest in the RAAS are continuously emerging, facilitating the understanding of mechanisms of diseases and development of specific drug targets.

ATRIAL NATRIURETIC PEPTIDE

Molecular and Biochemical Properties of Atrial Natriuretic Peptide

The cDNA for human ANP was isolated in 1984 and, shortly afterward, the gene was localized to the short arm of chromosome 1.[222,223] The chromosomal gene consists of three exons and two introns encoding for a mature mRNA transcript approximately 900 bases long.[223]

Translation of human ANP mRNA results in a 151-amino acid preprohormone.[224] Pro-ANP, a 126-residue molecule, is formed after cleavage of the signal peptide sequence of prepro-ANP and represents the major storage form of the hormone in atrial granules.[225] The circulating, biologically active form of ANP, often referred to as $AN_{99–126}$ or ANP_{1-28}, is a peptide comprising the 28 carboxy-terminal amino acids of the parent molecule.[224] The amino acid sequence of $ANP_{99–126}$ is highly conserved among mammalian species.[224] A disulfide bond between cysteine residues 105 and 121 gives $ANP_{99–126}$ its ring structure, which is essential for biologic activity.[226]

Pro-ANP (1–30), (31–67), and (68–98) are secreted from the heart and circulate in the plasma. Pro-ANP (1–30) and (31–67) increase sodium and water excretion and binding sites have been found in the proximal tubules and collecting ducts. Pro-ANP (31–67) inhibits the Na^+-K^+ pump at the medullary collecting duct through a prostaglandin-dependent mechanism and no effect on cGMP production. Pro-ANP (1–30) infusion in rats clearly increases urine output, sodium, and potassium excretion, the mechanism of which still needs to be elucidated.[227]

Secretion and Physiologic Regulation of Atrial Natriuretic Peptide$_{99–126}$

Cardiac atria contain the highest concentrations of ANP and serve as the major source of circulating hormone.[224] $ANP_{99–126}$ secretion from cardiomyocytes occurs largely in response to atrial stretch resulting from increased atrial transmural pressure.[228] Physiologic stimuli for the release of $ANP_{99–126,}$ include acute salt and volume loading, supine posture (head-down tilt), and head-out water immersion.[228–230] An increased rate of atrial contraction has also been shown to stimulate $ANP_{99–126}$ secretion.[231,232] Ang II, vasopressin, epinephrine, and phenylephrine stimulate $ANP_{99–126}$ secretion from the heart largely because of their systemic vasopressor effects.[233] On the other hand, glucocorticoids and endothelin raise $ANP_{99–126}$ levels possibly by acting directly on atrial myocytes.[234] Leptin decreases ANP secretion via an NO-mediated mechanism.[235] Physiologic and pathologic conditions in which elevated plasma levels of $ANP_{99–126}$ have been detected are summarized in Table 8.3.

The local synthesis of natriuretic peptides is increased in the kidney and in the vasculature in obstructive uropathy.[236] The activation of the renin-angiotensin system during low sodium intake antagonizes the biologic effect of ANP by interfering in the intracellular metabolism of cGMP.[237]

8.3 Conditions Associated with Increased Levels of Circulating Atrial Natriuretic Peptide

Physiologic	Pathologic
Acute volume expansion	Congestive heart failure
Supine posture	Atrial tachycardias
Head-out water immersion	Myocardial ischemia
Mineralocorticoid escape	Acute and chronic renal failure
Exercise	Postobstructive diuresis
Neonatal period	Nephrotic syndrome (subset) Cirrhosis with ascites Severe hypertension Primary hyperaldosteronism Hypoxia Other (SIADH myxedema)

SIADH, syndrome of inappropriate antidiuretic hormone secretion.

Physiologic Actions of Atrial Natriuretic Peptide$_{99–126}$

Three subtypes of ANP receptors (NPRs) have been identified.[238–240] NPR-A and -B have intrinsic guanylate cyclase activity that catalyzes production of cGMP after ligand binding. cGMP then serves as an intracellular second messenger that mediates the biologic activities of $ANP_{99–126}$.[241] NPR-B appears to have 50-fold higher affinity for a related natriuretic factor originally purified from porcine brain, known as C-type natriuretic peptide (CNP).[242] NPR-C, previously known as the (clearance) receptor, is devoid of guanylate cyclase activity and, therefore, does not confer biologic activity and is thought to mediate clearance of circulating ANP along with metalloendoprotease (E.C.3.4.24.11)[242] and of other related hormones, such as brain natriuretic peptide (BNP).[243] Selective downregulation of NPR-C in the kidney in response to dietary salt supplementation may contribute to local elevation in ANP levels and may be functionally significant in attenuating the development of salt-sensitive hypertension.[244] NPR density is decreased in diabetic rats with significant increase in plasma ANP levels.[245] ANP also antagonizes AVP-mediated increases in water permeability in IMCD cells.[246]

The major sites of action of $ANP_{99–126}$ are the kidneys, adrenal glands, and vascular smooth muscle.[247] Short-term administration of $ANP_{99–126}$ in laboratory animals and in humans induces pronounced natriuresis, diuresis, alteration in renal hemodynamics and tubular function, suppression of renin release, inhibition of aldosterone secretion by the adrenal glands, and decreased vasomotor tone, resulting in transient drop in systemic blood pressure. From these actions it has been postulated that ANP plays an important physiologic role in protecting against extracellular volume overload.[248]

Renal Actions of Atrial Natriuretic Peptide$_{99–126}$

ANP-induced increase in the GFR is well established.[249–251] $ANP_{99–126}$ increases efferent arteriolar resistance, resulting in increased P_{GC} and filtration fraction.[249] In addition, $ANP_{99–126}$ relaxes mesangial cells in vitro, suggesting that it can increase filtration area by cGMP generation in podocytes[252] and K_f in vivo.[253] Indeed, when baseline K_f is low, as in water-deprived animals, $ANP_{99–126}$ enhances GFR mainly by increasing K_f.[253] If preexisting vascular constriction is present in isolated perfused kidney, ANP tends to vasodilate renal vessels and increase renal blood flow (RBF).[254] In whole animals, however, $ANP_{99–126}$ infusion causes either a decline or no change in RBF.[254] The effects of $ANP_{99–126}$ on RBF are influenced by its systemic actions on blood pressure and the renin-angiotensin system. ANP seems to increase NO production at both renal and cardiac levels, further explaining its natriuretic and diuretic effects.[255] Finally, ANP has been reported to induce redistribution of blood flow from the cortex to the medulla and to increase vasa recta flow, leading to dissipation of the medullary solute gradient.[256,257]

$ANP_{99–126}$ has both direct and indirect effects on tubular transport of Na, chloride, and water.[258] In the proximal tubule, $ANP_{99–126}$ antagonizes Ang II-induced Na reabsorption[259] and, along with endothelin-3, inhibits the sodium-glucose transporter[260] and, like urodilatin, inhibits Na^+-ATPase activity.[261] In the IMCD, it directly inhibits Na transport by binding to ANP-R1 receptors and influencing amiloride-sensitive Na channels and the activity of apical Na-K-2Cl cotransporters.[262] Other mechanisms by which $ANP_{99–126}$ induces natriuresis and diuresis include suppression of renin and aldosterone release,[263] inhibition of the tubular actions of AVP,[246] and dissipation of the medullary solute gradient by impairing the increase in intracellular Ca^{2+} concentration. ANP blocks both the stimulatory and inhibitory effects of AVP on Na^+-dependent pHi recovery.[264]

Atrial Natriuretic Peptide Transgenic Mice

Transgenic mice over-express pro-ANP in hepatocytes. These transgenic animals have a hypotensive phenotype (20 to 30 mm Hg lower than control littermates) without compensatory tachycardia. GFR remained normal despite hypotension. Moreover, significant diuresis or natriuresis during steady state was not detected. Contrary to observations made after short-term infusion of $ANP_{99–126}$, plasma renin activity also did not change while aldosterone levels were elevated.[265–267]

Physiologic Consequences of Interrupting the Atrial Natriuretic Peptide Pathway

Disruption of the gene that encodes pro-ANP results in knockout mice that lack expression of ANP. Homozygous ANP knockout mice fed a standard diet have both mildly elevated blood pressure (average increase of 8 mm Hg) and cardiac hypertrophy compared to wild type mice, suggesting that ANP has a physiologic role in maintaining the normotensive state. This hypertension appears to be sensitive to dietary salt intake as feeding the homozygous ANP knockout mice a diet with an intermediate salt content (2%) would further increase blood pressure by an average of 20 mm Hg compared to wild type mice.[268] Knockout mice lacking ANP-R1 activity (known as GC-A null mice) have both elevated blood pressure and cardiac hypertrophy, similar to pro-ANP knockout mice, but the GC-A null mice have a salt-insensitive form of hypertension.[267] It is unclear why there should be a difference in the phenotype of these two types of knockout mice. It is possible that other guanylyl cyclase receptors, such as guanylyl cyclase C, can help regulate blood pressure in the face of changes in dietary salt intake and compensate for the lack of GC-A receptors.[268] ANP (−/−) mice have a blunted pressure-natriuresis response and suppressed expression of local renal renin-angiotensin system.[269]

Atrial Natriuretic Peptide-Related Peptides[149,212]

BNP is a 32-amino acid peptide with structural homology to $ANP_{99–126}$. Although originally isolated from porcine brain,[270] it is also secreted by cardiac ventricles and, to a lesser extent, from atria. The biologic effects of BNP infusion are similar to those of $ANP_{99–126}$. Unlike ANP, BNP secretion seems to be constitutive and unrelated to myocyte stretch. The kidney-specific degradation of ANP provides a mechanism for preferential regulation of kidney function by BNP, independent of peripheral ANP concentration.[270] In 1990, another homologous peptide, CNP, was isolated from porcine brain.[271] CNP is produced in the brain, where it achieves concentrations much higher than those of ANP and BNP. In contrast, circulating levels of CNP are lower. CNP lacks natriuretic, diuretic, and hypotensive effects and probably acts in a paracrine fashion in the CNS. The physiologic significance of BNP and CNP remains unclear.

Clinical Use of Atrial Natriuretic Peptide and Atrial Natriuretic Peptide Analogs in the Diagnosis and Treatment of Kidney Injury and Congestive Heart Failure

Measurement of circulating ANP and ANP analogs is now a well established marker in assessment of volume overload and left ventricular dysfunction and for predicting mortality, in the presence or absence of renal dysfunction.[272–276] A number of studies, reviewed recently,[277] provide support for the use of ANP/ANP analogs in protection against and treatment of acute kidney injury (AKI).[278–281] In these studies, low (but not high)-dose ANP infusions reduced the need for renal replacement therapy and hospital and intensive care unit length of stay, but did not appear to improve mortality in the management of cardiac surgery–associated AKI. Hyporesponsiveness to ANP in congestive heart failure may be due to reduced NPR-A expression.[282] In dogs, maximizing cGMP action with type V phosphodiesterase inhibitors augments the natriuretic responses to exogenous ANP.[283] Animal studies have shown the usefulness of encapsulated ANP gene transfected cells as a new tool for ANP gene delivery with possible implication for future therapy.[284]

URODILATIN OR RENAL NATRIURETIC PEPTIDE

Urodilatin is best described as a paracrine renal natriuretic peptide (RNP).[285] It was first isolated from human urine in 1988.[286] Its amino acid sequence is identical to $ANP_{99–126,}$ except for an additional four amino acids at the amino terminal. Despite its high degree of homology to $ANP_{99–126,}$ specific antihuman RNP polyclonal antibody has been generated and RNP levels can be measured by radioimmunoassay.[287] To date, RNP has not been detected in the circulation and the kidney is presumed to be its site of synthesis and action.[285]

RNP binds to ANP receptors in the kidney and stimulates cGMP production.[288] Its renal actions parallel those of $ANP_{99–126}$ and include more potent hyperfiltration, diuresis, and natriuresis.[288] RNP, like $ANP_{99–126}$, inhibits sodium uptake by inner medullary duct cells by inhibiting entry of Na

through apical sodium channels.[289] It appears that the natriuretic effect of RNP is more potent than that of ANP, possibly because it is resistant to degradation by renal cortical metalloendopeptidase.[290] Systemic infusion of RNP results in effects similar to those of $ANP_{99–126}$.

Several physiologic studies suggest that RNP functions as a paracrine hormone that regulates renal Na excretion. Drummer and coworkers[291] demonstrated in the human that urinary excretion of RNP, but not plasma ANP concentration, correlates with circadian variation in sodium excretion over a 9-day period. Moreover, acute infusion of normal saline in healthy subjects, balloon dilatation of left atrium, and water immersion induce a significant increase in urinary RNP.[292,293]

GUANYLIN AND UROGUANYLIN

Guanylin and uroguanylin are cGMP-regulating agonists isolated in 1994, respectively, from rat intestine and human/opossum urine that appear to have natriuretic properties.[294,295] In humans and mice, earlier studies reported that both genes for guanylin and uroguanylin are located close to each other on chromosomes 1 and 4, respectively, near the ANP A and B genes and probably arising from an ancestral uroguanylin/guanylinlike gene.[296] Preproguanylin and preprouroguanylin probably derived from a common precursor gene, as they share approximately 35% homology.[297] Bioactive uroguanylin can be found in the urine at higher concentrations than guanylin, suggesting that uroguanylin may be a hormonal link between the intestine and the kidney.[298] Uroguanylin, prouroguanylin, and proguanylin peptides have been shown to circulate in the plasma of humans and other animals.[299]

CORTICOSTEROIDS

Corticosteroids are steroid hormones synthesized by the adrenal cortex. On the basis of their physiologic functions, corticosteroids are traditionally divided into two groups—glucocorticoids (cortisone and cortisol) and mineralocorticoids (aldosterone)[300]—based on their potency in electrolyte and metabolism regulation.

Corticosteroids bind to intracellular receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). They are both part of the nuclear receptor family that also includes receptors for steroid and thyroid hormones, vitamin D_3, and retinoic acids. These receptors translocate to the nucleus after ligand binding[301] and regulate nuclear gene transcription at specific DNA response elements located in the five regulatory regions near the promoters of specific target genes (Fig. 8.4).[302] Both glucocorticoids and mineralocorticoids modulate renal function. Aldosterone-sensitive tissues include the distal parts of the nephron (distal tubule, connecting tubule, and all along the collecting duct), the surface epithelium of the distal colon (where it increases sodium absorption and potassium excretion), and other specific nuclear-binding sites for aldosterone in the thick ascending limb of Henle's loop and salivary and sweat glands. All MR-expressing tissues also express GR. In the kidney, evidence exists for distribution of the GR receptors along the whole parts of the nephron, except for the proximal tubule. No evidence exists for a glucocorticoid role in the colon.[303–305]

MR has the same affinity for both aldosterone and glucocorticoids. Glucocorticoids concentration in plasma is 100- to 1,000-fold higher than aldosterone and only 10% of it circulates as free, whereas all circulating aldosterone is free. MR selectivity exists to prevent complete occupancy of the MR receptor by glucocorticoids at physiologic concentrations. This is mainly mediated by the colocalization of the 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) enzyme in the distal nephron, along with MR. This enzyme transforms glucocorticoids (cortisol in humans) into metabolites (cortisone) that have weak affinity to MR.[301,305–307] 11β-HSD has two main isoforms. 11β-HSD1 acts predominantly as a reductase in vivo in many tissues, regenerating biologically active glucocorticoids (mainly cortisol in human and corticosterone in rodents) from their inactive forms (cortisone in human and 11-dehydrocorticosterone in rodents). 11β-HSD2, on the contrary, is a dehydrogenase that inactivates glucocorticoids.[308] The major role of 11β-HSD2 is highlighted in clinical situations, such as the syndrome of apparent mineralocorticoid excess where it is inactivated or after ingestion of excessive amounts of licorice where glycyrrhetinic acid, a derivative of licorice, has been described to inhibit 11β-HSD2.[309,310] Both of these conditions lead to hypokalemic hypertension with low renin and aldosterone levels. In cirrhosis also, there is MR activation by cortisol explained by a reduced activity of 11β-HSD2, which allows promiscuous activation of MR by the glucocorticoid cortisol as suggested by Frey.[311]

GRs have approximately equal affinities for aldosterone and endogenous glucocorticoids, but have the highest affinity for dexamethasone, a synthetic glucocorticoid.[301] Because mineralocorticoids circulate at much lower concentrations than glucocorticoids, significant binding of mineralocorticoids to GR does not occur under physiologic conditions.

Nongenomic Actions of Aldosterone

The effects of aldosterone on its target cells have long been considered to be mediated exclusively through the genomic pathway and were characterized by a 45-minute lag period; however, evidence has been provided for rapid effects of the hormone that may involve nongenomic mechanisms.[5–7,19,32] On the other hand, in a series of in vitro studies, aldosterone was shown to have a half maximal effect on both rapid (15 minutes) and delayed (120 minutes) Na flux.[4,32] The Na/H exchanger has been identified as a target for nongenomic regulation. Aldosterone rapidly increases Na/H exchanger activity in a variety of cells, including distal colon and renal epithelial cell lines,[3] but rapidly inhibits apical NHE_3 and HCO_3^- reabsorption in medullary thick ascending limb (MTAL) and has a dose-dependent biphasic effect on the Na/H exchanger (low doses stimulate and high doses inhibit it).[312]

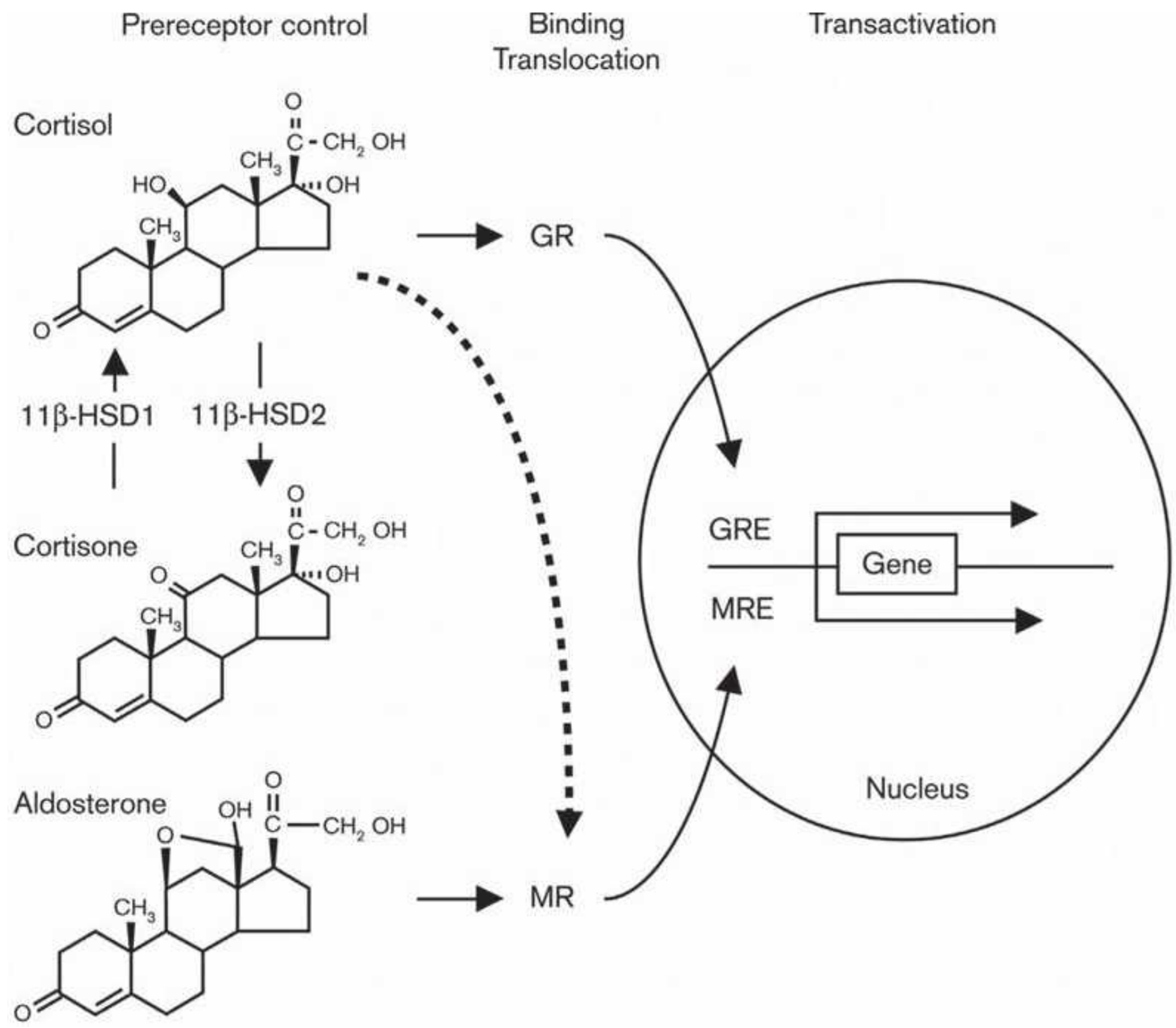

FIGURE 8.4 The intracellular concentrations of steroid molecules available for binding to glucocorticoid receptor (GR) or mineralocorticoid receptor (MR) depend on the free extracellular concentrations available for diffusion into the cytoplasm and the intracellular prereceptor control mechanism constituted by the 11β-hydroxysteroid dehydrogenase type 1 and 2 (11β-HSD1, 11β-HSD2) enzymes. Whereas 11β- HSD1 acts predominantly as a reductase and converts the 11-ketosteroid cortisone with virtually no affinity for MR and GR into the 11β-hydroxyglucocorticoid cortisol with a high affinity for both GR29,58 and MR, 11β-HSD220 is exclusively an oxidase and inactivates cortisol into cortisone, which allows protection of MR-expressing cells from promiscuous activation of MR by the glucocorticoid hormone cortisol. *GRE*, glucocorticoid-response element; *MRE*, mineralocorticoid-response element. (Frey FJ, Odermatt A, Frey BM. Glucocorticoid-mediated mineralocorticoid receptor activation and hypertension. *Curr Opin Nephrol Hypertens*. 2004;13:451–458.)

Aldosterone acts through nongenomic pathways to regulate many different ion transport proteins and signaling pathways in a variety of renal epithelial cells, such as proximal tubule cells derived from human renal cortex, MDCK-C11 cells (a cell line that exhibits properties of collecting duct intercalated cells), and principal cells isolated from rabbit cortical collecting duct and both M-1 and RCCD2 cortical collecting duct cell lines.[6] Studies using isolated perfused tubules also demonstrate that aldosterone, via nongenomic mechanisms, regulates the transepithelial transport function of different nephron segments, such as proximal S3 segment,[21] renal MTAL, type A intercalated cells of outer medullary collecting ducts, and principal cells in the connecting tubule and inner medullary collecting ducts.[6,313] In renal epithelial cells, an elevation in cytosolic Ca_2 serves as a second messenger for the nongenomic Na^-/H exchanger activation initiated by aldosterone.[2] The existence of a nongenomic action of aldosterone is in general attributed to conditions where this action is observed over a short period of time (minutes) against the much longer time (hours, days) needed for a genomic action.[313]

Rapid nongenomic aldosterone effects are characterized by their rapid onset of action (within minutes) and an insensitivity to inhibitors of transcription (e.g., actinomycin D) and of protein synthesis. Aldosterone also acts via rapid nongenomic effects in vivo in humans at the renal vasculature. Antagonizing the endothelial NO synthase unmasks these effects. Therefore, rapid nongenomic aldosterone effects increase renal vascular resistance and thereby mediate arterial hypertension if endothelial dysfunction is present.[314] Evidence suggests that there is a nonclassical membrane-bound aldosterone receptor.[315] These data come from kinetic studies that demonstrate saturable, radiolabeled binding of aldosterone to cell surface membranes that have kinetics compatible with physiologic activity.[316,317] In some cell lines, aldosterone can have very rapid physiologic effects that are not blocked by inhibitors of cell transcription and translation. For instance, in human mononuclear leukocytes, aldosterone can stimulate release of IP_3 or calcium within 30 seconds of exposure.[318,319] These membrane-bound receptors may explain some of the effects of aldosterone that occur prior to gene transcription, such as early stimulation of

sodium reabsorption[320] or early stimulation of salt intake,[321] possibly via phospholipase C/PKC signaling pathways.

Mineralocorticoid Actions in the Kidney

Aldosterone originates primarily from the zona glomerulosa of the adrenal glands. Some recent studies have suggested that the heart, vasculature, and brain can also synthesize aldosterone in response to local tissue injury, although extraadrenal synthesis in humans is still debated.[6–8,11,17,322] It is the chief mineralocorticoid of the body. Its physiologic role as a regulator of sodium and volume balance also allows aldosterone the potential to play a pathologic role in the development of hypertension in patients with renal disease.[322] It is also implicated in the pathophysiology of cardiac fibrosis and cardiac hypertrophy in end-stage heart failure.[3,4,323]

The major action of aldosterone in the kidney is regulation of Na, K, and H handling by the distal part of the nephron. Mineralocorticoid deficiency is associated with volume depletion, hyperkalemia, and mild metabolic acidosis. Conversely, mineralocorticoid excess leads to Na retention, hypokalemia, and metabolic alkalosis.

Ang II, high serum K^+ levels, and ACTH stimulate aldosterone secretion from the adrenal gland.[324] ANP and dopamine, on the other hand, suppress aldosterone secretion. Dietary sodium also modulates aldosterone release through its effects on the renin-angiotensin system. In addition to sodium and volume homeostasis, other triggers for aldosterone release have been cited, including hyperglycemia, adrenocorticotropic hormone (ACTH), and, more importantly in patients with CKD, angiotensin II and potassium.[49]

Sodium Reabsorption

One of the best-documented functions of aldosterone is its ability to increase Na reabsorption in the distal tubule and collecting duct.[325–327] The rate-limiting step to sodium reabsorption across tight epithelia is the permeability of the apical membrane of the transporting cell. Aldosterone increases apical Na permeability of tight epithelia, such as those found in the mammalian distal tubule and descending colon, by increasing the activity of the amiloride-sensitive epithelial sodium channel (ENaC). ENaC is formed by three subunits (alpha, beta, and gamma) and, based on coexpression studies in Xenopus oocytes, these subunits assemble into a complex heterooligomer forming the amiloride-sensitive pore.[328–330]

Other characterized aldosterone-induced targets include the serum and glucocorticoid-regulated kinase-1 (SGK-1), an important mediator of renal sodium homeostasis; corticosteroid hormone-induced factor (CHIF), which regulates the activity of the sodium and potassium-dependent adenosine triphosphatase pump (Na-KATPase); the glucocorticoid-induced leucine zipper protein; a transcription factor; and the G protein K-Ras2.[10–12] SGK-1 is thought to regulate Na^+ flux by increasing ENaC activity at the apical surface of epithelial cells. Aldosterone treatment of a rat collecting duct cell line as well as an adrenalectomized rat model demonstrated a rapid induction of SGK-1 mRNA within 30 minutes of treatment.[13,14] However, SGK-1 null mice only show mild abnormalities in sodium homeostasis, suggesting that other genomic targets are important for overall regulation of sodium transport.[15] Wong et al. demonstrated that ET-1 is a direct aldosterone gene target in the kidney and colon in Sprague dawly rats and may play an important role in aldosterone-regulated ion homeostasis.[323]

Aldosterone also enhances Na reabsorption by increasing Na–K-ATPase activity in basolateral membranes of principal cells in mammalian collecting duct and distal tubule.[327] Studies in toad bladder and mammalian nephron suggest that aldosterone upregulates Na–K-ATPase activity by at least three mechanisms: increased Na influx due to opening of amiloride-sensitive Na channels, induction of Na–K-ATPase subunit expression at the gene level, and induction of intracellular alkalosis, which occurs in tissues that contain aldosterone-sensitive Na/H exchangers.[331] Other hormones also modulate aldosterone's action on Na transport; for example, ANP is inhibitory and vasopressin is stimulatory (Table 8.3).[332]

Hypersecretion of endogenous mineralocorticoids or the administration of mineralocorticoids lead to transient sodium retention followed by a return to Na balance within a few days.[333] The return to Na balance despite elevation of circulating mineralocorticoid levels is referred to as aldosterone or mineralocorticoid "escape." During mineralocorticoid escape, increased Na reabsorption by the distal tubule and collecting duct remains unchanged, but is offset by decreased Na reabsorption in other nephron segments.[332,334] The latter results from increased renal arterial pressure and elevated plasma ANP levels, both of which suppress proximal tubule transport of Na. Mineralocorticoid escape is also, in part, mediated by decreased Na and water reabsorption in the loop of Henle.[334] Other factors, such as TGF-β and interleukin-1 (IL-1), may play a role in regulation of mineralocorticoid escape. These factors have been recently found to inhibit the action of aldosterone on the cells of the IMCD.[335]

Ang II binds to Ang II type 1 (AT-1) receptors in the kidney, which leads to glomerular hypertension, sclerosis, renal fibrosis, and cardiac remodeling, possibly through a TGF-β-mediated pathway.[30–32] ACE inhibitors provide renoprotection by inhibiting the conversion of Ang I to Ang II and precluding the negative effects of AT-1 receptor activation. Studies have shown that Ang II can be generated by ACE-independent pathways such as chymase in the heart,[33] which leads to "angiotensin escape." Angiotensin escape is one of the factors frequently cited to explain aldosterone breakthrough, which may be secondary to the generation of non-ACE mediated angiotensin II.[34] Potassium and adipocyte-released factors may also contribute to the phenomenon of aldosterone breakthrough. Continual dietary intake of potassium in the setting of reduced GFR may promote elevated potassium levels that subsequently trigger aldosterone secretion, as seen in the rat remnant kidney model.[28] ACE inhibitors and ARBs are also known to reduce

potassium excretion, so prolonged use of such agents may enhance potassium retention and predispose a patient to aldosterone breakthrough. Recent studies in rat models of metabolic syndrome with early nephropathy have shown enhanced aldosterone secretion due to adipocyte activity that was not abolished by candesartan administration.[35] These results suggest that adipocyte-released factors outside of Ang II may enhance aldosterone secretion and lead to increased proteinuria and podocyte injury in rats.[35] Thus, potassium, angiotensin II, and adipocyte-released factors may all contribute to the increase in aldosterone secretion in patients on prolonged ACE inhibitors or ARB therapy. The exact definition of aldosterone breakthrough has been a subject of controversy, as there is no current consensus on its precise definition. One of the common definitions is a rise in plasma aldosterone concentration, often past baseline values, following an initial decrease after the initiation of ACE inhibitor or ARB therapy.[322]

Potassium Secretion

Mineralocorticoids are the predominant hormonal influence on K secretion by principal cells of the collecting duct and connecting segment of the distal tubule.[335,336] Although mineralocorticoids always increase K secretion by these nephron segments, this does not necessarily translate into a kaliuresis because of the strong dependence of K excretion on distal Na delivery and urinary flow rate.[337] For example, in conditions of decreased Na delivery and urinary flow to the distal nephron, the kaliuretic effect of aldosterone is either diminished or abolished. The mechanisms by which aldosterone stimulates K secretion by principal cells overlap with those responsible for its Na-retaining action. The late distal convoluted and connecting tubules (CNTs) and cortical collecting duct (CCD) of the distal nephron mediate, in large part, the final regulation of urinary K^+ excretion.[19] The traditional model by which K^+ secretion is accomplished in these segments can be summarized as follows. Na^+ enters the CNT and principal cell from the urinary fluid through the apical amiloride-sensitive ENaC and is then transported out of the cell at the basolateral membrane in exchange for uptake of K^+ via the basolateral Na^+-K^+-ATPase. The high K^+ concentration within the cell and lumen-negative voltage, established by electrogenic Na^+ reabsorption, create a favorable electrochemical gradient for K^+ to diffuse into the urinary space through apical K^+-selective channels. Thus vectorial K^+ secretion in these segments requires a favorable electrochemical gradient and an apical permeability to K^+ increases in extracellular K^+ concentration directly stimulate aldosterone production in zona glomerulosa cells of adrenal glands.[6,59,338]

Aldosterone-induced Na influx through the apical membrane leads to the generation of a lumen-negative potential difference that favors K secretion.[326,327] In addition, although mineralocorticoids do not increase the density of active K channels in the apical membrane, they increase the conductance of apical and basolateral K channels independent of Na flux.[327,339] Physiologically, it is difficult to understand how one hormone can regulate the concentration of two different solutes that have varying levels of dietary intake. Patch-clamp experiments have demonstrated that other nonaldosterone circulating factors exist that can regulate K channel activity. Infusion of aldosterone by osmotic minipump will increase the density of EnaC, but not of K channels.[339] However, an increase in dietary K does increase the K-channel density.[340] Currently, it is unknown what other circulating factor controls K secretion.

A large body of evidence suggests that SGK1 mediates, at least in part, the effect of aldosterone on renal K secretion.[41,88,113,116] This notion is supported by studies performed in SGK1 knockout mice demonstrating that the phenotype of SGK1 deletion is similar to MR knockout mice and displays impaired renal K secretion in response to high dietary K intake.[41,341]

Renal Acidification

The role of mineralocorticoids in regulation of renal acidification is supported by several clinical observations. Syndromes of aldosterone deficiency are associated with metabolic acidosis because of reduced urinary acid excretion, whereas mineralocorticoid excess results in metabolic alkalosis. Aldosterone enhances urinary acidification through direct actions on epithelial cells in the collecting duct and indirectly by influencing various intrarenal and extrarenal factors.[342] Aldosterone increases H secretion by type A intercalated cells in the collecting duct via two mechanisms: direct stimulation of the proton pump (H-translocating ATPase), and indirectly by stimulating Na influx, which creates a lumen-negative potential difference.[342] As with K, the overall effect of aldosterone on renal acid excretion depends on Na delivery to the distal part of the nephron. Reduction of Na transport in the collecting duct, because of either decreased Na delivery or inhibition of distal Na reabsorption by amiloride, significantly attenuates the effect of aldosterone on net H excretion.[343] Aldosterone regulates the expression of aquaporin, AQP3, with no effect on AQP1 and AQP2, in the collecting duct, independently of Na intake.[344]

Effect on Inflammation

Besides its classical effects on salt homeostasis in renal epithelial cells, aldosterone promotes inflammation and fibrosis and modulates cell proliferation. As outlined by recent reports, mineralocorticoid also mediates inflammation and fibrosis through NF-κB activation in liver, heart, and glomerular mesangial cells[9–13] via a pathway involving the aldosterone early-induced gene, serum, and glucocorticoid-induced kinase 1 (SGK1).[11,12,345]

Studies in vitro revealed that aldosterone could induce proliferation of rat glomerular mesangial cells and promote collagen gene expression and synthesis via activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) mitogen-activated protein (MAP) kinase in renal fibroblast. Aldosterone

treatment of renal tubular epithelial cells increases calcium inflow and intracellular cyclic adenosine monophosphate levels. The results suggest that aldosterone plays a pivotal role in tubulointerstitial fibrosis by promoting tubular epithelial–mesenchymal transition and collagen synthesis in proximal tubular cells. The process is MR-dependent, and mediated by ERK1/2 mitogen-activated protein kinase pathway.[346]

Clinical trials have shown a potential role for MR blockers to further delay the development of end-stage renal disease by completing renin–angiotensin blockade. MR blockade produces a significant antiproteinuric effect and has minimal risk of causing hyperkalemia if the condition of the patient is closely monitored. As well as in the collecting ducts, mineralocorticoid receptors are distributed throughout the body, including the proximal tubule, thick ascending segment of the nephron, the heart, and the brain.[14,20,21,31] Aldosterone is thought to mediate fibrosis by activating factors such as reactive oxygen species; TGF-β; and increasing collagen synthesis, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and plasminogen activating factor I (PAI-1).[7,20,22,23] The fibrotic effect of aldosterone is often enhanced by high dietary salt intake.[24] Fibrosis and kidney damage induced in uninephrectomized rats placed on a high-salt diet and treated with angiotensin II were reduced by treatment with spironolactone and an aldosterone synthase inhibitor, FAD286.[32,322]

Quan et al. studied chronic kidney disease in rats subjected to subtotal nephrectomy.[37] Rats with intact adrenal glands were found to have increased proteinuria, renal histopathologic changes, and decreased inulin clearance when compared with rats subjected to adrenalectomy. Furthermore, the difference in the severity of kidney disease between rats that had undergone adrenalectomy and those that had not was not abolished with corticosteroid administration, which suggested that some other factor from the adrenal gland was responsible for the renal defects.[322]

Experiments conducted on saline drinking, stroke prone, spontaneously hypertensive (SHRSP) rats have shown that inhibition of mineralocorticoid receptor activation may delay renal disease. Spironolactone, a mineralocorticoid receptor blocker, implanted into SHRSP rats results in a reduction in proteinuria. The expression of TGF-β, NADPH oxidase, and collagen I/IV was increased in the untreated diabetic rats, but was reduced in spironolactone-treated rats. Aldosterone was found to increase collagen expression in rat renal fibroblasts, which may contribute to fibrosis.[20] Studies of humans have also shown that the deleterious effects of aldosterone are enhanced by high dietary salt intake, which is typical of the Western diet. A study involving 2,700 participants in the Framingham Offspring Study has shown that greater excretion of urinary sodium (a marker for dietary salt intake) correlates strongly with elevated urinary albuminuria and weakly (albeit nonlinearly) with serum aldosterone levels.[52] In a subsequent study conducted on patients with resistant hypertension despite the use of three antihypertensive medications, elevated dietary salt intake correlated with higher levels of proteinuria in patients with elevated urinary aldosterone levels.[53] This suggests that aldosterone may work in concert with dietary salt to accelerate kidney injury.[322] Studies designed to delineate the factors responsible for the renal injury associated with aldosterone have also been performed in humans. Sato and Saruta measured the urinary excretion of collagen type IV (a turnover product of fibrosis) by patients with diabetes and found that those on spironolactone showed a significant decrease in urinary collagen excretion, which suggests that collagen may be one factor associated with aldosterone-mediated fibrosis.[57] Because aldosterone is a major regulator of sodium and volume balance, it could also, theoretically, mediate its negative effects by increasing blood pressure.[322]

Glucocorticoid Actions in the Kidney

Glucocorticoids appear to be important for the normal maintenance of GFR.[347] In both adrenalectomized animals and in humans with adrenal insufficiency, GFR is reduced compared to controls. Adrenalectomized rats given physiologic doses of glucocorticoids regain a normal GFR.[348] Furthermore, short-term administration of pharmacologic doses of glucocorticoids has been reported to increase inulin clearance in both normal animals and humans.[349,350] Micropuncture studies in normal rats indicate that glucocorticoids enhance GFR by increasing glomerular plasma flow.[347,351] The latter results from selective vasodilation of both afferent and efferent renal arterioles.[351] The mechanisms by which glucocorticoids alter the glomerular microcirculation remain obscure. Because amino acid infusion causes similar glomerular hemodynamic changes, Baylis and colleagues suggest that glucocorticoids may increase GFR through their effects on catabolism of proteins to free amino acids.[347]

Glucocorticoid actions on the proximal tubule include enhancement of gluconeogenesis, ammoniagenesis, and Na reabsorption.[352] Lag in ammonium excretion resulting in acid retention is well described in subjects in a glucocorticoid-depleted state.[353] In adrenalectomized animals, glucocorticoid replacement restores proximal tubule ammoniagenesis and the ability of the kidney to respond to the chronic phase of acidosis.[354] Furthermore, in whole animals, glucocorticoid excess accelerates renal base generation, resulting in metabolic alkalosis.[355] Glucocorticoids regulate ammoniagenesis possibly through altering glutamine uptake and metabolism by proximal tubule cells.[355] Glucocorticoids increase proximal tubule Na reabsorption by at least two mechanisms: enhanced Na–K-ATPase activity and Na–H exchange.[356,357] Several experiments suggest that glucocorticoids inhibit Na-dependent phosphate and sulfate reabsorption in the proximal tubule.[352] These observations are supported by clinical reports of phosphaturia and lower serum phosphate levels in patients with Cushing disease and subjects given high doses of glucocorticoids.[358]

Both patients with Addison disease and adrenalectomized animals have decreased urinary concentrating ability,[359] due in part to reduction in RBF, GFR, and hydroosmotic

permeability of the collecting tubule.[360] In addition, adrenal corticosteroids contribute to urinary concentration by stimulating Na, K, and HCO_3 transport in the thick ascending limb of Henle's loop.[361] It is not entirely clear whether glucocorticoids, mineralocorticoids, or both mediate the effects of corticosteroids on renal-concentrating mechanisms.

Systemic excess of glucocorticoids is known to cause hypertension. The effect of glucocorticoids may be in part mediated by the suppression of endothelial nitric oxide synthase (eNOS).[2] Acute administration of glucocorticoids, however, may have beneficial effects on the cardiovascular system in part through nontranscriptional activation of eNOS.[308]

CATECHOLAMINES

Structure and Biosynthesis of Catecholamines

Norepinephrine (noradrenaline), epinephrine (adrenaline), and dopamine are collectively called catecholamines. These endogenous amines are derived from the amino acid tyrosine and the enzymes involved in their biosynthesis have been identified, cloned, and characterized[362] and include: tyrosine hydroxylase, dopa decarboxylase, dopamine β-hydroxylase, and phenylethanolamine-N-methyltransferase. The first step in catecholamine synthesis involves the hydroxylation of tyrosine by tyrosine hydroxylase, which is regarded as a rate-limiting step. This enzyme is activated by stimulation of sympathetic nerves or the adrenal medulla and is subject to feedback inhibition by catechol compounds. Catecholamines are synthesized in nerve terminals of the postsynaptic neurons of the sympathetic nervous system and in the adrenal medulla. Most of the steps occur in the cytoplasm whereas some take place in storage vesicles.[363] Storage of catecholamines in vesicles ensures their regulated release, protects them from enzymatic degradation, and prevents their leakage outside the cell. Termination of action of norepinephrine and epinephrine includes reuptake by nerve terminals, reuptake by nonneuronal cells, and metabolic transformation. Major enzymes involved in catecholamine metabolism include monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). The main source of epinephrine in the body derives from the adrenal medulla whereas noepinephrine originates mostly from nerve terminals.[363] At the renal level, catecholamines derive from renal efferent nerves (norepinephrine and, to a lesser extent, dopamine), from the circulation (epinephrine and norepinephrine), from the adrenal medulla (epinephrine), and from renal proximal tubule cells (dopamine) via α_1, α_2, β_1, and β_2 receptors for epinephrine and norepinephrine and D_1- and D_2-like receptors for dopamine.[364–367]

Physiology of Catecholamine Action in the Kidney

Catecholamines play an important role in the regulation of RBF, GFR, renin secretion, and tubular transport. Their effects depend on site of action and receptor type. The kidney is one of the major long-term regulators of blood pressure (BP). This is achieved by the interplay of several hormonal, neural, and humoral factors that would regulate principally two parameters: sodium homeostasis and peripheral vascular resistance.[368] In the following section, we discuss the renal functions of catecholamines separately on their receptors.

Alpha-adrenergic Stimulation in the Kidney

Both α_1- and α_2-adrenergic receptors have been localized to vascular smooth muscle and tubule cells of the nephron. Norepinephrine is the major agonist at these levels and adrenergic stimulation causes renal arteriolar vasoconstriction (increased afferent and efferent arteriolar resistance) by activating mainly α_1 receptors on vascular smooth muscle cells.[369] This α_1-mediated renal vasoconstriction results in decreased RBF and GFR. Recent evidence suggested that norepinephrine increases afferent arteriolar sensitivity to angiotensin II through activation of α receptors and secondary increase in calcium sensitivity of mouse afferent arteriole.[370]

In the proximal convoluted tubule, where α_1- and α_2-adrenergic receptors are expressed in high density, norepinephrine increases Na and water reabsorption, in part, by stimulation of Na^+-K^+-ATPase activity.[371–373] This activity is partially dependent on the cosecretion of neuropeptide Y that acts to synergize the stimulatory α-adrenergic effects of norepinephrine and to antagonize the inhibitory β-adrenergic effects. This is demonstrated by the fact that norepinephrine alone does not affect Na^+-K^+-ATPase activity in the proximal convoluted tubule unless neuropeptide Y or other β-adrenergic inhibitors are present.[374] In isolated rat and rabbit proximal convoluted tubule cells, α_1 and α_2 agonists stimulate Na^+–H^+ exchange, the overall effect of which is enhanced Na^+ and fluid absorption.[375,376]

In the loop of Henle, experimental evidence showed that α-adrenergic stimulation increases sodium and water reabsorption at the thick ascending limb level through α_1- and α_2-mediated activation.[377,378] In the collecting duct, Krothapalli and Suki[379] report that α_2 agonists inhibit vasopressin-stimulated water reabsorption by inhibiting adenylate cyclase activity.[379] Other investigators, however, challenge this observation.[380]

Beta-adrenergic Stimulation in the Kidney

β-adrenergic receptors have been identified in the glomerulus, juxtaglomerular apparatus, thick ascending limb of loop of Henle, distal convoluted tubule, and collecting duct.[380,381] β_1 stimulation enhances renin release from the juxtaglomerular cells of the afferent arterioles. Otherwise, there are few β receptors in renal vessels. Although β receptors have not been localized to the proximal tubule, physiologic studies suggest that β-adrenergic stimulation increases Na^+ and fluid transport in this nephron segment independently of enhanced renin secretion and angiotensin II production.[380] In the thick ascending limb, β-adrenergic receptor activation stimulates cAMP production and NaCl reabsorption.[380] β agonists also

increase Cl^-–HCO_3^- exchange and H^+-K^+-ATPase activity in the collecting duct.[371] The latter effect results in enhanced K^+ reabsorption by type A intercalated cells (and an apparent decrease in K^+ secretion).[371] A potential mechanism of action involves β-adrenergic stimulation of cAMP production and subsequent conversion to adenosine.[381]

Dopamine and the Kidney

Dopamine is the immediate metabolic precursor of norepinephrine and epinephrine. Locally, dopamine is synthesized by proximal tubule cells via enzymatic decarboxylation of L-dopa by aromatic amino acid decarboxylase (AADC). L-dopa reaches the tubule cell after filtration and Na^+-coupled reabsorption because renal proximal tubule cells lack tyrosine hydroxylase. The contribution of presynthesized and stored dopamine to local renal physiology is not yet clearly established.[368,382] High salt intake increases AADC activity and dopamine synthesis in proximal tubule possibly by enhancing Na^+-coupled uptake of L-dopa.[383,384] Regulation of intrarenal dopamine concentrations can occur at several levels: synthesis, storage, or degradation. Dopamine is a substrate for both MAO and COMT. Synthesis and metabolism of dopamine differs between neural and nonneural cells. Dopamine synthesized in the proximal tubule does not undergo further metabolism to norepinephrine and epinephrine because of the lack of dopamine β-hydroxylase[385]; instead, it diffuses to peritubular space and tubular lumen where it acts locally on its receptors.

Dopamine receptors are members of the G protein-coupled superfamily of heptahelical receptors. At least five receptors have been identified, subclassified into D_1- and D_2-like subfamilies based on their molecular structure and pharmacology. Both of the cloned members of the D_1-like receptor group (D_1 and D_5, also known as D_{1A} and D_{1B} in rodents) are coupled with the stimulating G protein, $G_s\alpha$, and stimulate adenylyl cyclase. All three of the cloned D_2-like receptors (D_2, D_3, and D_4) are associated with the inhibitory G protein, G_i/G_0, and inhibit adenylyl cyclase. Both families of receptors are expressed in the kidney. D_1 and probably D_5 are localized in the smooth muscle layer of renal arterioles, juxtaglomerular cells, proximal tubules, and cortical collecting duct. The D_3 receptor is present in arterioles, glomeruli, proximal tubules, MTAL of loop of Henle, and the collecting duct. The D_4 receptor is localized in the cortical collecting duct.[386,387]

Locally produced dopamine plays a central role in the regulation of sodium excretion. Circulating dopamine concentrations are in the picomolar range, hence not sufficiently high to activate dopamine receptors. The major signaling mechanism by which dopamine induces natriuresis is by inhibiting Na^+-K^+-ATPase in all the segments of the nephron via D_1-like receptors, whereas D_2-like receptors stimulate this enzyme. The end result of Na^+-K^+-ATPase inhibition is a dopamine-induced natriuresis. Several investigators suggest that the role of dopamine is to counterbalance the effects of antinatriuretic factors in the kidney.[383] Interestingly, Kuchel and Kuchel[388] point out that dopamine is the predominant catecholamine in fish, in which salt excretion is a priority. On the other hand, norepinephrine predominates in terrestrial animals, in which salt retention is essential for survival. In addition to inhibition of Na^+-K^+-ATPase activity, other studies suggest that dopamine suppresses Na^+–phosphate cotransporter, antagonizes the stimulatory effect of Ang II on Na^+–H^+ exchange in cortical brush-border membranes mediated by cAMP and PKA,[389,390] and stimulates renin synthesis in cultured rat juxtaglomerular cells.[391] Studies in humans have shown that dopamine does not induce natriuresis in Na^+-depleted subjects and that its natriuretic effect is more pronounced during conditions of volume expansion.[368,392] This demonstrates again that the natriuretic and diuretic effects of D_1-like receptors are dependent on sodium balance.

In the whole kidney, dopamine increases RBF and GFR through its D_1 receptor-mediated vasodilatory effects.[383,392] Supraphysiologic concentrations of dopamine, however, stimulate α-adrenergic receptors, which lead to vasoconstriction and decreased RBF. The natriuretic and vasodilating effects of dopamine have suggested a therapeutic role in patients with volume expansion, particularly when administered in low doses that do not activate adrenergic receptors. However, several studies have shown that dopamine has no major role as a therapeutic strategy in the prevention of further renal damage in acute kidney injury.[393,394] Moreover, dysfunction of the renal dopamine system has been postulated to contribute to the pathogenesis of systemic hypertension.[368,383] Results from at least two studies suggest that defects in renal generation of dopamine are common in patients with essential hypertension.[395,396]

The ability of the D_1 receptor to induce both natriuresis and vasodilation makes D_1 agonists, such as fenoldopam, potential therapeutic agents for the treatment of both hypertensive urgencies and acute renal failure. In healthy normotensive volunteers, fenoldopam has been shown to significantly increase renal plasma flow while only minimally reducing systemic blood pressure.[397] In people with hypertensive urgencies, fenoldopam has been shown to reduce systemic blood pressure by 23% while increasing natriuresis by 200%, diuresis by 46%, and renal blood flow by 42%.[398] Although the selective increase in renal plasma flow could be advantageous in the treatment of certain forms of acute renal failure, further studies must be done to define the specific utility of fenoldopam in this setting.

THE RENAL KALLIKREIN-KININ SYSTEM

In 1909, Abelous and Bardier reported for the first time the hypotensive effect of human urine when injected into the bloodstream of dogs.[399] Further studies by Werle and colleagues from 1926 to 1939 attributed these effects to the kallikrein-kinin system (KKS) and described its basic components: kallikreins, kinins, kininogens, and kininases. The

major actions of this system are mediated by bradykinin, a peptide hormone that exerts potent proinflammatory and vasodilatory effects. Interestingly, all components of the KKS are also expressed in the kidney, especially in the distal convoluted and connecting tubule as well as in the collecting duct, and have been shown to regulate renal hemodynamic and tubular function. In addition, in the last few years, studies have linked the KKS to different pathologic states including the diabetic nephropathy as reviewed here.

Structure and Synthesis of Kinins

The KKS is a complex multienzyme system that can be divided into two types: (1) a circulating KKS that belongs to the coagulation system and (2) a tissue KKS that acts in a paracrine or autocrine fashion. The KKS leads to the production of kinins, namely bradykinin and lys-bradykinin (kallidin), from kininogens through the action of kininogenase, namely kallikrein (Fig. 8.5).[399] Two types of kallikreins have been identified—a plasma and a tissue kallikrein—both of which are serine proteases that are encoded by different genes and differ in their distribution and regulation.[399] Mice lacking tissue kallikrein show dramatically reduced levels of renal kinin, suggesting that tissue kallikrein is the main system involved in the kidney,[400] although little is known regarding the putative role of plasma kallikrein under pathologic conditions. Renal tissue kallikrein is synthesized in large amounts by connecting tubule cells and is mainly secreted into the urinary fluid and to a lesser extent to the peritubular interstitium.[399]

In humans, two types of kininogens have so far been described: a high molecular weight (HK) form present in blood and a low molecular weight (LK) form present in various tissues. It is generally accepted that tissue kallikrein prefers LK but is capable of cleaving HK, whereas plasma kallikrein cleaves HK exclusively.[399] Kininogens are synthesized in the liver and circulate in blood plasma at concentrations of 45 to 120 μg per mL for LK and of 65 to 115 μg per mL for HK, but are also found in other body fluids and organs such as kidney.[401]

Novel functions of kininogens have been recently discovered. Derivatives of HK have been shown to be involved in the regulation of endothelial cell proliferation, angiogenesis, and apoptosis.[402] In addition, some seem to possess potent and broad-spectrum microbicidal properties against both gram-positive and gram-negative bacteria, and thus may represent an alternative to conventional antibiotic therapy.[402]

Physiology of Kallikrein-Kinin System in the Kidneys

Tissue kallikrein is secreted by many cells throughout the body but some tissues produce particularly large quantities such as the kidney, lung, intestine, brain, and glandular tissues (salivary and sweat glands and pancreatic exocrine gland). This enzyme is activated intracellularly from a precursor, prokallikrein, to produce tissue kallikrein.[403] The enzyme responsible for this conversion has not yet been identified.

Regulatory mechanisms of tissue kallikrein remain partly unknown. Aprotinin, a polypeptide purified from the lung, and kallistatin have been shown to inhibit the activity of renal and other tissue kallikreins.[404] In addition, it has been shown that salt restriction stimulates the synthesis of renal kallikrein through an unclear mechanism, possibly implicating aldosterone.[405] Interestingly, it was also recently shown that potassium intake triggers an increase in renal kallikrein secretion through an aldosterone-independent mechanism

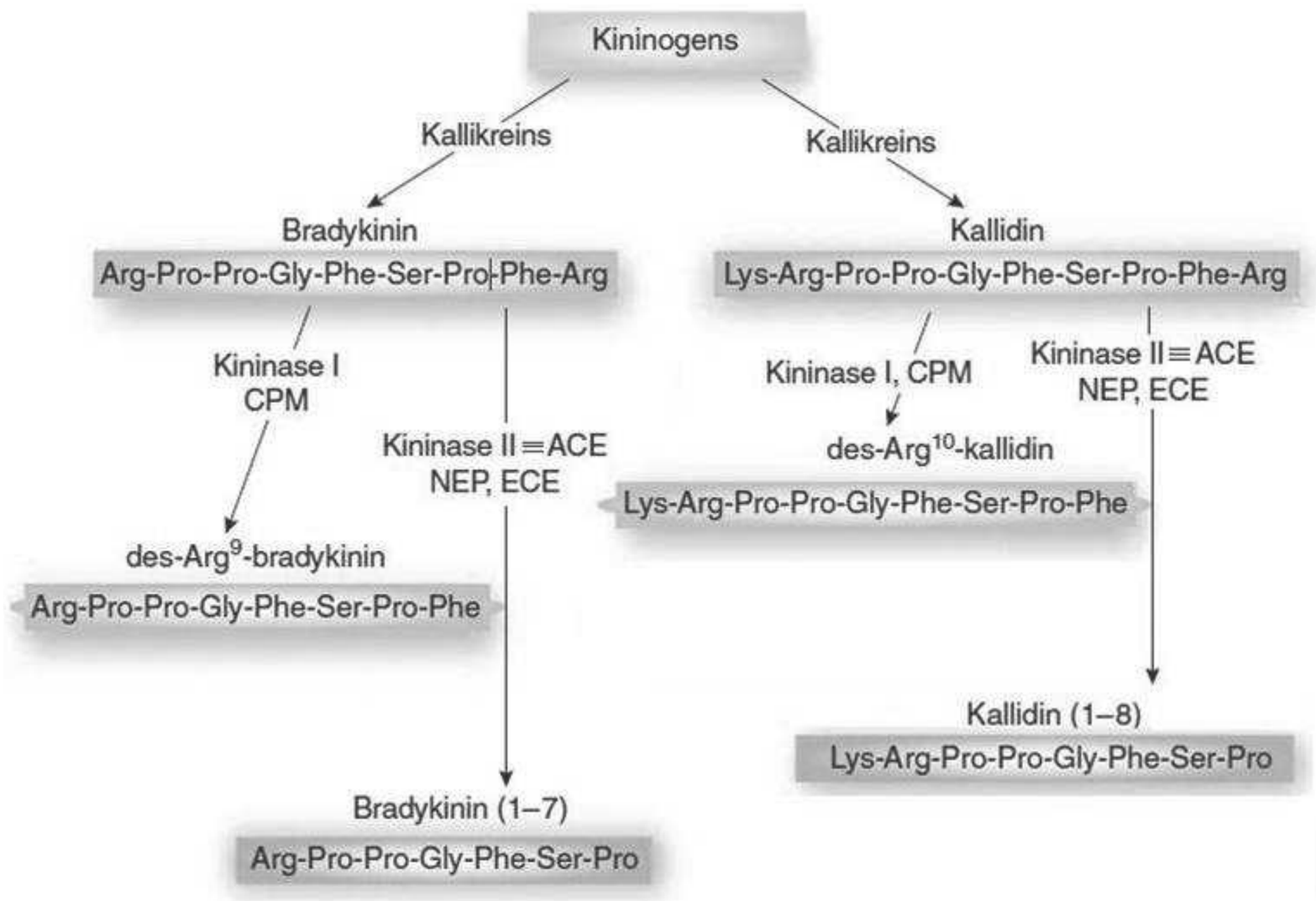

FIGURE 8.5 Biosynthesis and metabolism of kinins. *CPM*, carboxypeptidase-M; *ACE*, angiotensin I-converting enzyme; *NEP*, neprilysin (endopeptidase 24.11); *ECE*, endothelin-converting enzyme; *red*, active peptides; *blue*, inactive peptides. (Adapted from Kakoki M, Smithies O. The kallikrein-kinin system in health and in diseases of the kidney. *Kidney Int.* 2009;75(10):1019–1030.) (See Color Plate.)

involving membrane depolarization of kallikrein-secreting cells in the renal connecting tubules, followed by enhanced calcium influx.[406,407] In addition to dietary sodium and potassium intake, hereditary factors may determine tissue kallikrein activity. In fact, a loss of function polymorphism in the human tissue kallikrein gene (R53H) has been identified with a frequency of 0.03 in Caucasians.[408] Interestingly, these partially tissue kallikrein-deficient subjects develop a form of arterial dysfunction characterized by remodeling of the brachial artery, which is not adapted to a chronic increase in wall shear stress.[408]

Although of little relevance for the kidney, it is noteworthy that plasma kallikrein is regulated through a different, more complex, mechanism. Plasma kallikrein is synthesized and secreted by the liver as an inactive zymogen. It is activated by the intrinsic coagulation cascade whereby contact of plasma with negatively charged macromolecular surfaces initiates a proteolytic cascade that ultimately converts prekallikrein to plasma kallikrein.[409]

The half-life of bradykinin in plasma is short (~30 seconds), suggesting that its actions are regulated locally through its production and degradation within tissues. In fact, both bradykinin and kallidin can be metabolized through two pathways.[410] Kininase I (also called carboxypeptidase-N), as well as carboxypeptidase-M, remove the C-terminal arginine from the kinins to generate their des-Arg derivatives, which are agonists of B1R (see later). Kininase II (also known as ACE), neprilysin (endopeptidase 24.11), and endothelin-converting enzyme cleave off the two C-terminal amino acids (Phe and Arg) of the kinins, thereby inactivating them.[411,412] It is noteworthy that kallidin can be converted into bradykinin by a plasma aminopeptidase.

Kinins exert their biologic effects by acting on two types of G protein-coupled receptors called bradykinin B1 receptor (B1R) and B2 receptor (B2R) (Fig. 8.6). Although B2R is ubiquitous and expressed throughout the kidney, B1R is mainly expressed after induction by endotoxin, cytokines, ischemia, and other noxious stimuli.[411] Interestingly, treatment with an inflammatory signal (lipopolysaccharide) induces the expression of B1R mRNA in all renal segments except the outer medullary collecting ducts. B1R, once induced by inflammatory mediators and tissue damage, assumes some of the physiologic roles of B2Rs.[413] Bradykinin and kallidin are equipotent and both have higher affinity for B2R. Interestingly, metabolites (des-Arg9-BK [DABK] and Lys-DABK) of bradykinin and kallidin, which result from the action of carboxypeptidases, have higher affinity for B1R.

Both receptors activate similar intracellular signaling cascades involving phospholipase C activation and intracellular calcium mobilization. In addition, through calcium-dependent and -independent mechanisms, KKS increases NO and prostaglandin synthesis, both of which seem to mediate some of the effects of kinins.[410] Alternative mediators of kinins also include endothelium-derived hyperpolarizing factor (EDHF), norepinephrine, substance P, cytokines, and tissue plasminogen activator.[410]

Experimental evidence suggests that kinins regulate renal blood flow and renal excretion of sodium and water.[414,415] Studies on the role of the renal KKS, using congenitally kininogen-deficient Brown-Norway Katholiek rats and B2R knockout mice, amongst other models, revealed that this system starts to induce natriuresis and diuresis when sodium accumulates in the body as a result of excess sodium intake or aldosterone release, for example, by angiotensin II. Thus, it is hypothesized that the system works as a safety valve for sodium accumulation. In fact, mice lacking B2R and/or B1R develop salt-sensitive hypertension.[416] Interestingly, humans with essential hypertension have low levels of urinary

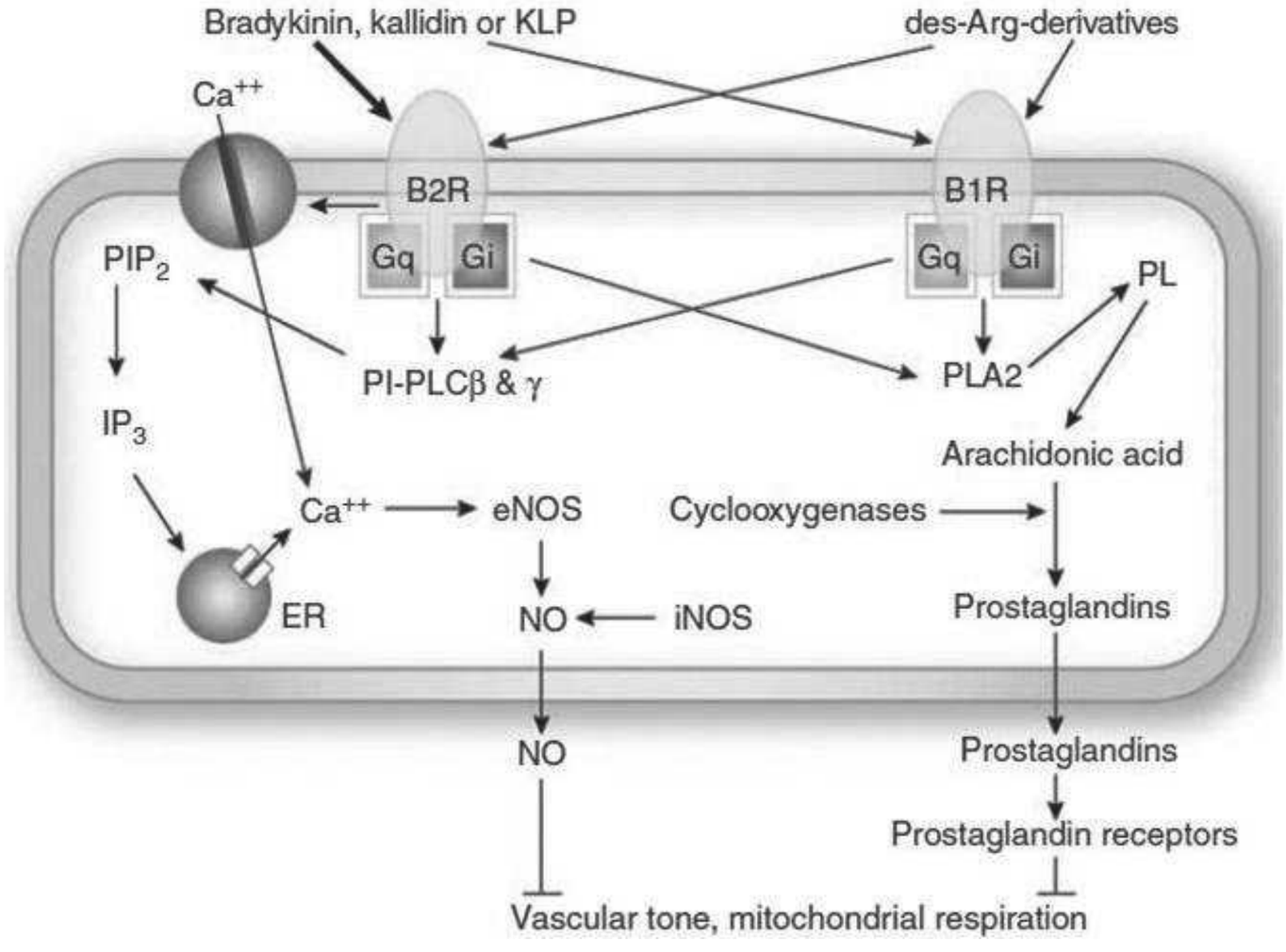

FIGURE 8.6 Bradykinin intracellular signaling cascade. The thickness of arrows arising from the kinins indicates the relative potency of each peptide to elevate intracellular calcium concentrations. *PIP_2*, phosphatidylinositol-4,5-bisphosphate; *PI-PLC*, phosphatidylinositol-specific phospholipase C; *IP_3*, 1,4,5-inositol triphosphate; *ER*, endoplasmic reticulum; *PL*, phospholipids; *PLA2*, phospholipase A2. (Reproduced from Kakoki M, Smithies O. The kallikrein-kinin system in health and in diseases of the kidney. *Kidney Int.* 2009;75(10):1019–1030.)

kallikrein.[405] Conversely, hypertensive mice and rats over-expressing the human kallikrein develop hypotension.[417] However, the renal KKS seems to be involved only in the long-term regulation of blood pressure under conditions of hypertensive insult such as high salt intake.[418] Evidence suggests that bradykinin promotes natriuresis, in part, by decreasing sodium reabsorption in the distal part of the renal nephron. Bradykinin has been shown to inhibit the amiloride-sensitive component of conductive sodium uptake in inner medullary collecting duct cells[419] and to cause a reversible inhibition of sodium reabsorption ex vivo in rat cortical collecting ducts.[420] Furthermore, inhibition of B2Rs in rats fed a normal salt diet decreases fractional sodium excretion with little effect on medullary and cortical perfusions or on GFR.[421] Recently, Zaika et al. confirmed that bradykinin inhibits ENaC in the aldosterone-sensitive distal nephron.[422] It is noteworthy that during volume expansion, a condition that requires further sodium reabsorption, at least one additional mechanism is activated: kinins mediate the increase in papillary blood flow, thereby indirectly inhibiting further tubular electrolyte reabsorption.[421]

The renal KKS is believed to operate in concert with the renin-angiotensin system to physiologically regulate the distribution of RBF and the excretion of water and electrolytes. It has been confirmed that the effects of kinins antagonize the effects of RAAS on blood pressure and sodium handling. In fact, reduced KKS activity may contribute to the establishment of a pathophysiologic state characterized by unopposed hyperactivity of the renin-angiotensin system, resulting in salt retention.[423] It has been shown that this reduced activity of the KKS predominates over renin-angiotensin system overactivity in all conditions of sodium balance in essential and family-related hypertension.[424] KKS and the renin-angiotensin system are functionally coupled at the level of ACE. Inhibition of this enzyme not only blocks angiotensin II production but also increases bradykinin production. It was shown that the beneficial effects of ACE inhibitors were mainly due to NO production following B2R activation.[425] Interestingly, chronic treatment with ACE inhibitors induces B1R expression in vasculature and in the kidney, which could mediate the renoprotective effects of ACE inhibition.[426]

Interestingly, the contribution of the KKS to renal handling of potassium has been recently addressed by Chambrey's group.[407] Usually, potassium is almost completely reabsorbed by the end of the loop of Henle and active secretion in the late distal nephron determines the fraction that needs to be excreted in the urine. Thus, except in a situation of potassium depletion, in which net potassium absorption also occurs in the collecting system, the latter is generally a site of net potassium secretion. It was recently shown that cortical collecting ducts isolated from mice with tissue kallikrein gene disruption do not secrete potassium but instead exhibit net transepithelial potassium absorption due to abnormal activation of H^+-K^+-ATPase.[407] This suggests that potassium absorption is constantly under the negative control of tissue kallikrein. However, the mechanism or signaling pathway by which tissue kallikrein controls H^+-K^+-ATPase remains to be determined.

In addition to sodium and potassium, the KKS seems to regulate the metabolism of calcium, through a B2R/B1R-independent mechanism. Picard et al. showed that knocking out tissue kallikrein in mice leads to hypercalciuria of renal origin.[427] In fact, the distribution of tissue kallikrein overlaps that of the epithelial calcium channel (TRPV5) in the distal nephron. Furthermore, kallikrein gene expression was increased by a low-calcium diet.[427] Interestingly, no such abnormalities were observed in B2R knockout mice with or without treatment with B1R antagonist. This suggests that the effect of kallikrein on calcium excretion is independent of kinin production.

Furthermore, products of the KKS may modulate cell growth. Experimental evidence suggests that while under certain conditions, in vitro bradykinin may inhibit growth of normal renal fibroblasts.[428,429] It can also stimulate the growth of fibroblasts, mesangial cells, and arterial smooth muscle cells in other conditions.[430] Over-expression in hypertensive Dahl salt-sensitive (DSS) rats of the human kallikrein gene by adenoviral delivery results in reversal of the changes associated with hypertensive nephrosclerosis.[430] Chao et al. further confirmed that kinin exerts its renoprotective effects against salt-induced renal injury in the DSS rat model by inhibiting cellular apoptosis, inflammatory cell recruitment, and fibrosis through suppression of oxidative stress, TGF-β expression, and MAPK activation, with no effect on blood pressure.[431] To a large degree, the benefits of kinins in that study were dependent on the elevated levels of renal NO.[431] NO has been shown to play an important role in protection against hypertension and glomerulosclerosis in DSS rats.[432] These renoprotective effects of NO may be attributed to a decrease in oxidative stress. In fact, NO can scavenge superoxide anions. It can also inhibit the assembly of a functional NADH/NADPH oxidase, thereby attenuating the production of superoxide.[433] In addition, given that oxidative stress can stimulate the expression of proinflammatory and profibrotic molecules, the increase in NO production triggered by kinins could not only decrease oxidative stress but consequently attenuate the inflammatory and fibrotic responses.[434] Tissue kallikrein has also been reported to attenuate salt-induced renal damage by activation of B2R.[435,436] The protective effect of the KKS against renal injury was also confirmed in B2R knockout mice.[437] In addition, a human B2R gene polymorphism has been demonstrated in patients with chronic renal failure, suggesting a role of this receptor in the early development of this pathology.[438]

Furthermore, as mentioned previously, bradykinin, through its receptors, increases prostaglandin production in at least three ways. First, it promotes translocation into the cell membrane and phosphorylation of cytosolic phospholipase A2 through a Ca^{2+}-dependent phosphorylation. Second, it activates membrane-associated phospholipase A2 through a Ca^{2+}-independent mechanism. Phospholipase A2 frees arachidonic acid from membrane phospholipids.

This arachidonic acid gets converted to prostaglandins by cyclooxygenase. The third mechanism by which bradykinin increases the production of prostaglandins is by inducing cyclooxygenase-2.[403,433] It has been shown that the prostaglandins formed following stimulation of the bradykinin receptors act through their specific receptors to mediate some of the effects of kinins on vascular tone and on mitochondrial respiration. Of potential relevance to the kidney, Kopp and Smith[439] demonstrated in a rat model of unilateral ureteral obstruction that indomethacin abolished BK-induced natriuresis and diuresis in the contralateral kidney.[439] Further studies are required to determine the role of bradykinin-induced prostaglandins in the healthy and diseased kidney, especially because renal diseases present with several characteristics including inadequate filtration of proteins and inflammatory cell recruitment.

Finally, recent evidence suggests that bradykinin participates in the regulation of neonatal glomerular function and acts as a growth regulator during renal development. An intact KKS is necessary for the normal functional development of the kidney.[440] During nephrogenesis, tubular and glomerular growth and differentiation, and acquisition of specialized functions, are coordinated in time and space with renal vasculogenesis, glomerulogenesis, and regional hemodynamic changes. The end result ensures that tubular structure and function are tightly coordinated with glomerular filtration during normal kidney development. To achieve this delicate task of glomerulotubular balance, the developing kidney produces growth factors and vasoactive hormones that act in a paracrine manner to regulate nephrovascular growth, differentiation, and physiologic functions. One such paracrine system is the KKS, which generates bradykinin. Bradykinin activates B2R to regulate RBF and salt and water excretion. Gene-targeting studies indicate that the fetal KKS plays an important role in the maintenance of terminal epithelial cell differentiation.[441] In fact, gestational salt loading induces abnormal renal development in B2R knockout mice.[442] It was also recently shown that B1R is unlikely to play a role in early nephrogenesis but its enrichment in the maturing proximal tubule suggests a potential role for this receptor in terminal differentiation of the proximal nephron.[443]

Clinical Pathophysiologic Role of the Kallikrein-Kinin System in the Kidneys

Diabetic nephropathy occurs in ~30% of all patients with diabetes. ACE inhibition is protective in many models of diabetic nephropathy, a protection partly mediated by the KKS. In fact, it has been shown in different diabetic models that the beneficial effects of ACE inhibitors were attenuated by a B2R antagonist.[444–447] The same findings were observed in diabetic mice lacking B2R.[448,449] Albuminuria, glomerular sclerosis, interstitial fibrosis, lipofuscin accumulation in proximal tubules, and lifespan shortening were enhanced in this model. Corroborating these findings, human tissue kallikrein gene delivery efficiently attenuated insulin resistance and prevented diabetic nephropathy in streptozotocin-induced diabetic rats.[450] Once again, NO seems to be mediating this renoprotective role. In fact, L-arginine, a substrate of NO synthase (NOS), reduces proteinuria associated with streptozotocin-induced diabetes.[451] Conversely, an NOS inhibitor as well as an endothelial NOS (eNOS) deficiency accelerates the severity of diabetic nephropathy.[452–455] Interestingly, cicaprost, a prostacyclin analog, also delays the pathogenesis of the diabetic nephropathy, suggesting that prostaglandins in addition to NO mediate the beneficial effects of the KKS.[456] It is noteworthy that some groups have reported that deletion of B2R protects against the streptozotocin-induced diabetic nephropathy.[457] In addition, it has been shown that glomerular kinin receptors are induced by diabetes and this may contribute to the development of glomerular injury and to the development of microvascular complications of diabetes.[458] Bradykinin also regulates the expression of connective tissue growth factor (CTGF), TGF-β receptor II, and collagen I in mesangial cells, which provides a mechanistic pathway through which B2R activation contributes to the development of diabetic nephropathy.[459] In addition, Christopher et al.[460] demonstrated that glucose by itself regulates the expression of B2R in vascular smooth muscle cells. These contradictory results may be attributed to the differences between strains of mice and/or the methods of induction of diabetes.

Furthermore, immune-mediated nephritis plays a major role in the pathogenesis of systemic lupus erythematosus (SLE) and Goodpasture syndrome, which is the experimental model of glomerulonephritis caused by antibodies against glomerular basement membrane (anti-GBM). Liu et al. recently showed that mouse strains with upregulated expression of renal and urinary kallikreins are less susceptible to anti-GBM antibody-induced nephritis and lupus nephritis.[461] In addition, administration of kallikreins decreased the anti-GBM antibody-induced nephritis, demonstrating a link between KKS and immune-mediated renal damage.[461] These findings corroborate the aforementioned results that attribute a renoprotective role for KKS in nephritis following insults such as salt imbalance and diabetes.

The KKS has also been shown to protect against aminoglycoside-induced renal injury. Aminoglycoside antibiotics have been used to treat infections by aerobic gram-negative bacteria. However, they cause ototoxicity and nephrotoxicity. Interestingly, administration of tissue kallikrein is protective against gentamicin-induced renal injury in rats partly by inhibiting the inflammatory response and apoptosis through suppressing of oxidative stress.[462] In addition, L-arginine also prevents gentamicin-induced tubular damage in rats, whereas a NOS inhibitor aggravates the renal failure.[463] Similarly to the diabetic nephropathy, endogenous prostacyclin production also prevented the apoptosis and oxidative stress induced by gentamicin.[464] Thus, the renoprotective effects of tissue kallikrein against gentamicin toxicity seems to be once more mediated by NO and prostacyclin.

Furthermore, KKS seems to play a protective role in ischemic acute renal failure (IARF). As previously discussed,

NO production induced by B2Rs is an important player in the protective effects of ACE inhibitors.[425] In addition, ARBs seem to be less protective than ACE inhibitors against ischemia-reperfusion injury,[465] suggesting that ACE inhibitors may partly mediate their beneficial effects by inhibiting the inactivation of the kinins than by suppressing angiotensin II formation. In addition, studies have reported that deleting B1R and/or B2R aggravates renal damage following IARF; however, the double knockout had more detrimental effects than a deficiency in B2R only, emphasizing that both B1R and B2R are necessary for protection in IARF.[466] In addition to NO, older studies have shown that prostaglandins E1, E2, and I2 are also protective mediators of the KKS in IARF.[467] Contrary to these findings, results from exogenously administered bradykinin on IARF are conflicting and suggest that it aggravates IARF, although suppression of the endogenous KKS is also detrimental.[468] Thus, it seems that the physiologic levels of bradykinin produced endogenously are important for the functional recovery after IARF, but the higher levels achieved with exogenously administered bradykinin are detrimental.

In support of the aforementioned results in animal models, genetic studies in humans revealed that polymorphisms in several KKS-related genes are associated with higher incidence of renal problems. In fact, ACE polymorphism seems to influence diabetic nephropathy[469] and a polymorphism in the human B2R gene has been correlated with a higher susceptibility for nephropathy in diabetic patients.[470] Both human SLE and spontaneous lupus nephritis were also recently found to be associated with polymorphism in the kallikrein gene family, particularly KLK1 and KLK3.[471] Other studies have shown that the ACE D/D genotype is a risk factor for progression to chronic renal failure in patients with IgA nephropathy.[472,473] In addition, this ACE D/D genotype is also a risk factor for patients with autosomal dominant polycystic kidney disease (ADPKD) to develop ESRD at an earlier age.[474] In fact, polymorphisms in most of the genes in the KKS have been associated with the progression of chronic renal failure to ESRD, including ACE,[475] B1R and B2R,[476] and eNO.[477]

ADENOSINE

Adenosine, a purine nucleoside, is a paracrine hormone that regulates cellular and physiologic functions in many tissues. Three major pathways produce adenosine: the intracellular pathway, the extracellular ATP pathway, and the transmethylation pathway. Intracellular generation of adenosine results from the action of 5′-nucleotidase on adenosine monophosphate (AMP) during hypoxia (sequential dephosphorylation of intracellular ATP to adenosine). In cells that rapidly consume ATP, enhanced utilization of ATP increases the intracellular production rate of adenosine. In the kidney there are two prime examples of the intracellular ATP pathway. Increased delivery of Na^+ to the thick ascending limb of loop of Henle stimulates Na^+-K^+-ATPase activity in the basolateral membrane of epithelial cells through the increased flux of Na^+ across the luminal membrane. Oxygen availability in the renal medulla is marginally adequate; consequently, increased Na^+-K^+-ATPase activity may deplete ATP levels and lead to dephosphorylation of adenine nucleotides to form adenosine. Reactive ischemia is a second example of the intracellular ATP pathway in the kidney. A short period of ischemia in the kidney triggers a brief increase in renal vascular resistance, a phenomenon known as reactive ischemia. Renal ischemia activates the intracellular ATP pathway of adenosine production and adenosine causes reactive ischemia via activation of A_1 receptors in the preglomerular microvessels.[478] Adenosine, produced intracellularly, can traverse cell membranes by facilitated diffusion and function in a paracrine or autocrine fashion.

The extracellular ATP pathway is yet another mechanism of adenosine production in the kidney. Extracellular production of adenosine from AMP is possible because of the presence of ecto-5′-nucleotidase on the surface of many cell types. Release of adenine nucleotides into the extracellular compartment from renal sympathetic nerve terminals, intrarenal platelets, renal endothelial cells, renal vascular smooth muscle cells, and/or renal epithelial cells exposes extracellular ATP to ecto-ATPases, ecto-ADPases, and ecto-5′-nucleotidases, and these enzymes metabolize adenine nucleotides to adenosine.[478] In the kidney, ecto-5′-nucleotidase activity is expressed on tubular luminal membranes, fibroblasts, and mesangial cells and is believed to be the major source of renal adenosine.[479]

When oxygen supply is adequate, enzymatic hydrolysis of S-adenosyl homocysteine (SAH) to L-homocysteine and adenosine constitutes the major production pathway. It is the transmethylation pathway of adenosine production. Approximately one-third of the adenosine release to the extracellular space by cardiomyocytes is through the transmethylation pathway, but the importance of this pathway in the kidney is unclear.[478]

Adenosine Receptors

Purinoceptors or adenosine receptors, also called P_1 receptors, are classic G protein-coupled receptors with four known subtypes (A_1, A_2A, A_2B, and A_3 receptors).[478,480] A_1 receptors (A1Rs) have a high affinity for adenosine, whereas the affinity of A_2A receptors for adenosine is approximately threefold less compared with that of A1R. A_2B and A_3 receptors are low-affinity adenosine receptors.[478,480–482]

A1Rs are present in afferent arterioles, glomeruli including mesangial cells, juxtaglomerular cells, vasa recta, as well as in various segments of the tubular and collecting duct system including proximal tubule, thin limbs of Henle, TAL, and collecting ducts.[483] They evoke vasoconstriction by inhibiting adenylate cyclase activity (via activation of G_i and G_o), thereby reducing cAMP generation in vascular smooth muscle and signaling.[478] In renal epithelial cells and isolated rabbit afferent arterioles, A1R activation also appears to stimulate phospholipase C activity. A_2A and A_2B receptors produce vasodilation by stimulating adenylate cyclase

activity to increase cAMP generation; A_2A signal by engaging G_s, G_{olf}, and p21ras and A_2B receptors are also known to stimulate phospholipase C via G_q. A_3 receptors are thought to exert their physiologic effects through activation of calcium signaling pathways (phospholipase via G_q families) and perhaps inhibition of cAMP accumulation (via G_i), but their role in regulating the renal microvascular and epithelial function has not been extensively examined.[478,480]

P_2 receptors were originally described as purinergic receptors, reflecting the idea that they responded to ATP released as a neurotransmitter from peripheral sympathetic nerves. Currently P_2 receptors are divided into two distinct families: P_2X and P_2Y.[480]

Distinct genes code for each receptor family and there are marked differences in the structural and signal-transduction characteristics between the two families.

Renal Actions of Adenosine

Adenosine regulates a wide array of physiologic functions, including cardiac rate and contractility, vascular smooth muscle tone, neurotransmitter release, lipolysis, leukocyte function, platelet function, and renal hemodynamics and electrolyte transport.[481,484,485]

Adenosine is produced in the kidney and acts in an autocrine or paracrine fashion.[479,481] Both high-affinity A1R and low-affinity A2R are widely distributed throughout the renal vasculature and the nephron.[481,486] The renal effects of adenosine are diverse and include alterations in RBF, GFR, hormone production, neurotransmitter release, and tubular absorption (Table 8.4).

8.4 Renal Actions of Adenosine

Function	Effect	Receptor
Renal blood flow	Transient ↑ Delayed slight ↑	A_1 A_2
GFR	↓↓	A_1 and A_2
Renin production	↓↓ ↑	A_1 A_2
Erythropoietin production	↓ ↑	A_1 A_2
Sodium excretion	↑↑ ↓	A_2 A_1
GMC production and proliferation	↓	A_2B

↓, decrease; ↑, increase; GFR, glomerular filtration rate; GMC, glomerular mesangial cells.

Effect on Renal Blood Flow and Glomerular Filtration Rate

Infusion of adenosine into animals' renal artery results in transient reduction of RBF secondary to A1R mediated afferent arteriolar vasoconstriction, followed by a delayed A2R mediated postglomerular vasodilation and return of RBF to normal.[487–489] Selective A2R agonists increase RBF via vasodilation of the medullary renal microcirculation. A1R importantly regulate renal function. Infusion of A1R agonists into the renal interstitium diminishes blood flow to both superficial and deep nephrons. In the outer cortex, A1R-induced vasoconstriction is most likely caused by contraction of preglomerular, rather than postglomerular, microvessels; however, in juxtaglomerular nephrons, A1R mediate vasoconstriction by contracting preglomerular microvessels, efferent arterioles, and outer medullary descending vasa recta. Ang II strongly enhances A1R-induced preglomerular vasoconstriction. Contraction of preglomerular microvascular smooth muscle cells by A1R underlies the mediator role of adenosine in tubuloglomerular feedback (TGF) (Fig. 8.7), explains the ability of adenosine to decrease GFR, and potentiates postjunctional vasoconstrictor responses to renal sympathetic neurotransmission in the kidney.[478] It is conceivable that A1R are present in endothelial cells along the renal vasculature and that adenosine causes the release of NO and perhaps other endothelial vasodilators when administered from the vascular, but not from the interstitial, aspect of the vessel. The resulting A1R-induced constriction would, therefore, be blunted by endothelial factors only when adenosine is given intravascularly. In a study in dogs, the administration of NOS inhibitors caused a marked augmentation in the constrictor response of RBF to bolus injections of adenosine, whereas the dilator effect of the A_2 agonist CGS-21680 was unaffected, indicating that adenosine may cause NOS activation through an A1R-mediated mechanism. It is now well recognized that the majority of vasodilator agents act by binding to their receptors on endothelial cells and by eliciting the generation and release of endothelial relaxing factors, most notably NO, endothelial hyperpolarizing factor, and prostaglandins. However, in a number of studies, adenosine appears to augment NOS activity and NO release through an A2R-mediated process, an action that would enhance the dilator component rather than diminish the constrictor component of the adenosine actions. Also adenosine has been shown to consistently stimulate the production of NO in cultured endothelial cells, usually through an A2AR-dependent mechanism.[489]

Adenosine induces a sustained decrease in GFR secondary to reduced P_{GC}.[490] Infused in humans, it results in an insignificant increase in RBF and a significant, moderate decrease in GFR.[491,492] It is postulated that adenosine-mediated reduction in GFR constitutes the underlying mechanism of TGF.[493,494] The hypothesis states that increased solute delivery to the MD stimulates sodium transport, resulting in ATP hydrolysis and generation of adenosine. Adenosine, in turn, completes the feedback loop by decreasing GFR and

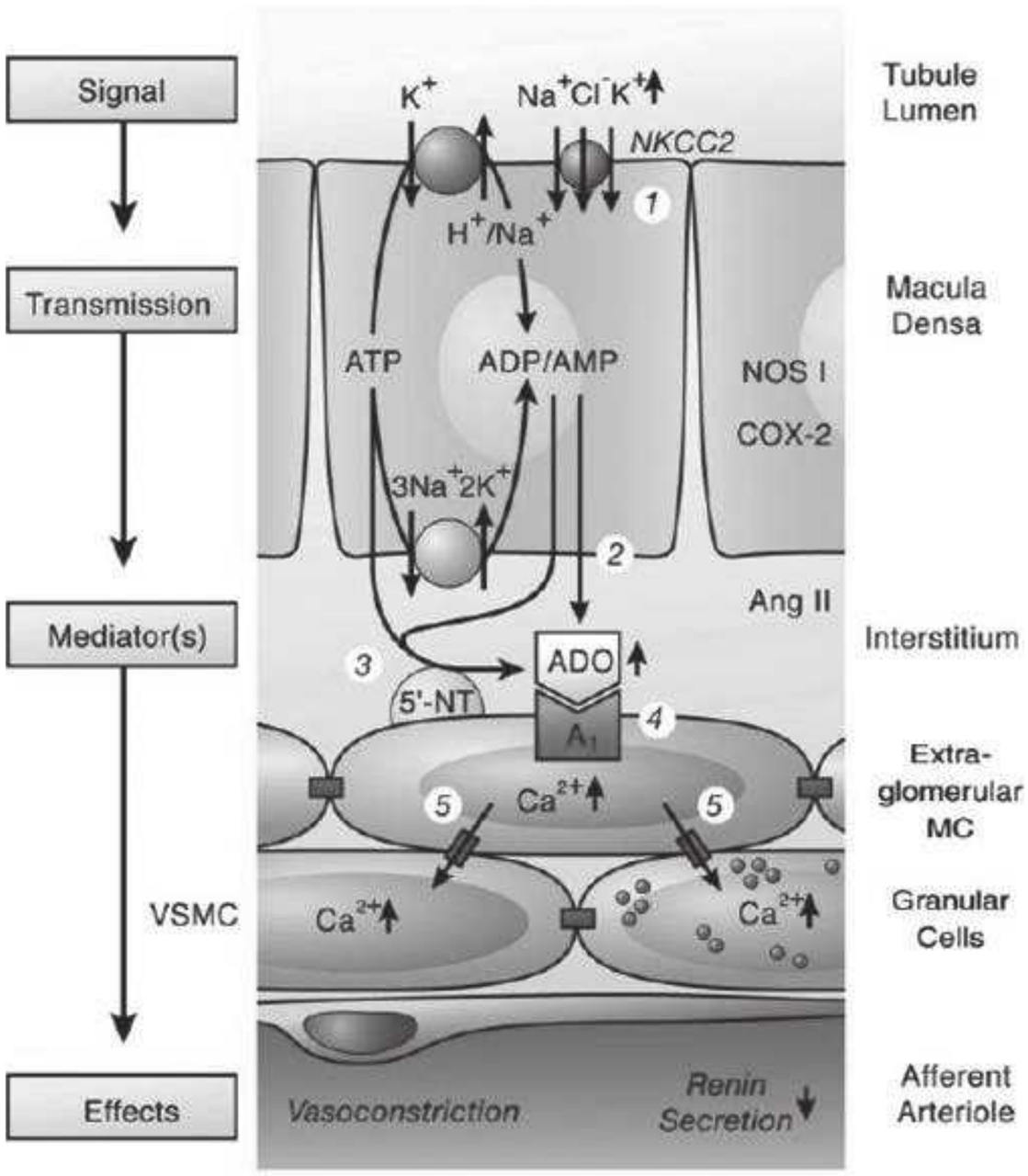

FIGURE 8.7 Proposed mechanism of adenosine acting as a mediator of the tubuloglomerular feedback. Numbers in circles refer to the following sequence of events. *1*, Increase in concentration-dependent uptake of nitrogen (N), potassium (K), and chlorine (Cl) via the furosemide-sensitive Na-K-2Cl cotransporter (NKCC2); *2* and *3*, transport-dependent, intra- and/or extracellular generation of adenosine (ADO); the extracellular generation involves ecto-5′-nucleotidase (5′-NT); *4*, extracellular ADO activates adenosine A1 receptors triggering an increase in cytosolic Ca^{2+} in extraglomerular mesangium cells (MC); *5*, the intensive coupling between extraglomerular MC, granular cells containing renin, and smooth muscle cells of the afferent arteriole (VSMC) by gap junctions allows propagation of the increased Ca^{2+} signal resulting in afferent arteriolar vasoconstriction and inhibition of renin release. Factors such as nitric oxide, arachidonic acid breakdown products, or angiotensin (Ang) II modulate the described cascade. *NOS I*, neuronal nitric oxide synthase; *COX-2*, cyclooxygenase-2. See text for further explanations. (Adapted from Vallon V, Muhlbauer B, Osswald H. Adenosine and kidney function. *Physiol Rev*. 2006;86(3):901–940.)

normalizing solute delivery to the distal nephron. The hypothesis is supported by experiments demonstrating that A1R blockade inhibits TGF.[495,496,497]

Effect on Sodium Reabsorption and Renal Tubular Transport

In contrast to A1R, which directly stimulate Na^+ reabsorption by increasing epithelial transport mechanisms, activation of A2R in microvessels of juxtaglomerular nephrons and in the medullary microcirculation enhances medullary blood flow, thus altering peritubular forces that modulate Na^+ reabsorption. The net result is an increase in Na^+ excretion.[478] Adenosine-induced decrease in solute excretion, particularly sodium, has been generally attributed to the concomitant reduction in GFR and urine flow. The presence of A1R and A2R on renal epithelial cells, however, suggests that adenosine has direct effects on tubular transport.[481] In rats, for example, infusing adenosine or adenosine receptor agonists in a dose that does not alter systemic blood pressure, RBF, or GFR still leads to sodium and water retention.[498,499] This effect was independent of renal enervation, at least for A1-specific agonists, and suggested a direct tubular effect of adenosine on A1R to stimulate sodium reabsorption.[499] A1R in the nephron are linked to Na^+ transport in several segments. However, the natriuresis and diuresis associated with systemic inhibition of A1R are due primarily to reduced proximal tubule (PT) reabsorption. Studies on kidney fluid and electrolyte transport show that activation of A1R stimulates NaCl reabsorption in cortical proximal tubule, which is a tubular segment with relatively high basal oxygen supply. In contrast, adenosine inhibits NaCl reabsorption in medullary TAL and IMCD—that is, nephron segments with relatively low oxygen delivery.[483] Caffeine and theophylline are nonselective adenosine antagonists, and reports suggest high levels of each induced diuresis.[500]

Adenosine acts as a mediator of the TGF, which establishes an inverse relationship between GFR and the NaCl concentration at the MD. In the juxtaglomerular apparatus, extracellular formation of adenosine by ecto-5′-nucleotidase contributes to the adenosine pool that mediates TGF-induced afferent arteriolar vasoconstriction. The TGF mechanism stablizes the NaCl load to the distal nephron, which facilitates fine regulation of body NaCl balance at these sites. Endogenous adenosine, the synthesis of which is increased by enhanced NaCl transport, limits via adenosine A1R activation the oxygen demand in relatively hypoxic nephron segments by directly and differentially affecting reabsorption along the nephron.[483]

Activation of basolateral P2 receptors in the collecting duct inhibits vasopressin-stimulated water reabsorption, and stimulation of apical P2 receptors can affect solute transport in both the proximal and the distal nephron: an in vivo micropuncture study in the rat showed that stimulation of apical P2Y1 receptors inhibits proximal tubular bicarbonate reabsorption through suppression of NHE3 activity,[1] and in vitro and in vivo evidence indicates that stimulation of apical P2 receptors in the distal nephron inhibits amiloride-sensitive sodium reabsorption[2,3] and reduces the activity of apical K secretory channels.[4,501]

Effect on Renin

Adenosine suppresses renin release by the kidney.[481] In sodium-depleted animals, renin release is inhibited by maneuvers that increase renal adenosine production,[502,503] an effect that results from direct action of adenosine on renin-producing cells.[504] Inhibition of renin release is most likely mediated by binding of adenosine to high-affinity A1R.[505] In this regard, A1R restrain renin release responses, a theory known as the adenosine-brake hypothesis. A1R are coupled to G_i and, therefore, inhibit adenylyl cyclase. Because stimulation of renin release from juxtaglomerular cells by many stimuli involves activation of adenylyl cyclase, activation of juxtaglomerular A1R attenuates renin release and antagonism of renal A1R increases renin release.[478] In contrast, agonists selective for the low-affinity A2R stimulate renin release, particularly when administered in high doses.[481,506] This suggests that adenosine regulates renin release by exerting either an inhibitory or stimulatory effect, depending on its local concentration. Studies employing acute blockade or chronic deficiency of adenosine A1R receptors rather indicate a modulating, tonic inhibition of the renin system by adenosine. In addition, an MD-dependent source of adenosine and activation of adenosine A1R contribute to renin release inhibition under conditions of high NaCl concentrations at the MD. An increase in intracellular cAMP is an important stimulator of renin release. Part of the cAMP could be released by renin-secreting cells and is extracellularly converted to adenosine, which acts as a negative feedback control or brake when renin secretion is stimulated.[483]

Effect on Renal Medullary Oxygenation

Renal medullary hypoxia is an obligatory part of the process of urinary concentration. When O_2 supply is further impaired, however, medullary hypoxic injury can develop. Various mechanisms act in concert to minimize medullary hypoxia. Adenosine-mediated actions like maintaining high proximal reabsorption, inhibiting reabsorption in the medullary TAL, and reducing GFR by the TGF mechanism, when distal NaCl concentrations increase, can be part of these mechanisms. Furthermore, in the deep cortex and medulla, adenosine via A2R activation causes vasodilation, which increases medullary blood flow and medullary oxygenation. Thus, adenosine through distinct actions on the vasculature and tubular transport system contributes to the stabilization of the O_2 demand-to-supply ratio particularly in the renal medulla.[483] Nishiyama and associates[480,507] demonstrated that hypoxia-induced renal vasoconstriction was associated with elevated interstitial adenosine levels and could be blocked by adenosine A1R antagonists. Adenosine plays a role in balancing oxygen supply and demand during renal hypoxia by regulating RBF, GFR, renin secretion, and solute transport.[493]

Pathophysiologic conditions associated with increased renal production of adenosine include ARF, myoglobinuric ARF, and mercuric chloride-induced ARF.[508–510] A1R may also be involved in other drug-induced nephrotoxicity. For example, selective antagonism of A_1 receptors attenuates nephropathy caused by nephrotoxins such as cisplatin, gentamicin, cephaloridine, glycerol, and radiocontrast media.[478]

A2R and Diabetic Nephropathy

Awad et al. showed that streptozotocin (STZ)-induced diabetes in rodents leads to marked proteinuria and decreased renal function that is attenuated with continuous subcutaneous administration of A2R agonists. Both nephrin and podocin mRNA levels were reduced after STZ-induced diabetes, an effect completely restored with A2R agonist treatment.[511] They also demonstrated that chronic administration of selective A2A agonists attenuates renal lesions and functional abnormalities characteristic of diabetic nephropathy. The renal tissue protective effect of the A2A agonist is believed to be mediated primarily by abrogating the inflammatory response associated with diabetes. Chronic A2R activation in diabetic rats ameliorates histologic and functional changes in kidneys induced by diabetes and causes reduced inflammation associated with diabetic nephropathy.[511]

Ischemia and Reperfusion Injury

Activation of A1R in many organs, including the kidney, initiates several cytoprotective kinase cascades including ERK MAPK, Akt, and PKC. Activation of cell surface A1R produces cytoprotective effects against ischemia and reperfusion (IR) injury in many organ systems including the heart, kidney, and brain. Joo et al. demonstrated that transient A1R activation produces both acute and delayed protective effects in the kidney including reduced renal cortical necrosis and apoptosis after IR injury. Renal apoptosis is an important component in the development of ARF after IR injury.[512] A2R exert important anti-inflammatory actions that may protect the kidneys from injury (Fig. 8.8). Their activation strongly inhibits neutrophil endothelial cell interactions in vitro and, in vivo, and markedly decreases the renal infiltration of neutrophils and attenuates renal dysfunction following IR injury.[478]

Adenosine 5′-tetraphosphate

Adenosine 5′-tetraphosphate (AP4) is the most potent vasoactive purinergic mediator identified to date, exerting the vasoconstriction predominantly through P2X1 receptor activation. The fact that AP4 is more resistant to degradation further favors potent and prolonged vasoconstrictive effects. The vasoconstrictive in vitro effects of AP4 are paralleled by hypertensive effects in vivo. Therefore, it may be speculated that AP4 is a vasoconstrictor secreted by human endothelial cells, which also plays a role in the regulation of systemic blood pressure under physiologic and pathologic conditions.[513]

Adenosine and Angiotensin II

Certain actions of adenosine, such as vasoconstriction of the renal afferent arteriole, are either dependent on or significantly enhanced by Ang II.[481,514] Evidence suggests that a reduction in, or prevention of, Ang II formation and action

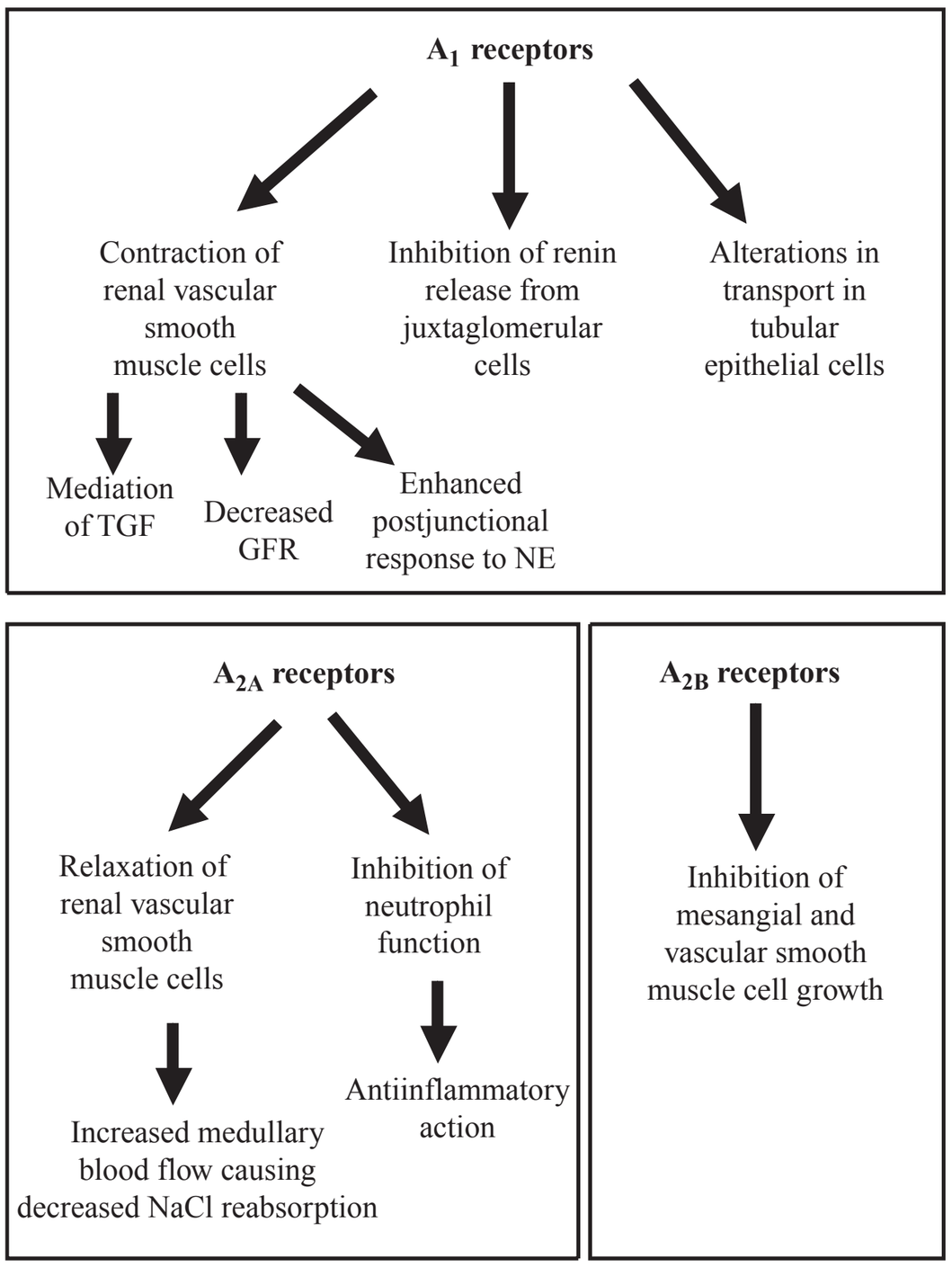

FIGURE 8.8 Regulation of renal function by adenosine. *TGF*, tubuloglomerular feedback; GFR, glomerular filtration rate; *NE*, norepinephrine. (From Bulut OP, Dipp S, El-Dahr S. Ontogeny of bradykinin B1 receptors in the mouse kidney. *Pediatr Res.* 2009;66(5):519–523.)

cause a marked attenuation of the vasoconstrictor response of the intact kidney to adenosine. Conversely, an elevation of ambient Ang II concentrations enhances the constrictor effect of A1R activation.[489]

Effects on Glomerular Mesangial Cells

A2B receptors' activation attenuates vascular smooth muscle cell proliferation and collagen and protein synthesis (Fig. 8.8). Studies with a number of adenosine receptor agonists and antagonists and with antisense oligodeoxynucleotides against the A2B receptor indicate that the growth inhibitory effects of adenosine on vascular smooth muscle cells are mediated by these receptors.[478]

Effect on Erythropoietin

A1R stimulation inhibits erythropoietin (EPO) synthesis, whereas that of A2R enhances EPO synthesis.[515]

Effect on Norepinephrine

Activation of A1R on sympathetic neurons in the kidney causes presynaptic inhibition of norepinephrine (NE) release.[516] Postjunctionally, however, adenosine seems to enhance sensitivity to NE.[516] Because renal denervation does not alter adenosine-induced changes in RBF and GFR, the hemodynamic actions of adenosine in the kidney are most likely independent of its effect on neurotransmitter release.[481]

Pancreatohepatorenal Extracellular cAMP-Adenosine Pathway

In addition to an autocrine/paracrine role in the kidney, the extracellular cAMP-adenosine pathway may also function as an endocrine system by which the pancreas and liver regulate renal function. The pancreas releases glucagon directly into the portal circulation in response to appropriate stimuli. Glucagon in the portal circulation stimulates hepatic adenylyl cyclase, which results in secretion of cAMP by hepatocytes into the venous circulation. Liver-derived cAMP circulates to the kidney, where it is filtered into the proximal tubule and then metabolized to adenosine. Adenosine would then engage A1R in epithelial cells to enhance electrolyte transport. It is important to note that, in contrast to cAMP, which is stable in blood, adenosine has a half-life in human blood of less than 1 second. The liver has the capacity to release significant amounts of cAMP into the hepatic vein.[478]

The most recent studies strongly suggest that an autocrine/paracrine extracellular cAMP-adenosine pathway, in fact, does not participate in the regulation of Na^+ transport by proximal epithelial cells. It appears that the pancreatohepatorenal extracellular cAMP pathway is more important in modulating proximal tubular function. Although intrarenal infusions of glucagons do not reduce Na^+ excretion, intraportal infusions of glucagon in sheep cause a marked antidiuresis. Although speculative, it is conceivable that the pancreatohepatorenal cAMP-adenosine pathway participates in physiologic adjustments of renal transport, as well as in pathophysiologic processes. In normal mammals, both hypoglycemia and exercise are powerful stimulants to glucagon release. Activation of the pancreatohepatorenal cAMP-adenosine pathway by glucagon in response to hypoglycemia might increase Na^+ glucose symport in proximal tubules and, thus, increase the efficiency of glucose transport, an adaptive mechanism to combat hypoglycemia. Activation of the pancreatohepatorenal cAMP-adenosine pathway by glucagon during exercise might enhance Na^+ transport in the proximal tubules and thus increase the efficiency of Na^+ reabsorption, an adaptive mechanism to avoid volume depletion during sustained physical exertion. The pancreatohepatorenal cAMP-adenosine pathway might be overly activated in the metabolic syndrome. Although oral glucose normally strongly inhibits glucagon secretion by the pancreas, in animals and people with the metabolic syndrome, an oral glucose challenge markedly stimulates pancreatic glucagon secretion by approximately 200%. If the pancreatohepatorenal cAMP-adenosine pathway exists, each time such a patient ingests a high carbohydrate meal the renal tubules would be exposed to a wave of excess adenosine

production. Because adenosine causes increased reabsorption of Na^+ and vasoconstriction of the preglomerular microcirculation, this could contribute to the pathophysiology of hypertension in the metabolic syndrome. Importantly, adenosine receptors also inhibit lipolysis in fat cells and may reduce insulin sensitivity in skeletal muscle. However, at this time, both the physiologic and pathophysiologic roles of the putative pancreatohepatorenal cAMP-adenosine pathway are speculative.[478]

PARATHYROID HORMONE AND PARATHYROID HORMONE–RELATED PEPTIDE

In response to low levels of extracellular Ca, parathyroid glands secrete parathyroid hormone (PTH), an 84-amino acid polypeptide hormone.[517] PTH is initially synthesized as a 115-amino acid polypeptide, pre-pro-PTH, which is cleaved within parathyroid cells at the N-terminal portion first to pro-PTH (90 amino acids) and then to PTH (84 amino acids). PTH-related peptide (PTHrP) was first identified as a cause of humoral hypercalcemia of malignancy and is secreted predominately as a 141-amino acid peptide. PTH and PTHrP have sequence homology in the first 13 amino acids (the amino terminus).[518] Increased circulating PTH or PTHrP leads to mobilization of Ca from bone, enhancement of Ca reabsorption in the renal tubule, and increased production of 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$) from 25 hydroxyvitamin D_3(25-OH D_3) by proximal tubule cells through 1α hydroxylase stimulation. $1,25(OH)_2D_3$, in turn, increases Ca absorption by the intestine and possibly Ca reabsorption by the kidney. The combined actions of PTH and $1,25(OH)_2D_3$ result in normalization of the extracellular Ca concentration. In addition to its regulatory actions on calcium balance, PTH can regulate phosphorus balance by inhibiting its reabsorption in the proximal and distal tubules of the nephron.

PTH synthesis and secretion by the parathyroid gland is tightly regulated.[517] Although a decreased extracellular Ca level stimulates PTH synthesis and secretion, increased extracellular levels of either Ca or $1,25(OH)_2D_3$ are inhibitory. The extracellular phosphorus level regulates PTH production directly at a posttranscriptional level[519] and indirectly by altering circulating Ca and $1,25(OH)_2D_3$ concentrations. Increased serum phosphorus secondary to renal insufficiency, for example, decreases Ca concentration and $1,25(OH)_2D_3$ production, leading to stimulation of PTH release, but can itself increase PTH level without alterations in ionized calcium or serum $1,25(OH)_2D_3$ levels.[520,521]

Magnesium also regulates PTH secretion and its depletion can decrease PTH secretion. Fibroblast growth factor 23 (FGF23) decreases PTH mRNA and secretion. Ben-Dov et al. showed that FGF23 acts directly on the parathyroid through the MAPK pathway to decrease serum PTH.[522]

The biologic activity of PTH resides in its amino-terminus. There is increasing evidence that the C-terminal fragment of PTH, PTH(7-84), exerts a hypocalcemic effect that is reversed by PTH(1-34) and PTH(1-84). PTH(7-84) inhibits PTH(1-84)-induced bone resorption. Nakajima et al. studied the effect of PTH(7-84) on PTH(1-34)-induced production of $1,25(OH)_2D_3$ in primary cultured murine renal tubules. PTH(1-34) stimulated the conversion of 25-OH D_3 to $1,25(OH)_2D_3$, and PTH(7-84) dose-dependently inhibited this process. Real-time polymerase chain reaction (PCR) revealed that PTH(1-34) increased the expression level of 1α-hydroxylase mRNA, whereas PTH(7-84) did not. This may at least partly account for the decreased serum level of $1,25(OH)_2D_3$ in patients with severe primary hyperparathyroidism with renal failure.[523]

Once secreted, PTH is rapidly cleared from plasma through uptake principally by the liver and kidney, where PTH(1-84) is cleaved into amino- and carboxyl-terminal fragments that are then cleared by the kidney. Hepatic clearance of PTH involves rapid proteolysis by Kupffer cells to N-terminal fragments and C-terminal fragments (CPTH). CPTH fragments can exert direct effects on bone cells via CPTH receptors. They are cleared predominantly by the kidney and accumulate disproportionately during renal failure.[524] Intact PTH has a plasma half-life of 2 to 4 minutes. In comparison, the C-terminal fragments, which are cleared principally by the kidney, have half-lives that are five to ten times greater.

Parathyroid Hormone and Parathyroid Hormone–Related Peptide Receptors

Two types of receptors exist. Type 1 PTH receptors (PTH1R) bind PTH and PTHrP. Binding to PTH1R occurs in the 15- to 34-amino acid region of both hormones (N-terminal sequence). It is interesting that these two peptides bind with almost equal affinity and yet do not share sequence homology in this region. The PTH1R mediates the biologic activity of PTH and PTHrP. PTH1R is heavily expressed in bone and kidney, and is also present in other tissues such as breast, skin, heart, blood vessels, pancreas, and other tissues. It activates multiple cellular signaling pathways including cAMP, PLC pathway, PKC, and release of intracellular calcium stores. Muller et al. showed also that activation of PTH1R engages major apoptosis signaling pathways, namely in apoptosis of differentiating embryonic cells.[525] Type 2 PTH receptors (PTH2R), which share 51% homology with PTH1R, can bind PTH but they do not bind PTHrP with high affinity. PTH2Rs are expressed in only a few tissues—their biologic significance is unknown.[518] Increasing evidence points to the presence of novel PTH receptors (CPTHR) with specificity for the carboxyl-terminal region of PTH. This portion of the hormone was previously thought to be biologically inert but has now been shown to possess hypocalcemic activity. The CPTHRs are present in various tissues but are most heavily expressed in bone.

Although PTH is a well-characterized endocrine regulator of mineral homeostasis, PTHrP is a key regulator of placental calcium transport in the fetus, and it appears to

be a physiologic modulator of smooth muscle tone. Current concepts indicate that PTHrP is a developmental and/or growth-regulating factor, much more similar to other known cytokines and growth factors than to PTH.[526] Under physiologic conditions, PTHrP levels are increased during pregnancy and lactation.[527] However, as described earlier, it appears to play a predominately pathophysiologic role in the adult, causing hypercalcemia.

Renal Actions of Parathyroid Hormone

PTH receptors and PTH-sensitive adenylate cyclase have been identified in glomeruli and basolateral membranes of epithelial cells in the proximal tubule, thick ascending limb of Henle's loop, and distal convoluted tubule (DCT).[7,528] PTH has three major effects on the kidney: increased Ca reabsorption, inhibition of phosphate reabsorption, and stimulation of $1,25(OH)_2D_3$ synthesis. Its other actions on the kidney include modulation of GFR, gluconeogenesis, magnesium reabsorption, and acid-base handling.

PTH decreases renal Ca excretion through multiple mechanisms. Ichikawa et al.[529] demonstrated that PTH infusion in rats decreases GFR by reducing K_f. A decreased GFR leads to decreased filtered load of Ca and, therefore, Ca excretion. PTH also enhances tubular Ca reabsorption by stimulating active Ca transport in the thick ascending limb of Henle's loop and distal tubule.[530,531] The effect of PTH on Ca transport in the proximal tubule varies and is probably related to Na and water reabsorption in this nephron segment.

PTH causes phosphaturia primarily by inhibiting phosphate transport in the proximal tubule, specifically by inhibiting sodium-phosphate (Na-P) cotransport.[532] Two different renal Na-P cotransporters have been identified and have been termed type 1 (Npt1) and type 2 (Npt2). Npt2 is a target for regulation by PTH and decreases Na-P transport by endocytic retrieval and lysosomal degradation of the Npt2 protein.[533] This endocytic retrieval can occur either from proximal tubules exposed to apical or basolateral PTH and can signal via either the cAMP-PKA or the PLC-PKC pathway.[534] Mice that are Npt2 null have profound phosphate wasting.[535] Npt2-null mice are resistant to further phosphaturic effects from exogenous PTH.[536] α_2-Adrenergic receptor stimulation blunts the phosphaturic response to PTH.[537] PTH-stimulated synthesis of $1,25(OH)_2D_3$ in the proximal tubule is discussed in the next section.

Although no single hormone has been specifically shown to regulate Mg homeostasis, PTH appears to increase Mg reabsorption in the kidney.[538] PTH also plays a role in acid–base homeostasis by enhancing urinary acid excretion.[539] Although PTH inhibits bicarbonate reabsorption in the proximal tubule, it indirectly stimulates distal hydrogen ion secretion and titratable acid excretion by increasing phosphate delivery to the distal nephron. PTH has also been shown to enhance proximal tubular gluconeogenesis[540] and renin secretion by juxtaglomerular cells.[541]

Renal Actions of Parathyroid Hormone–Related Peptide

In the kidney, PTHrP appears to modulate RPF and GFR, and induces proliferative effects on both glomerular mesangial and tubuloepithelial cells. PTHrP is known to be upregulated in several experimental nephropathies such as acute renal failure, obstructive nephropathy, as well as diabetic nephropathy. In transgenic mice models and in the former condition, PTHrP appears to contribute to the progression of renal damage by increasing tubulointerstitial cell survival, inflammation, and renal fibrogenesis. In diabetic nephropathy, PTHrP can promote renal hypertrophy and proteinuria. Ang II, a critical factor in the progression of renal injury, appears to be, at least in part, responsible for endogenous PTHrP upregulation in these pathophysiologic settings.[526] PTHrP also participates in the hypertrophic signalling triggered by high glucose on podocytes. In this condition, Ang II induces the upregulation of PTHrP, which in turn might induce both TGF-β1 and p27Kip1 expression and thereby promotes the hypertrophy of podocytes.[542]

Conventional renal cell carcinoma (CRCC) originates from the renal proximal tubular epithelium, a target tissue for PTHrP proliferation effects.[543] It was shown that PTHrP, acting through its receptor PTH1R, is an essential growth factor for CRCC in vitro and in vivo and a new target for the VHL gene products.[544,545] It seems that PTHrP induces tumor cell survival through inhibition of cell apoptosis.[546]

VITAMIN D

Along with PTH, vitamin D plays a central role in calcium and phosphate homeostasis. The active form of vitamin D, $1,25(OH)_2D$, is a steroid molecule synthesized from either vitamin D_3 (cholecalciferol) or vitamin D_2 (ergocalciferol). These two forms of vitamin D differ only by the side chain to the sterol skeleton. Vitamin D_3 is present in the diet (animal sources, mainly fish oil) and is also synthesized by the skin from 7-dehydrocholesterol upon exposure to ultraviolet light. Vitamin D_2 is available only from dietary sources (plants, yeast, and fungi). Vitamins D_3 and D_2 are biologically inactive. They require activation in the liver and kidney. After binding to carrier proteins, in particular, vitamin D-binding protein (DBP), vitamin D is transported to the liver where it is enzymatically hydroxylated to 25-hydroxyvitamin D (calcidiol, 25[OH]D) through the action of hepatic microsomal and mitochondrial cytochrome P450 vitamin D–25-hydroxylase. Subsequently, 25(OH)D, bound to DBP, is transported to the kidneys where it is hydroxylated exclusively in the proximal tubule by the mitochondrial 25(OH)D–1α-hydroxylase.[547] 1α-Hydroxylation is the rate-limiting step in the formation of the most abundant active metabolite $1,25(OH)_2D_3$.[548–550] This step is tightly regulated through multiple feedback mechanisms,[549,550] mainly PTH, calcium, phosphate, fibroblast growth factor 23 (FGF23), and $1,25(OH)_2D_3$ itself (Fig. 8.9). An increase

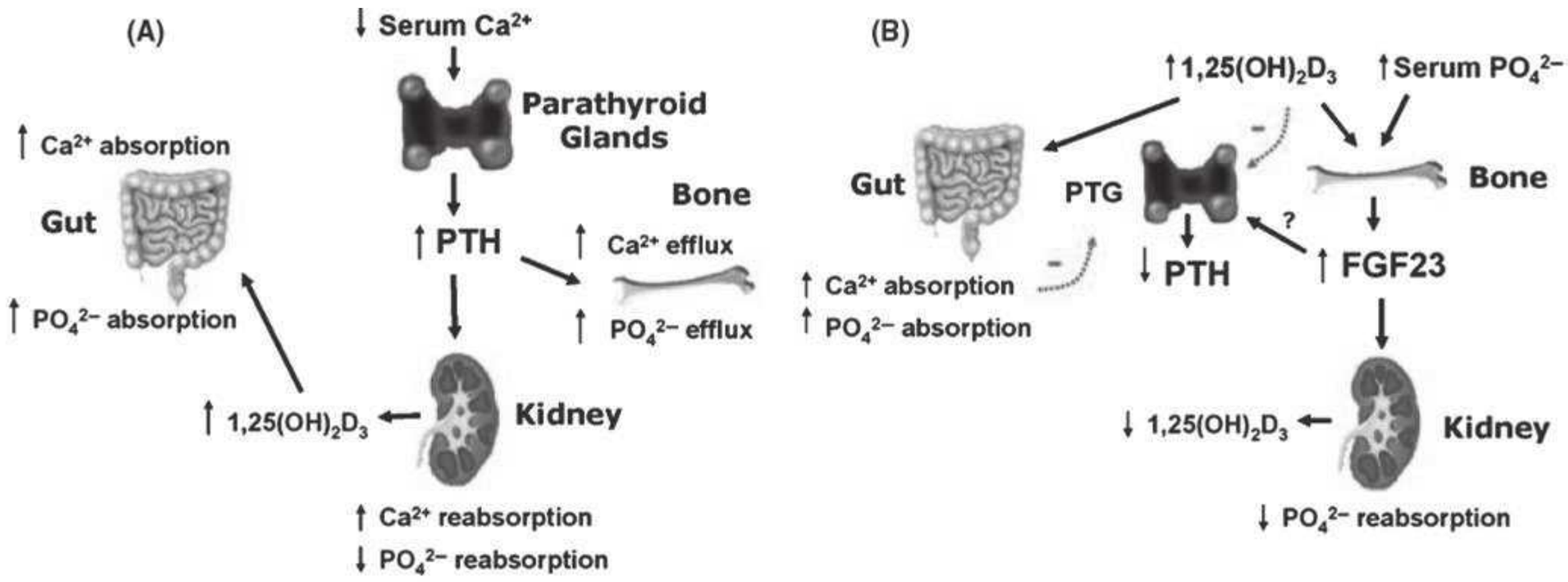

FIGURE 8.9 **A:** Calcium–parathyroid hormone (PTH)–vitamin D axis. The calcemic hormone PTH produced by the parathyroid gland targets kidney to increase calcium absorption and $1,25(OH)_2D_3$ production and bone to increase calcium efflux. $1,25(OH)_2D_3$ stimulates calcium absorption from the gut, which, along with renal and bone calcium, restores serum calcium to normal. **B:** Fibroblast growth factor 23 (FGF23)–bone–kidney axis. FGF23 produced by bone osteocytes has phosphaturic effects and suppresses $1,25(OH)_2D_3$, thereby providing a means to lower serum phosphate in a PTH-independent manner. (From Stubbs J, Liu S, Quarles LD. Role of fibroblast growth factor 23 in phosphate homeostasis and pathogenesis of disordered mineral metabolism in chronic kidney disease. *Semin Dial*. 2007;20(4):302–308.)

in PTH levels, secondary to decreased serum Ca, stimulates 1α-hydroxylase activity in the kidney. A low serum phosphorous or $1,25(OH)_2D_3$ concentration also activates 1α-hydroxylase, whereas elevated $1,25(OH)_2D_3$ levels and the phosphaturic FGF23 are inhibitory. Other less established stimulators of 1α-hydroxylase activity include calcitonin, growth hormone, insulin, insulin-like growth factor, estrogen, and prolactin.[548–550]

In addition to the kidney, extrarenal sites of 1α-hydroxylase activity have been identified. These include macrophages, keratinocytes, hepatocytes, and human placenta, as well as skeletal muscle cells[551] and various bone cell preparations,[552,553] although the regulatory processes for this conversion are not well understood. Extrarenal production of $1,25(OH)_2D_3$ can lead to hypercalcemia in certain pathologic situations, as in patients with active sarcoidosis or in patients with lymphoma.

The first step in the inactivation and catabolism of vitamin D is hydroxylation of 25(OH)D and $1,25(OH)_2D$ by 24-hydroxylase, which is present in the kidney, intestine, and several other tissues that possess $1,25(OH)_2D_3$ receptors.[547,550] Importantly, kidney 24-hydroxylase and 1α-hydroxylase are reciprocally regulated.[547,550,554] $1,25(OH)_2D_3$ and hypophosphatemia increase 24-hydroxylase activity whereas vitamin D deficiency and PTH (via CAMP) suppress it.[554]

25(OH)D has a long half-life (approximately 3 weeks) and is the best measure of vitamin D status. Calcitriol has a short half-life (4 to 6 hours) and exists at circulating levels 1/1,000 of those of 25(OH)D. $1,25(OH)_2D_3$ exerts its biologic actions by binding to intracellular vitamin D receptors (VDR), which in the unliganded form are located in the cytosolic and nuclear compartments.[555] Like other members of the steroid-thyroid family of receptors, the liganded form of VDR functions as a transcription factor in the nucleus. The VDR–$1,25(OH)_2D_3$ complex regulates transcription of more than 60 genes by interacting with DNA sequences known as vitamin D response elements. Central to its role in Ca homeostasis, $1,25(OH)_2D_3$ induces the transcription of genes coding for Ca-binding proteins.[556] Vitamin D-dependent Ca-binding proteins (CaBP-Ds) are found in high concentrations in Ca-transporting tissues, such as the kidney, intestine, and placenta. CaBP-Ds bind calcium with high affinity and are associated with Ca transport in these organs.

Another potential regulatory site for vitamin D signaling, other than the synthesis and degradation of $1,25(OH)_2D_3$, is by regulation of VDR expression. Receptor regulation has been shown to be physiologically important, as $1,25(OH)_2D_3$ signaling is dependent on both the number and occupancy of VDRs on the cell surface membrane.[557]

Aside from its biologic effects in the kidneys, calcitriol is transported by DBP to other vitamin D receptor VDR-positive target tissues (mainly bone, intestine, and parathyroid gland) to act in a genomic or nongenomic manner. Regulation of gene expression by calcitriol is mediated by VDR and takes place within hours. By contrast, nongenomic responses of calcitriol are probably mediated by a specific membrane-bound VDR and occur within seconds to minutes. Nongenomic effects of calcitriol include rapid changes in phosphoinositide metabolism, increases in intracellular calcium levels, stimulation of intestinal calcium transport and phosphate fluxes, elevation in cyclic guanosine monophosphate (cGMP) levels, and activation of protein kinase C.[558] These activities have been found in many cells, including keratinocytes, enterocytes, muscle cells, osteoblasts, and chondrocytes. VDR seems to be necessary for some of these

nongenomic transduction processes; however, another protein named 1a,25-dihydroxy-membrane associated rapid response steroid binding (MARRS) is also seemingly involved in these rapid nongenomic actions.[558]

Physiologic Actions of $1,25(OH)_2D_3$

$1,25(OH)_2D_3$ participates in a hormonal system that tightly regulates the extracellular Ca concentration.[556] A decline in serum Ca stimulates PTH release, which acts on the kidney to increase production of $1,25(OH)_2D_3$. $1,25(OH)_2D_3$, in turn, stimulates intestinal absorption of Ca and decreases its excretion by the kidneys. $1,25(OH)_2D_3$ also acts on bone to stimulate Ca mobilization.[559] Normalization of serum Ca shuts off this cascade by suppressing 1α-hydroxylase activity in the kidney and release of PTH from the parathyroids. Increased $1,25(OH)_2D_3$ also contributes to turning off Ca-correcting mechanisms by inhibiting its own production.[560] In addition to its effects on Ca homeostasis, $1,25(OH)_2D_3$ also enhances phosphate absorption in the intestine and kidney.

About 50% to 60% of filtered Ca is reabsorbed in the proximal tubule. This process is Na-dependent, and the majority of Ca is reabsorbed via a paracellular pathway.[561,562] Approximately 20% of filtered Ca is reabsorbed in Henle's loop, 10% to 15% in the distal tubule, and 5% in the collecting duct. Unlike the proximal tubule, Ca reabsorption in the distal tubule appears to be Na-independent.[561] In addition to VDR, distal tubule epithelial cells contain CaBP-Ds and ATP-dependent plasma membrane Ca pumps.[563] It has been postulated, therefore, that $1,25(OH)_2D_3$ enhances renal Ca reabsorption by direct action on the distal tubule in a manner analogous to stimulation of Ca absorption by intestinal cells.[556] Several experimental studies support this hypothesis: Concentrations of CaBP-Ds and Ca transport rates in renal cells are increased by vitamin D, whereas vitamin D deficiency abolishes CaBP-D synthesis and decreases Ca absorption.[556] Experimental studies also suggest a role for $1,25(OH)_2D_3$ in phosphate handling by the kidney. In isolated perfused proximal tubule segments, low concentrations of $1,25(OH)_2D_3$ antagonize the phosphaturic action of PTH.[563] In rats in which vitamin D deficiency was induced, but the diet was manipulated to maintain normocalcemia, normophosphatemia, and normal PTH levels, $1,25(OH)_2D_3$ stimulated tubular reabsorption of phosphate.[564] It was thought that $1,25(OH)_2D_3$ regulates phosphate reabsorption by direct modulation of Na-P cotransport in renal tubule cells.[565] However, data on rats subjected to a low phosphate diet suggest that the Na-P cotransport in the kidney cannot be explained by the $1,25(OH)_2D$–VDR axis.[566]

In patients with renal failure requiring dialysis, serum phosphate levels increase as a result of the decreased capacity of the kidney to excrete phosphate; the elevated serum phosphate inhibits $1,25(OH)_2D_3$ formation and leads to decreased serum Ca levels and to increased PTH secretion (secondary hyperparathyroidism). PTH would lead to worsening of hyperphosphatemia with more phosphate released from bone than excreted in the kidney with diminished PTH sensitivity, ultimately leading to renal osteodystrophy and possible calcium phosphate precipitation in tissues and vessels with an associated increase in cardiovascular events. The use of active vitamin D analogs in an attempt to remedy secondary hyperparathyroidism may increase Ca while worsening hyperphosphatemia, therefore increasing the Ca × P product and rendering CaP precipitation more likely. Phosphate binders can help decrease serum phosphate levels.

A new set of agents called calcimimetics have been approved in this setting (e.g., cinacalcet). They bind to CaSR in the parathyroids and increase its sensitivity to Ca, therefore counteracting the excessive PTH secretion in a dose-dependent manner and decreasing the Ca × P product.[567]

The presence of VDR on monocytes/macrophages and activated lymphocytes suggests that $1,25(OH)_2D_3$ plays a role in regulating the functions of these cells.[568]

Renoprotective Effects of Vitamin D

It has been demonstrated that $1,25(OH)_2D_3$ possesses renoprotective property against hyperglycemia-induced renal injury by suppressing the renal RAS. Evidence suggests that $1,25(OH)_2D_3$ is a negative endocrine regulator of renin biosynthesis and directly transrepresses renin gene transcription. $1,25(OH)_2D_3$ suppresses renin biosynthesis in mice, and vitamin D deficiency stimulates renin production. VDR knockout mice and those missing the 1α-hydroxylase enzyme develop hypertension and cardiac hypertrophy and high renin levels.[569] Also, diabetic mice lacking VDR develop more severe renal damage than wild type mice because of more robust activation of the intrarenal RAS, including more induction of renin and angiotensinogen.[570] Deb et al. demonstrated that $1,25(OH)_2D_3$ suppresses hyperglycemia-induced angiotensinogen expression in the kidney by blocking NF-κB activation of the angiotensinogen gene transcription.[571] Forman et al. showed that, among normotensive individuals, lower 25(OH)D levels were associated with higher circulating Ang II levels and a blunted renal plasma flow response to exogenous Ang II infusion, both findings consistent with activation of the RAS in the setting of lower plasma 25(OH)D.[572] Melamed et al. showed that low 25(OH)D levels are associated with the development of ESRD.[573] In animal studies, vitamin D and VDR agonists (VDRA) were shown to ameliorate glomerulosclerosis, glomerular hypertrophy and inflammation, podocyte hypertrophy, mesangial proliferation, albuminuria, and interstitial fibrosis.[574,575] These effects may be independent of BP and PTH. In the kidney, vitamin D may be important for maintaining podocyte health, preventing epithelial-to-mesenchymal transformation, and suppressing renin gene expression and inflammation. In human studies, CKD is associated with a very high prevalence of 25(OH)D deficiency. Emerging evidence in patients with CKD show that vitamin D can reduce proteinuria or albuminuria even in the presence of ACE inhibition.

In addition to reducing proteinuria, VDRA may reduce also insulin resistance, BP, and inflammation and preserve podocyte loss providing biologic plausibility to the notion that the use of VDRA may be associated with salubrious outcomes in patients with diabetic nephropathy.[575]

Fibroblast Growth Factor 23

FGF23, a 30-kDa protein primarily synthesized by osteoblasts and osteocytes, controls renal phosphate excretion by regulating renal Na-dependent phosphate cotransporters (NaPi2a and NaPi2c). It is phosphaturic and decreases 1α-hydroxylase levels by a VDR independent mechanism, and induces 24-hydroxylase activity, therefore reducing $1,25(OH)_2D_3$ by a VDR-mediated mechanism.[576] In vivo studies have shown that FGF23 is one of the most potent phosphatonins that induces renal phosphate wasting and reduction of $1,25(OH)_2D_3$.[577] Another unique characteristic of FGF23 is that this molecule derives from bone and exerts its hormonal effects in the kidney despite the ubiquitous presence of its receptors (FGFRs).

FGF23 directly acts on parathyroid glands and attenuates secretion of PTH in the presence of Klotho, an antiaging protein, as a cofactor. Klotho mutant mice display a phenotype identical to that of FGF23 null mice, both of which are characterized by premature aging–related phenotypes associated with hypercalcemia, hyperphosphatemia, and paradoxically high $1,25(OH)_2D$ levels. Klotho is predominantly expressed in the distal tubule of the kidney. Aside from its function as a cofactor for the stimulation of FGF-23, it also colocalizes with epithelial Ca channel transient receptor potential vallinoid-5 (TRPV5). Mice overexpressing the Klotho gene age slowly through a mechanism that involves insulin and oxidant stress resistance.[574] Klotho is expressed in limited tissues such as the kidney, parathyroid, and pituitary gland.[577]

The identification of FGF23 and Klotho as a physiologic regulator of phosphate and vitamin D metabolism has considerably advanced the understanding of the mineral and bone disorder in CKD. It is now clear that FGF23 plays a central role in the pathogenesis of altered mineral metabolism and secondary hyperparathyroidism in CKD patients.[577] The primary systemic stimuli of FGF23 secretion are increased $1,25(OH)_2D$ levels and increased dietary phosphorus intake. In kidney failure, FGF23 levels increase early and steadily rise with progression of kidney disease, likely as an appropriate physiologic adaptation to maintain normal phosphorus balance by helping to augment urinary phosphate excretion in conjunction with increased PTH levels and by decreasing gut phosphorus absorption through decreased $1,25(OH)_2D$. In the long term, this compensation may become maladaptive by causing a progressive decline in $1,25(OH)_2D$ levels with attendant consequences such as secondary hyperparathyroidism. Moreover, excess FGF23 levels have been independently linked with cardiovascular disease and mortality, suggesting that chronically elevated FGF23 levels may directly contribute to adverse CKD outcomes.[578]

FGF23 has been also implicated in the pathogenesis of X-linked hypophosphatemic rickets/osteomalacia (XLH), tumor-induced osteomalacia (TIO), and autosomal-dominant hypophosphatemic rickets/osteomalacia (ADHR)—all entities with common features, including hypophosphatemia because of renal phosphate wasting and impaired mineralization of bone with normal serum Ca and PTH.[579,580]

EICOSANOIDS

The eicosanoids are a group of locally acting hormones or autacoids that are derived from dietary polyunsaturated fatty acids. In humans, arachidonic acid, an essential fatty acid esterified into cellular membrane phospholipids, is the most abundant and important precursor. After deesterification by phospholipases, free arachidonic acid may either rapidly reesterify into membrane lipids, avidly bind intracellular proteins, or undergo enzymatic oxygenation to yield the various biologically active molecules referred to as eicosanoids. The type of product formed depends on the enzymes involved in the oxygenation process (Fig. 8.10).[581] Oxygenation of arachidonic acid by cyclooxygenase results in prostaglandin and thromboxane (TX) synthesis. Oxygenation by lipoxygenase generates hydroxyeicosatetraenoic acids and leukotrienes. These two major enzymatic pathways are all expressed in the kidney.[582,583] The specific nature of the products generated varies with both cell type and initial stimulus for arachidonic acid release. Eicosanoids have diverse biologic effects in the kidney, the significance of which will be discussed later.

Cyclooxygenase Products

Prostaglandins

Prostoglandins (PGs) are a unique group of cyclic fatty acids with diverse biologic effects that are produced throughout the body. The kidney is a major site of PG production, metabolism, and action.[584,585] PGs are important modulators of renal function in both physiologic and pathophysiologic settings. The spectrum of their effects in the kidney encompasses modulation of RBF, GFR, salt and water transport, and the release of renal hormones. It is within the setting of compromised renal status that maintenance of renal function is most dependent on PGs. Under these circumstances, inhibition of PG synthesis with nonsteroidal anti-inflammatory drugs (NSAIDs) is likely to impair renal function.[586]

Structure and Synthesis of Prostaglandins Arachidonic acid (eicosatetraenoic acid) is the major substrate for the synthesis of PGs in humans. The initial step is catalyzed by cyclooxygenase (COX), a major therapeutic target of analgesic, antipyretic, and anti-inflammatory actions of NSAIDs. COX converts arachidonic acid to PGH_2, which is subsequently metabolized by various PG synthases to more stable, biologically active, prostanoids, namely PGE_2, prostacyclin (PGI_2), $PGF_{2\alpha}$, PGD_2, and thromboxane A_2 (TxA_2) (Fig. 8.10). For a detailed description of the biosynthetic pathways leading to the

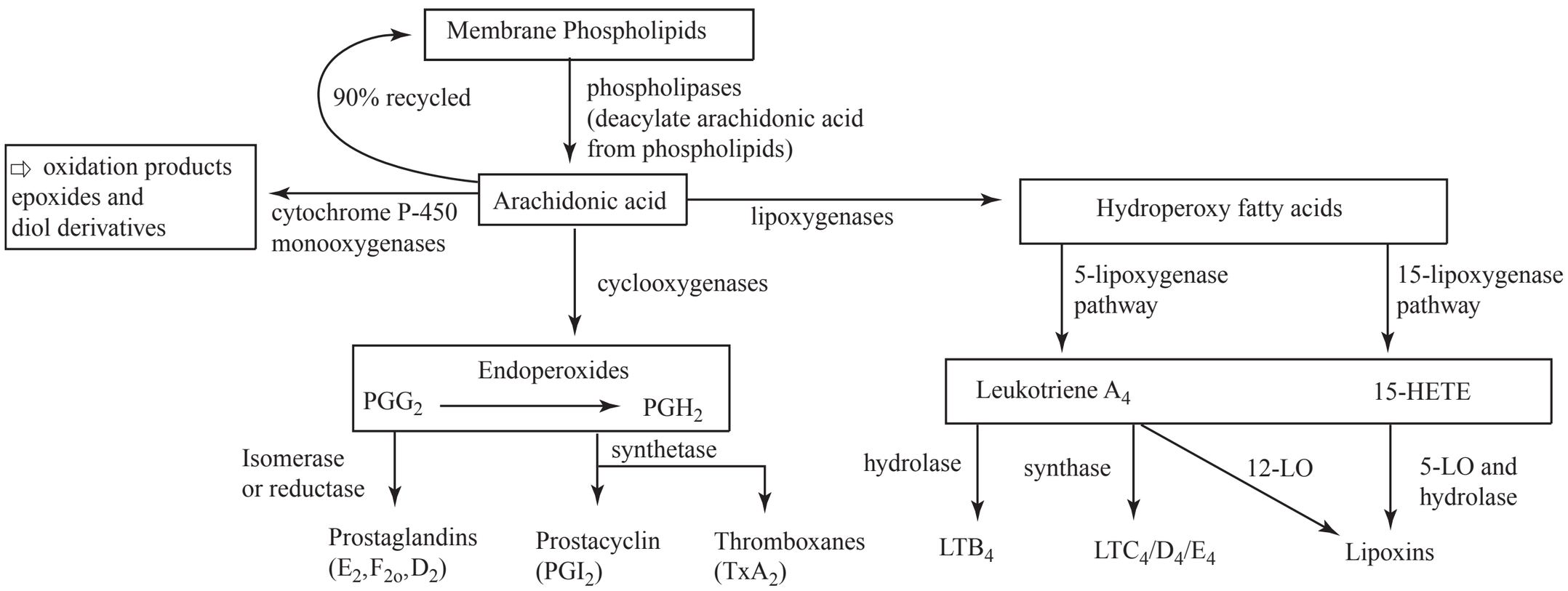

FIGURE 8.10 Renal eicosanoid synthesis. The oxygenated products of arachidonic-acid (eicosatetraenoic-acid) metabolism are referred to as eicosanoids. These include lipoxygenase, cytochrome P450 monooxygenase, and cyclooxygenase products. The lipoxygenase pathway yields hydroxy fatty acids and leukotrienes. The cytochrome P450 monooxygenase pathway yields ω-oxidation products and diol derivatives. The cyclooxygenase pathway yields the prostanoids, which include the prostaglandins (PGE_2, PGF_2, PGD_2, and prostacyclin) and thromboxane. The most important prostaglandins are dienoic (i.e., possessing two double bonds outside the ring structure)—hence the subscript 2.

different types of PGs and thromboxanes, collectively called prostanoids, the reader can refer to following reviews.[587,588]

The COX pathway is also the major pathway for arachidonic acid metabolism in the kidney.[582,583] In both animals and humans, two separate COX enzymes have been identified that are encoded by two separate genes: COX-1 (589) and COX-2 (590). The human COX-1 enzyme is constitutively present in the renal vasculature (arterial and arteriolar endothelial cells, mesangial cells),[583,587,591–593] glomerular epithelial cells,[594] renal interstitial cells,[587,595] along most segments of the tubule, although in markedly varying concentrations,[596–598] and in the medullary and papillary collecting ducts in humans, monkeys, dogs, rabbits, and rats.[587] The COX-2 enzyme, first thought to be only inducible in inflammatory responses, was found to be constitutive in the kidney.[599] Its expression is consistently focal and limited to the MD of the juxtaglomerular apparatus, epithelial cells of the thick ascending limb, and papillary interstitial cells of rats, rabbits, and dogs. In mice, COX-2 expression varied with developmental stages, with high levels of expression in the MD and thick ascending limbs of fetal kidneys and minimal expression upon renal maturation, suggesting a putative role for COX-2 in nephrogenesis.[600] In the adult human kidney, expression of the COX-2 protein has been observed in endothelial and smooth muscle cells of arteries and veins, and intraglomerularly in podocytes, but not in MD cells.[587] It is noteworthy that the distribution of prostanoid synthases within the kidney remains incompletely characterized.[601]

Physiology of Prostaglandins in the Kidneys The rate of PG production is dependent on the release of free arachidonic acid from tissue stores by phospholipase A_2 (PLA_2). Arachidonate tissue stores vary with dietary intake of essential fatty acids and can be depleted when intake is deficient.[602] Fish oil diets (rich in omega-3 polyunsaturated fatty acids) will compete for the arachidonate oxidation process and inhibit formation of active products.[603] Table 8.5 lists several modulators of the key steps involved in PG synthesis. Although under basal conditions both COX-1 and COX-2 contribute to prostanoid production in the kidney, some stimuli have been shown to initiate a more selective response. Evidence indicates that Ang II, through AT_1 receptors, increases MD COX-2 expression.[604,605] In addition, conditions associated with activation of the renin-angiotensin system, such as a low-salt diet or diuretic administration, all significantly increase MD COX-2 expression.[606,607] Recently, pressure was also shown to be an important promoter of renal COX-2 expression in renal medullary interstitial cells subjected to mechanical stress in vitro as well as in rats subjected to ureteral obstruction.[608] In contrast to COX-2, cortical expression of COX-1 is independent from Ang II but relies on hemodynamic changes.[604] Several renal pathophysiologic states such as glomerulonephritis, in addition to ureteral obstruction, are associated with increased prostanoid production.[609–611]

Prostanoids are rapidly degraded, which limits their effects to the immediate vicinity of their site of synthesis and accounts for their autocrine or paracrine function. They mediate diverse actions, in part related to their site of synthesis and the cells on which they act (Tables 8.6 and 8.7). Their principal physiologic role is mediation and/or modulation of hormone action at these locations.[583,587,592,612,613] Thus, cortical production by arterioles and glomeruli is related to regulation of RBF, GFR, and renin release. Other cortical sites of PG production affect ammoniagenesis[614] and calcium

8.5 Modulators of Prostaglandin Synthesis[a]

Modulator	Site of Action
Promoters	
Angiotensin II	PLA_2
AVP	PLA_2
Bradykinin	PLA_2
Norepinephrine	PLA_2
PAF	PLA_2
Interleukin-1	PLA_2 and COX
TNF-α	PLA_2
PDGF	COX
EGF	COX
Calcium	PLA_2
Diabetes	PLA_2
Ischemia	PLA_2
Chronic AVP therapy	COX
Ureteral obstruction	COX and TX synthase
Venous obstruction	COX
Glomerulonephritis	COX
Nephrotic syndrome	TX synthase
Inhibitors	
Glucocorticolds	PLA_2 and COX
Potassium	PLA_2
Urea	PLA_2
Mepacrine	PLA_2
NSAIDs	COX

[a]Note that hormones are important physiologic modulators of prostaglandin production. AVP, arginine vasopressin; PAF, platelet-activating factor; TNF-α, tumor necrosis factor-α; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; NSAIDs, nonsteroidal anti-inflammatory drugs; PLA, phospholipase A; COX, cyclooxygenase; TX, thromboxane.

and phosphate transport.[615] Medullary PG production is directed to regulating vasa recta blood flow, tubular sodium and chloride transport, and the response of the collecting duct to vasopressin. Inhibition of COX activity in the absence of exogenous administration or endogenous release of hormones such as Ang II, NE, or vasopressin has little effect on renal functional parameters.[616] Once their local release is enhanced, COX products may themselves stimulate the local generation of other hormones. Under pathophysiologic conditions, such as inflammatory injury, local release of prostanoids may mediate some of the functional derangements that characterize these conditions.[609–611]

Prostanoids act through specific and distinct receptors.[617,618] These receptors are members of the G protein-coupled family of receptors. In the kidney, they mainly include the E-prostanoid (EP), F-prostanoid (FP), I-prostanoid (IP), and T-prostanoid (TP) receptors, which respectively interact with PGE_2, $PGF_{2\alpha}$, PGI_2, or TxA_2 (Fig. 8.1). Multiple subtypes of each of these prostanoid receptors may exist, as in the case with the PGE_2 receptor (EP receptor), thus explaining the apparently contrasting effects mediated by PGE_2 on smooth muscle and collecting duct permeability to water.[619] The differential sensitivity of tissues to several structural PGE analogs has led to the identification of at least four distinct EP receptors: the two vasodilator receptors, EP_2 and EP_4, and the two vasoconstrictor receptors, EP_1 and EP_3.[620] EP_1 receptors signal mainly by IP_3-mediated increased intracellular Ca^{2+}.[621–623] In contrast, the vasodilator receptors EP_2 and EP_4, signal through increased cAMP.[624–626] EP_3 receptors constrict smooth muscle, probably by inhibiting cAMP generation via a pertussis toxin-sensitive, G_i-coupled mechanism.[627,628] In mesangial cells, the $PGF_{2\alpha}$ receptor (FP receptor) seems to be coupled to increased intracellular Ca^{2+}. At higher concentrations, $PGF_{2\alpha}$ also stimulates EP receptors.[629,630] The TXA_2 receptor (TP receptor) appears to signal via phosphatidylinositol hydrolysis, leading to increased intracellular Ca^{2+}.[631] There is pharmacologic evidence for existence of TP receptors in the glomerulus.[629] The PGI_2 receptor (IP receptor) signals via stimulation of cAMP generation.[632,633] PGI_2 has been demonstrated to play an important vasodilator role in the glomerular microvasculature, where the effects of PGI_2 and PGE_2 to stimulate cAMP generation were distinct and additive.[634] Thus, because multiple PGs can be synthesized through the COX pathway and because these PGs can interact with different receptors, their pathophysiologic effects are further diversified and depend on which prostanoid is produced and which receptor is available locally.

Renal Hemodynamics There are some species differences in the renal actions of PGs, and this must be taken into account when extrapolating data from animals to humans. Although acute inhibition of PG synthesis does not change arterial pressure in normal circumstances, it does produce both an increase in renal vascular resistance and a decrease in sodium and water excretion.[635–637] In general, PGE_2 and PGI_2 are vasodilators in most species, whereas TXA_2, $PGF_{2\alpha}$, and PGE_2 (in certain circumstances) are vasoconstrictors.[584,638,639] The contribution of these vasoactive properties of COX products to the regulation of renal vascular tone under normal physiologic conditions is probably minimal.[640–645]

In contrast, the local release of vasodilator PGs (PGE_2 and PGI_2) in response to renal vasoconstrictors plays an important role in maintaining RBF and GFR. There is compelling evidence indicating that mesangial cell synthesis and release of PGE_2 and PGI_2 modulate the constrictor actions of Ang II, NE, and AVP.[583,584,587,629,646] Activation of the renin-angiotensin and sympathetic nervous systems leading to enhanced release of angiotensin, catecholamines, and AVP occurs in conditions, such as hemorrhage, volume depletion, general anesthesia, cirrhosis, and cardiac failure. While serving to maintain the systemic blood pressure, these hormones constrict mesangial cells and glomerular

8.6 Renal Actions of Eicosanoids

	Product Pathway		
Action	**Cyclooxygenase**	**P450 Epoxygenase**	**Lipoxygenase**
Vascular			
Constriction	TXA_2	5,6-EET 20-HETE	LTD_4, LTC_4 LXA_4, LXB_4
Dilation	PGE_2, PGI_2	5,6-EET	LXA_4, cyclooxygenase-dependent
Mesangial			
Contraction	TXA_2, $PGF_{2\alpha}$	LTD_4, LTC_4	LTC_4
Relaxation	PGE_2, PGI_2		
Mitogenic	$PGF_{2\alpha}$, TXA_2		
Antimitogenic	PGI_2, PGE_2		
Na transport			
Inhibition	PGE_2	5,6-EET	
Na-K-ATPase			
Inhibition	PGE_2	11,12-DHT	
Stimulation		19-HETE	
Water transport			
Inhibition	PGE_2	EETs, DHTs	

TXA, thromboxane A; PGE, prostaglandin E; PGI, prostaglandin I; PGF, prostaglandin F; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; DHT, dihydrotestosterone; LTD, leukotriene D; LTC, leukotriene C; LXA, lipoxin A; LXB, lipoxin B. See text for references.

8.7 Renal Actions of the Different Prostanoids

Mediator	**Main Source**	**Primary Effects**
PGE_2	Tubular epithelial cells Interstitial cells	Renal vasodilation Relaxation of mesangial cells Modulation of glomerular capillary ultrafiltration coefficient Stimulates renin release from juxtaglomerular apparatus Antagonizes hydroosmotic effect of ADH in collecting tubular epithelial cells Inhibits sodium chloride reabsorption Mediates renal response to loop diuretics
PGI_2	Vascular and glomerular endothelial cells	Renal vasodilation Relaxation of mesangial cells Stimulates renin release from juxtaglomerular apparatus
$PGF_{2\alpha}$	Mesangial cells	Contracts smooth muscle Glomeruli
TXA_2	Glomeruli	Contracts smooth muscle Contracts mesangial cells

ADH, antidiuretic hormone; TXA, thromboxane A; PGE, prostaglandin E; PGI, prostaglandin I; PGF, prostaglandin F.

arterioles. Fortunately, their enhancement of renal PG release locally opposes their constrictor effects. The vasodilatory action of PGs on the afferent arteriole serves to maintain renal perfusion, whereas their relaxant effects on mesangial cells maintains the effective surface area for filtration.[638] Inhibition of PG generation in these circumstances is associated with a dramatic fall in RBF and GFR.[645–648] Vasodilator PGs, in particular PGI_2, may also counteract the vasoconstrictor responses to calcium in human subjects.[638] In addition to modulating the effects of vasoconstrictors, endogenous PGs mediate the actions of some vasodilator agents. These include a role for PGI_2 in mediating the vasorelaxant actions of dopamine[650] and magnesium[651] in humans.

Finally, PGs synthesized from the MD can trigger renin release,[652] which leads to increased Ang II levels. Ang II preferentially constricts the glomerular efferent arteriole, thus increasing intraglomerular pressure and, ultimately, maintaining GFR in volume-contracted conditions.[653] This response is further reinforced by the PGE_2-induced afferent vasodilation. In contrast, TXA_2 exerts a negative effect on renin release.[654] However, inhibition of COX activity reduces plasma renin activity, suggesting that the predominant influence of prostanoids is stimulatory. PG-mediated renin release is independent of β-adrenergic mechanisms.[655] It has been recently reported that a decrease in extracellular tonicity leads to renin release through a mechanism involving aquaporin 1–mediated water influx in juxtaglomerular cells and PG-dependent formation of cAMP and activation of PKA.[645]

Solute Excretion Infusion of arachidonic acid or the COX products PGE_2 or PGI_2 directly into the renal artery results in natriuresis.[656,657] Natriuresis is largely a direct tubular phenomenon originating in the distal nephron.[657] PGE_2 has mild or no effects on sodium transport in the proximal tubule and most segments of the ascending limb of Henle, with the exception of the medullary thick ascending limb in some species.[658] This lack of effect is in keeping with both the low rates of PG production and the low density of PG receptors in these nephron segments.[597,598] Under normal circumstances, inhibition of COX does not result in alteration of sodium delivery out of the loop of Henle to the early distal tubule.[659] PGE_2, however, has significant effects on sodium transport in the collecting duct, where it inhibits transepithelial sodium transport.[656,658] In fact, in most mammalian species, the collecting ducts are the major nephron segments responsible for PG synthesis[597,598] and, along with the MTAL, express the majority of receptors for PGE_2 in the kidney.[660,661]

There is evidence that PGE_2 exerts its inhibitory effect on rabbit CCD sodium transport by at least two mechanisms. The first involves inhibiting principal cell basolateral Na^+-K^+-ATPase activity[662–664] and the second by directly decreasing the open probability of the apical amiloride-sensitive sodium channels.[665,666] PGE_2 utilizes multiple signal transduction pathways in the CCD. These include increase in intracellular Ca^{2+}, activation of PKC, and modulation of cAMP levels.[584] The inhibitory effects of PGE_2 on sodium transport in the thick ascending limb probably involve inhibition of adenyl cyclase.[667] Through G_i-coupled EP_3 receptor expressed in the thick ascending limb,[668,669] PGE_2 also blocks the phosphaturic action of PTH in the proximal tubule.[670] Recent evidence obtained from a randomized, placebo-controlled, crossover study in healthy humans suggest that PGs may modulate sodium excretion through ENaC regulation in the distal nephron.[671] Natriuresis may also be regulated by renal medullary COX-derived prostanoids acting in a paracrine manner. The prostanoid receptors regulating renal medullary blood flow through the descending vasa recta seem to be EP2, EP4, and/or IP receptors.[672] The importance of EP2 and IP receptors has been confirmed to be associated with salt-sensitive hypertension in animal models of EP2 and IP receptor deficiency.[673,674] Expression of EP3 in the renal medulla was shown to be induced by hypertonicity, suggesting a role for this receptor as well in natriuresis and protection of the renal medulla. EP3 activation leads to inhibition of Na^+-K^+-$2Cl^-$ transporter and aquaporin 2.[675]

Sodium loading is associated with an increase in urinary PG excretion, yet PGs are not important in the regulation of sodium balance in normal euvolemic subjects. However, in circumstances associated with sodium retention and compromised renal function, PGs play a significant role. In fact, high-salt diet increases renal medullary COX-2 and microsomal prostaglandin E synthase-1 (mPGES-1) expression to maintain blood pressure homeostasis.[676–678] Inhibition of PG synthesis or blocking their effects in such conditions is associated with sodium retention.[647,679,680] Administration of furosemide is associated with increased PG excretion, which is, in part, mediated by a direct action on tubular cells.[681,682] Administration of NSAIDs diminishes the natriuretic action of furosemide and other loop diuretics, suggesting a role for PGs in mediating the action of these agents. This cannot be the sole mechanism, however, for the natriuretic response to diuretics outlasts the increase in PG excretion.

Water Excretion PGs, especially PGE_2, affect water transport in the collecting duct in many ways. PGs may indirectly regulate water excretion by a reduction of the corticomedullary osmotic gradient via inhibition of solute transport in the thick ascending limbs or by increase in renal medullary blood flow. In fact, during physiologic stress, PGE_2 dilates descending vasa recta, thereby buffering the constrictor effects of Ang II, AVP, and catecholamines, which is vital to prevent hypoxic damage to renal medullary cells.[683] Moreover, PGs of the E series blunt the hydraulic conductivity response of the collecting duct to AVP.[684,685] In fact, in vivo infusions of arachidonic acid or PGE_2 induce water diuresis, whereas inhibition of PG synthesis potentiates the urinary hyperosmolality caused by AVP.[686] However, in the absence of vasopressin, basolateral PGE_2 actually increases osmotic water reabsorption.[687,688] These effects on water conductivity in the collecting ducts have been explained by changes in cAMP accumulation. AVP mediates the increase in water conductivity in the collecting duct through increased cAMP generation.

Studies on the effect of PGE_2 on cAMP metabolism in this nephron segment demonstrated that PGE_2 could both stimulate basal cAMP generation and suppress AVP-stimulated cAMP generation.[689,690] The inhibitory effects of PGE_2 on AVP-stimulated cAMP generation and water conductivity in the collecting duct are probably mediated through the EP_3 receptor, as discussed previously.[675,690,691] In addition to affecting water flow via modulation of cAMP levels, PGE_2 has been shown to inhibit AVP-induced water conductivity by activation of PKC and elevation of intracellular calcium.[619,692]

Application of basolateral PGE_2 probably increases water absorption in the collecting duct by stimulating cAMP production.[687,688] The EP_4 receptor, which is found on the epithelial cells of the ureter, bladder, and collecting duct, is coupled to the G_s-stimulated cAMP signaling pathway.[625,693] This suggests that an EP_4 receptor mediates cAMP-stimulated water absorption in the collecting duct. Recently, PGE_2 was shown to mediate lithium-induced polyuria by downregulating the expression of aquaporin 2 and the Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) in the medulla but not in the renal cortex.[694]

Another facet of the PGE_2-AVP interaction is that AVP acutely stimulates endogenous PGE_2 production by the collecting duct. This effect has been demonstrated in rats[695,696] and the current consensus is that it also occurs in humans.[696,697] It also suggests that PGE_2 participates in a negative feedback loop, whereby endogenous PGE_2 production dampens the action of AVP. In agreement with a functional role for the increase in urinary PGE_2 production seen during AVP infusions are numerous observations of enhanced renal concentrating ability in animals or humans pretreated with inhibitors of PG production.[698,699] Given that the concentration of AVP needed to stimulate PGE_2 production is 10 to 100 times the concentration needed to maximally stimulate water conductivity, it is controversial whether AVP plays a physiologic role in PGE_2 generation.[700] At these concentrations, AVP has been shown to acutely increase intracellular calcium and activate PKC, via activation of phospholipase C.[700,701]

Other Effects on Renal Function The tubuloglomerular feedback (TGF) response is modulated by multiple autacoids and hormones including ATP, Ang II, NO, and PGs, including PGE_2, PGI_2, and TxA_2.[702,703] Under basal conditions, MD COX-2 directly modulates TGF response, particularly through TXA_2,[702] with little contribution of COX-1, as well as via inhibition of nNOS-dependent NO.[704] When subjected to low-dose chronic Ang II, the increase in TGF response is mediated by the local release of vasoconstrictive COX-1 PGs.[705] These studies suggest that: (1) both COX isoforms regulate TGF but (2) their contribution differs in various settings.

Clinical Pathophysiologic Role of Prostaglandins in the Kidneys

PGs, through their vasodilator effects, play a salutary role in maintaining RBF and GFR in several prerenal conditions such as hemorrhage, septic shock, cirrhosis, and low cardiac output states. Studies in patients with congestive heart failure have confirmed that enhanced PG synthesis is crucial in protecting kidneys from the effects of elevated vasoconstrictor levels in these patients.[706] Renal artery stenosis is another condition associated with increased renal PG secretion[707] that may locally act to enhance renal perfusion. Administration of COX inhibitors in these settings with renal hypoperfusion is associated with adverse effects on RBF and GFR.[708]

With regard to intrinsic renal diseases, COX products have been implicated in modulating or mediating renal injury (or both). Single nephron GFRs increase significantly after renal ablation and are associated with increased glomerular synthesis and urinary excretion of prostanoids by the remaining nephron.[709,710] In this model of renal ablation, increased expression of COX-2 has been described and inhibition of COX-2 was associated with amelioration of the renal functional changes associated with renal ablation.[709,710] Similar results were observed with omega-3 fatty acid[711] or flax oil[712] supplementation after renal ablation. Selective inhibition of TXA_2 synthesis is associated with an increase in GFR, lessening of proteinuria, and preservation of renal histology.[713,714] In addition, COX-2 inhibition was also found to slow progression in the rat model of polycystic kidney disease.[715] Dietary soy protein was recently shown to decrease production of prostanoids and expression of COX in a model of polycystic kidney disease and to be associated with reduced disease progression.[716]

Enhanced TXA_2 production has been implicated in the pathophysiology of the intense vasoconstriction that characterizes the obstructed kidney[717,718] and in mediating the decrease in RBF and GFR that occurs in the early phase of nephrotoxic serum nephritis.[609,610,719,720] In patients with lupus nephritis, an inverse relation between TXA_2 biosynthesis and GFR has been proposed.[721,722] In this setting, renal function improved after short-term therapy with a TX receptor antagonist, but not with aspirin.[721,723] In addition, administration of TXA_2 synthesis inhibitors or receptor antagonists has been associated with improved renal function in animals with allograft rejection and cyclosporine toxicity.[638] Although basal COX-2 levels are important for podocyte survival, overexpression of COX-2 in podocytes leads to increased albuminuria and glomerular injury, partly through activation of the thromboxane receptor.[724,725] Jia et al. also recently showed that cisplatin-induced renal injury is mediated by activation of the COX-2/mPGES-1 pathway, which may offer a new therapeutic target for management of the adverse effect of cisplatin chemotherapy.[726]

Ischemia/reperfusion injury has been shown to be worsened by COX-2 inhibitors or in COX-2 knockout mice, suggesting that PGs protect the kidney against acute renal injury.[727] Recent studies show that EP4 and EP2 receptor agonists improve renal function and/or increase survival rate of a rat model of acute renal failure, supporting a protective role of EP2 and EP4 receptors in preventing the progression of kidney failure.[728] Lithium treatment was also shown to improve outcome of renal ischemia/reperfusion

injury through NO and/or COX pathways and represents a promising therapeutic approach to boost renal viability and function after ischemia/reperfusion injury in the setting of transplantation.[729] However, other groups reported a beneficial effect of COX-2 inhibitors that could be acting through immune modulation.[730,731] These opposite findings could be partly explained by differences in the experimental models.

The role of COX products in mediating diabetic nephropathy remains controversial. Vasodilator PGs may contribute to the hyperfiltration that occurs in early stages of diabetic nephropathy, whereas TXA_2 may play a role in the subsequent development of albuminuria and basement membrane changes.[732,733] MD COX-2 expression is increased in models of hyperfiltration, such as high-protein diet and diabetes. COX-2 inhibition decreases hyperfiltration and proteinuria, and inhibits development of glomerular sclerosis in experimental diabetes.[734,735] Interestingly, inhibition of COX-2 in patients with type 1 diabetes mellitus reduces, but does not correct, hyperfiltration in subjects whose baseline GFR was $\geq$135 mL/min^{-1}/1.73 m^{-2}.[736] However, in response to COX-2 inhibition, women with type 1 diabetes mellitus exhibited a significant renal hyperfiltration response suggesting that women have a greater dependence on vasodilatory PGs than men.[737] A role for decreased PGI_2 synthesis in type IV renal tubular acidosis associated with diabetes mellitus has also been suggested.[738] The increased renal production of TXA_2 and PGI_2 in type 2 diabetes has also been suggested as a role for these compounds in the pathogenesis of diabetic nephropathy.[733,739,740] Recently, the lipocalin-type PGD2 synthase (L-PGDS) knockout mouse was shown to develop structural changes associated with diabetic nephropathy, such as glomerular hypertrophy, fibrosis, and basement membrane thickening.[741] Urinary excretion of L-PGDS, found at elevated levels in type 2 diabetic patients, correlates with the progression of diabetic nephropathy and reflects the underlying early increase in glomerular damage.[742]

Diminished vasodilator renal PG synthesis has also been implicated in the pathogenesis of the severe sodium retention that occurs in patients with the hepatorenal syndrome.[743] Pregnancy is associated with increased glomerular synthesis and urinary excretion of PGE_2, $PGF_{2\alpha}$, and PGI_2.[744] Augmented renal vasodilator PG production does not appear to regulate GFR and RBF in normal pregnancy; however, diminished synthesis of PGI_2 has been demonstrated in human and animal models with pregnancy-induced hypertension.[745–747] A beneficial effect of reducing TXA_2 generation, while preserving PGI_2 synthesis, by low-dose (60 to 100 mg per day) aspirin therapy has been proposed in patients at risk for pregnancy-induced hypertension.[748,749] In patients with hypertension, COX inhibition by NSAIDs is associated with increased salt retention and resistance to the diuretic action of thiazides and furosemide.[750,751] Short-term use of some NSAIDs was found to increase the mean arterial pressure of hypertensive patients.[752,753] On the other hand, attempts to treat hypertension with PG analogs have generally been disappointing.[754,755]

Finally, chronic inhibition of COX by regular use of NSAIDs leads to gastrointestinal toxicity and may increase the risk of chronic renal disease, especially in older patients and patients with heart disease.[756–758] Selective COX-2 inhibitors have been developed and have been shown to spare gastric PG production. These nontraditional COX-2 selective anti-inflammatory agents might have represented a significant advance for the treatment of acute and chronic inflammatory disorders[759–761]; however, the safety of their use has been placed into question after two large prospective cohorts in elderly patients showed an increased risk of cardiovascular events in patients on selective COX-2 inhibitors versus those on NSAIDs.[762,763] Clinical studies have also demonstrated that selective COX-2 inhibitors can even reduce GFR and RBF in physiologically stressed volunteers or patients.[764,765]

In summary, prostanoids exert diverse and complex functions in the kidney under physiologic and pathologic conditions. Further understanding of pathways involving and regulating COX, prostanoid synthases, and prostanoid receptors should provide targets for pharmacologic treatments of renal disease.

LIPOXYGENASE PRODUCTS

Biosynthesis and Metabolism

Lipoxygenases (LOs) comprise a family of enzymes capable of mediating selective lipid oxidation.[766] Enzymatic lipoxygenation of arachidonic acid leads to the generation of leukotrienes (LTs), lipoxins (LXs), and hydroxyeicosatetraenoic acids (HETEs). Formation of these compounds is initiated by 5-, 12-, or 15-lipoxygenase, whereby a hydroperoxy group is introduced onto arachidonic acid at carbon-5, carbon-12, or carbon-15, respectively, to yield the corresponding 5-, 12-, or 15-hydroperoxytetraenoic acid (HPETE). HPETEs are unstable compounds that are transformed into the corresponding 5-, 12-, and 15-HETE, which, in turn, undergo enzymatic modification leading to the generation of the various LTs and LXs. The 5-lipoxygenase pathway is a major route of arachidonic acid metabolism in the polymorphonuclear cells and macrophages leading to the formation of 5-HETE and LTs.[767–769] 5-Lipoxygenase requires activation by a cell membrane-bound protein called the 5-lipoxygenase-activating protein (FLAP).[770] The 15-lipoxygenase enzyme catalyzes the production of 15-HETE and initiates another major pathway of arachidonic acid metabolism in leukocytes. In activated neutrophils and macrophages, sequential lipoxygenation of arachidonic acid at carbons-15 and -5 yields trihydroxy derivatives, the LXs.

Renal Actions of Lipoxygenase Products

In the rat, 5-LOX FLAP + 12-LOX mRNA are present in the glomerulus as leukotriene B_4 (LTB_4) and leukotriene D_4 (LTD_4) receptors.[771] 15-LOX is localized to the distal nephron only.[771] Products of lipoxygenase are classically proinflammatory;

however, lipoxins, 15-HPETE, and 15-HETE exhibit anti-inflammatory activity.[674]

The LTs are potent proinflammatory molecules. LTB_4 has minimal spasmogenic properties, but is the most potent chemotactic substance yet described for polymorphonuclear cells, and promotes their activation and adhesion to the endothelium.[767] It has no significant effects on renal hemodynamics in normal animals, but amplifies glomerular inflammation and proteinuria in animals with glomerulonephritic injury.[773] The peptidyl LTs contract vascular, pulmonary, and gastrointestinal smooth muscle and increase vascular permeability to macromolecules.[767] LTC_4 and LTD_4 exert potent effects on glomerular hemodynamics. In rats, systemic administration of LTC_4 leads to reduction in RBF and GFR.[774] Similarly, infusion of either LTC_4 or LTD_4 in the isolated perfused kidney results in dramatic increase in renal vascular resistance and reduction in GFR.[775] LTD_4 mediates these effects by causing a significant increase in efferent arteriolar resistance, leading to a fall in glomerular plasma flow rate (Q_A), and a rise in glomerular capillary hydraulic pressure (P_{GC}). In addition, it markedly reduces the glomerular capillary ultrafiltration coefficient (K_f) and, therefore, its overall effect is to decrease single nephron GFR.[776] LTC_4 and LTD_4 contract mesangial cells[777,778] and LTD_4 stimulates neutrophil adhesion to these cells.[779] In both rats and humans, specific mesangial cell LTD_4 receptors have been identified. Intracellular signaling for LTD_4 in these cells involves receptor-activated phosphatidylinositol diphosphate (PIP_2) hydrolysis, release of inositol phosphates, and increased intracellular calcium concentrations.[780,781]

LXA_4 attenuates LTB_4-induced neutrophil chemotaxis and inhibits natural killer cell cytotoxicity.[767,769] The effects of LXA_4 are mediated primarily by functional high-affinity LXA_4 receptors.[782] In rat glomerular mesangial cells, LXA_4 competes with LTD_4 at a common receptor whereby LXA_4 mediates partial agonist–antagonist effects.[783] Different LXs display distinct effects on renal hemodynamics.[769,784,785] In rats, LXA_4 causes a selective decrease in afferent arteriolar resistance, thereby increasing RBF, glomerular capillary pressure, and GFR. The LXA_4-induced increase in GFR, however, is partially offset by its mild effect in decreasing K.[783,785] The vasodilator actions of LXA_4 are mediated by prostaglandins.

Role of Lipoxygenase Products in Kidney Disease

LTs are increasingly recognized as major mediators of glomerular hemodynamic and structural deterioration during the early phases of experimentally induced glomerulonephritis.[784,786,787]

Mesangial cell (MC) proliferation is a central event in the pathogenesis of glomerulonephritis. LTD_4-induced proliferation of mesangial cells is modulated by LXA_4.

Increased glomerular generation of LTB_4 and peptidyl-LTs has been demonstrated in several models of glomerular injury.[784,786,787] LTB_4 probably worsens glomerular injury by augmenting leukocyte recruitment and activation and the peptidyl LTs, by depressing K_f and GFR.[773–779] Selective blockade of the 5-lipoxygenase pathway, in the course of glomerular injury, is associated with significant amelioration of the deterioration of renal hemodynamic and structural parameters.[788,789] In addition, dietary deprivation of essential fatty acids, which results in arachidonic acid and eicosanoid deficiency, confers protection against the histopathologic and the functional consequences of immune-initiated injury in the glomerulus.[790] In hemodialysis patients, 5-LOX activity and expression are significantly increased in peripheral blood monocytes, and can be markedly suppressed by polyunsaturated fatty acid supplementation.[1] In human glomerulonephritis, 5-lipoxygenase and 5-LO-activating protein (FLAP) mRNA expression have been detected in kidney biopsy specimens from patients with immunoglobulin A (IgA) nephropathy and mesangial proliferative glomerulonephritis and were associated with a clinically worse renal status.[791] Urinary LTE_4 levels are also elevated in patients with active SLE.[792] A pathophysiologic role for LTs has also been described in experimental acute allograft rejection,[793] cyclosporine toxicity, and acute ureteral obstruction.[787] LXA_4 and 15-S-HETE are also generated during experimental glomerular injury and may exert salutary effects on glomerular function by antagonizing the proinflammatory actions of LTs.[784,794–797] In animals with experimental glomerulonephritis, 5-lipoxygenase inhibition results in marked reduction in proteinuria and preservation of GFR.[786,798] The binding of 5-lipoxygenase to FLAP is a prerequisite for subsequent formation of leukotrienes from arachidonic acid.[799] The use of a FLAP antagonist has been shown to reduce proteinuria and restore glomerular size selectivity in human glomerulonephritis.[800] 12/15-lipoxygenase (12/15-LO) expression is increased in high glucose (HG)-stimulated MC and in experimental diabetic nephropathy.[801–803]

ENDOTHELIN

Endothelin (ET), originally isolated from porcine aortic endothelial cells, is an extremely potent and long-lasting vasoconstrictor.[804] Initially, ET's effect on vascular tone regulation was the focus of research. Later, other actions—such as ET effects on cell growth and proliferation, ion transport, eicosanoid synthesis, renin and ANP release, fibrosis, and inflammation—emerged as important factors linking ET to diseases.[771,805,806] The kidney is an important site of ET production and expresses a high density of ET receptors.[807] Endothelin may, therefore, act in an autocrine and paracrine manner to influence renal hemodynamics, tubular function, and mesangial cell biology.

Biochemistry, Synthesis, and Receptor Biology

The term endothelin refers to a family of homologous 21-amino acid vasoconstrictor peptides found in three distinct isoforms: ET-1, ET-2, and ET-3. In humans, ET isoforms

are encoded by three separate genes located on chromosomes 6, 1, and 20, respectively.[808,809] The initial ET peptide translation product is a large (approximately 200 amino acids) isopeptide-specific prohormone named preproendothelin. Posttranslational processing of this prohormone to mature ET requires two steps. The first involves its proteolytic cleavage by dibasic pair-specific endopeptidases on Lys-Arg and Arg-Arg pairs, which respectively flank the N- and C-terminals of the preproendothelin molecule, to yield an intermediate 38- or 39-amino acid proET polypeptide. The subsequent step is accomplished by proteolytic cleavage of proET between Trp^{21} and Val^{22} by a putative endothelin-converting enzyme (ECE).[810] All ET isopeptides have a hairpin loop configuration structure imparted by two intrachain disulfide bonds bridging amino acid residues 1 through 15 and 3 through 11, the reduction of which leads to a twofold loss of biologic activity.[811] The two endothelin isoforms ET-2 and ET-3 differ from ET-1 in 2 and 6 amino acid residues, respectively. The three ET isoforms are highly homologous in their amino acid sequences and tertiary structure to certain scorpion and snake venoms, the sarafotoxins, which suggests common genetic evolutionary origins.[812] Although all isoforms of ET are potent vasoconstrictors, there are significant cell- and tissue-specific differences in the secretion of, and biologic responses to, different isoforms.[813,814]

ECEs are metalloproteases that belong to the M13 group of proteins, a family including several proteins such as neutral endopeptidases, X-converting enzyme, ECEs, and others.[815] Three major ECE isoforms have been identified to date and named ECE-1, ECE-2, and ECE-3, of which ECE-1 and ECE-2 are the most prominent.[820] The ECEs differ from each other regarding cell/tissue distribution, localization, pH of optimal activity, and substrate specificity.[815] Four variants of ECE-1 have been reported in humans (ECE-1a, ECE-1b, ECE-1c, and ECE-1d), and two ECE-2 variants (ECE-2a and ECE-2b).[809] Both ECE-1 and ECE-2 cleave big ET-1 more efficiently than either big ET-2 or big ET-3. ECE-1 is expressed ubiquitously with highest expression in endothelium, lung, ovary, testis, and adrenal medulla, whereas ECE-2 is expressed in neural tissues. ECE-3, which selectively cleaves ET-3, has been found in the bovine iris.[816] ECE-1 was located at the cell surface and on intracellular vesicles.[817] ECE-1 also hydrolyzes BK, substance P, and insulin.[809] Not all the enzymes responsible for the final step of posttranslational processing of ET-1 have been identified. Current evidence suggests that other proteases may be involved in the final processing of ET, because mice lacking ECE-1 and ECE-2 can still produce mature ET-1.[818] In addition, alternative, ECE-independent pathways have been suggested possibly involving tissue chymases and non-ECE metalloproteinases.[819] Recently, several inhibitors specific for the ET-converting enzyme have been reported.[820]

Initial studies identified ET on the basis of its release from large-vessel endothelial cells. Since then, ET immunoreactivity has been detected in the kidney, spleen, skeletal muscle, and lung.[821] In the kidney, the arcuate arteries, veins, glomerular arterioles, and capillaries are a rich source of ET.[20] In the glomerulus, there is evidence for ET secretion by mesangial, endothelial, and epithelial cells.[823] In the rest of the nephron, the internal medullary collecting duct (IMCD) has been demonstrated to be a major site of ET-1 and ET-3 production.[823–825]

Normally, blood vessels produce very little ET, and the normal circulating level of ET is extremely low.[24] Secretion of ET by endothelial cells is controlled at the level of transcription and these cells do not store ET for future release.[810,827] Thus ET-1 probably acts primarily as a paracrine/autocrine mediator and not as a circulating hormone.[809] ET peptide secretion is upregulated by various humoral mediators, such as thrombin, BK, insulin, Ang II, AVP, endotoxin, IL-1, TGF-β, and tumor necrosis factor (TNF).[828] These mediators may be responsible for the increase in ET observed in various pathophysiologic states. Hypoxia is also an important stimulus for ET production.[829] In the kidney, increasing osmolarity serves as a stimulus for tubular production of ET.[830] On the other hand, NO, ANP, and prostacyclin exert inhibitory influences on ET synthesis and release.[831] In summary, mesangial cells' ET-1 release is under complex regulation, with vasoconstrictor, profibrotic, inflammatory, and proliferative agents augmenting its release, whereas vasorelaxant agents tend to inhibit its production.[832]

Endothelins exert their actions through two major receptor subtypes known as ET_A and ET_B receptors. These belong to the superfamily of G-protein coupled receptors and have been identified in a variety of tissues.[833] A third type of ET receptors, ET_C, has been cloned from Xenopus laevis.[834] Both ET_A and ET_B receptors have widespread distribution and are abundantly expressed in the kidneys. The ET_A receptor is believed to be involved in vasoconstrictive and proliferative responses to ET-1 and binds endothelins with the following affinity: ET-1 > ET-2 > ET-3.[809] ET_B receptor, on the other hand, is a nonisofrom selective receptor and recognizes all three ET isoforms with equal affinity. Its activation induces transient vasodilation. The recently cloned ET_C receptor binds preferentially ET-3 and its stimulation causes NO release.[823,833,834] Both ET_A and ET_B receptors are expressed on vascular smooth muscle.

The kidney expresses abundant mRNA transcripts for ET_A and ET_B receptors.[823,833,835] Expression of ET receptors is especially prominent in the renal artery, glomerular arterioles, endothelium, mesangium, vasa recta bundles, and collecting duct.[771,823,833] Both receptor subtypes are expressed in the glomerulus. Vascular smooth muscle cells of the arcuate arteries and the renal medullary interstitial cells display ET_A receptors. Epithelial cells of the cortical, inner medullary, and outer medullary collecting ducts have ET_B receptors.[823]

ET receptor activation leads to diverse cellular responses involving a chain of receptor-mediated amplification of effectors. At the ET_A receptor, these responses include the phospholipase pathway (PLC) leading to the formation of inositol triphosphate and diacylglycerol and to the release of

stored calcium into the cytosol. This causes cellular contraction and vasoconstriction. ET-mediated effects persist after dissociation of ET from its receptor, probably because of persistently high intracellular calcium or prolonged activation of signaling pathways.[815] Endothelial cells can express ET_B receptors linked to formation of NO and prostacyclin and mediate endothelium-dependent vasorelaxation.[836] In nephron segments and other renal structures, ET mediates its effects via a multiplicity of intracellular signal-transduction pathways that involve phospholipase activation, tyrosine phosphorylation of proteins, and elevation of intracellular free calcium.[837]

Biologic Effects of Endothelin in the Kidney

Endothelin effects on the kidney are broadly divided into vascular and tubular. Endothelin is a potent renal vasoconstrictor, as much as 30-fold more potent in this regard than Ang II.[771,804,805,836,837] Indeed, the renal vasculature is more sensitive than other vascular beds to the vasoconstrictive effects of ET-1.[838] In the isolated perfused kidney, ET-1 administration reduces GFR and causes a dose-dependent increase in renal vascular resistance. In whole animals, systemic ET infusion induces a decline in cortical blood flow, GFR, and urine volume[771,805]; however, some studies have shown differential regulation of blood flow between the cortex and the medulla with exogenous ET-1 infusion, showing cortical vasoconstriction and NO-dependent medullary vasodilation.[839,840] The direct effects of ET-1 on preglomerular and postglomerular resistances are quantitatively similar at lower doses, so the glomerular transcapillary hydraulic pressure and GFR are maintained.[841] However, at higher doses, a greater increase in preglomerular resistance occurs that, in addition to a decrease in glomerular capillary ultrafiltration coefficient (K_f), leads to a decline in GFR.[841,842] Dihydropyridines inhibit ET-mediated vasoconstriction exclusively in the afferent arterioles.[841] Renal hemodynamics may also be influenced by indirect effects of ET, such as modulation of arachidonic acid metabolism and renin release.[842] Local generation of prostaglandins (PGs), such as $PGF_{2\alpha}$, may mediate some of the vasoconstrictor effects of ET.[843] ET-1 directly inhibits renin release, but the net renin secretory response in vivo varies with the ET-1 dose, as well as with the state of activation of intrarenal baroreceptors and the MD-mediated pathway.[842] In humans, similar effects have been shown on renal vasculature, with renal vasoconstriction and reduction in RBF and GFR, whereas no studies addressed the effect of ET-1 on intrarenal distribution of blood flow.[838]

At the tubular level, ET-1 seems to play an important role in volume regulation. Despite compromise of RBF and GFR, infusion of nonpressor doses of ET in animals is associated with an increase in urinary flow and Na^+ excretion.[771,805] In addition, studies in the isolated perfused kidney have shown that ET increases Na^+ excretion despite a dramatic decline in GFR.[805] These effects on Na^+ and water balance are largely due to the ability of ET to reduce Na^+-K^+-ATPase activity and reversibly inhibit AVP-stimulated cAMP generation and water transport in the IMCD.[805,844] In addition, a natriuretic role for ET-1 is demonstrated in animals through ET_B receptors engaging NO and leading to inhibition of chloride transport in the MTAL of Henle's loop.[845] Furthermore, ET effect on epithelial amiloride-sensitive sodium channel is dose-dependent including activation and inhibition through both ET_B-mediated and non–ET_B-mediated effects.[846,847] The high concentration of ET receptors in the human inner medulla[835] and the recent observations suggesting ET production by human IMCD cells[825] justify the assumption that a similar physiologic role for intrarenal ET may also be operative in humans. ET also affects Na^+ balance indirectly by stimulating release of ANP.[848]

ET is well known to induce contraction of MCs in culture and results of micropuncture experiments demonstrate that ET-1 directly reduces the coefficient of ultrafiltration.[849] ET is also recognized as a growth factor with mitogenic effects on MCs in culture, inducing changes in MC phenotype and gene expression.[771,805,833] Abundant evidence points to a pivotal role for ET-1 in the biology and, particularly, the pathology of the renal mesangium. The peptide is produced by MC and can, in turn, act on MCs to elicit proliferation, hypertrophy, contraction, and/or extracellular matrix accumulation. These effects are mediated in large part through activation of ET_A and particularly involve PKC and MAPK. Excessive ET-1 production by, and action on, MCs is of pathogenic importance in glomerular damage in animal models of glomerulonephritis (GN), diabetes, and hypertension (Fig. 8.11).[832]

Studies with ET receptor antagonists demonstrated that, in the animal kidney, ET-1-induced reductions in total RBF are mediated by ET_A receptor, whereas selective medullary vasodilation is mediated by ET_B receptor.[850,851] In humans the situation is less clear. Antagonism studies have suggested that ET-1 effects through ET_A receptors may not be major contributors to the maintenance of renal vascular tone in health, whereas ET-1 vasodilatory effects through ET_B receptors are important.[852]

Endothelins in Kidney Disease

Endothelins have been implicated in a variety of diseases including cardiovascular (CV) and renal diseases, neoplasia, wound healing, and others. ET-1 has been implicated in the pathophysiology of congestive heart failure, showing cardiac pro-arrhythmic effects and promoting fibrogenesis and ventricular remodeling.[853,854] Despite its central role in CV disease pathogenesis, antagonism did not show any clear beneficial effect.[815]

The role of ET-1 in the pathogenesis of hypertension has been evoked by several studies showing a cross-talk between all pressor and vasoconstrictor systems including ET, the renin-angiotensin system, and the sympathetic nervous system.[815] Collectively, study results suggest that dysregulation of the endothelin system contributes to multisystem complications of hypertension.[855–857]

At the renal level, various diseases were associated with a dysfunctional endothelin system, including progression

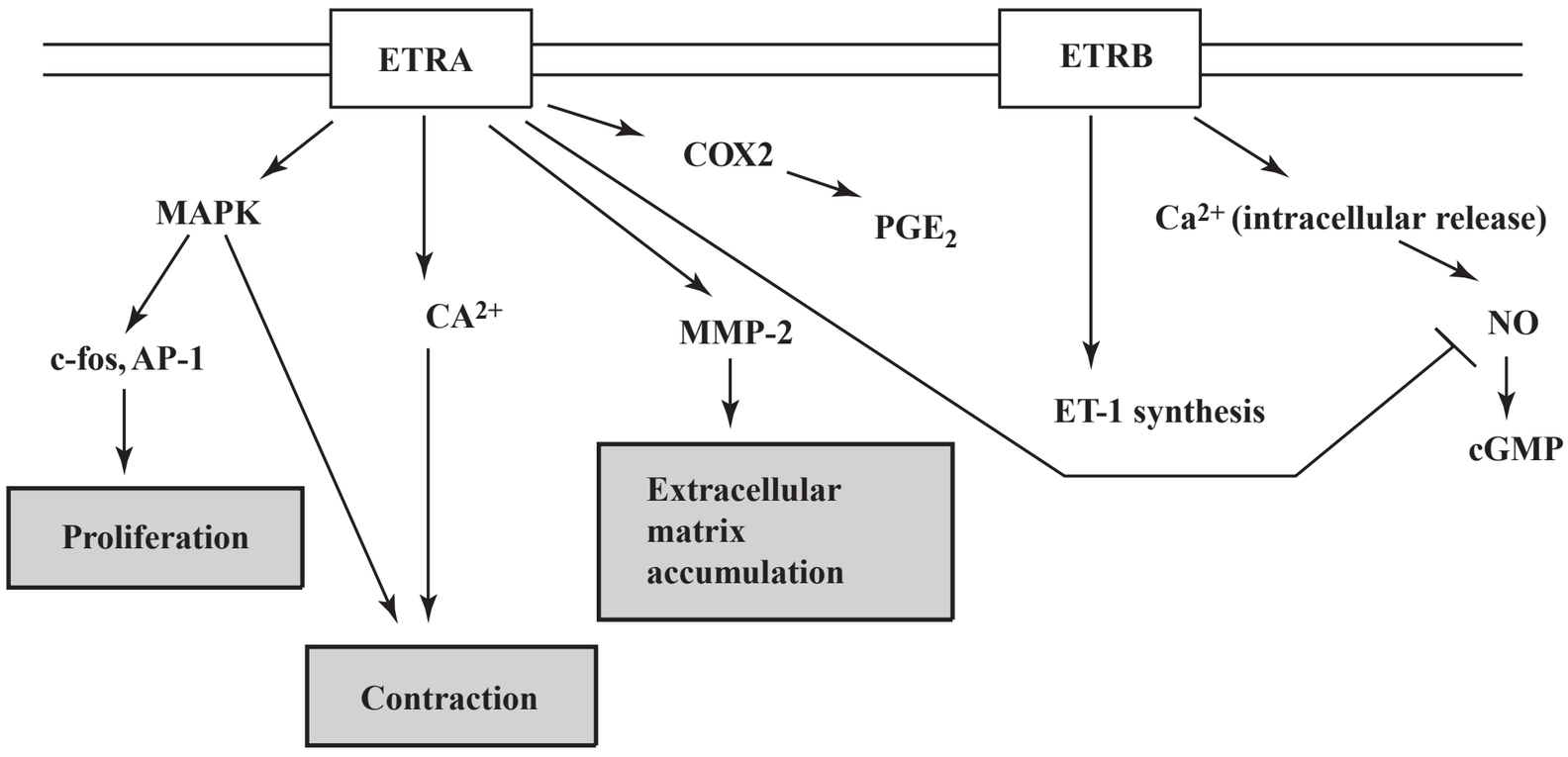

FIGURE 8.11 Schematic representation of biologic effects mediated by endothelin receptor A (ETRA) and endothelin receptor B (ETRB) in mesangial cells. The net effect of ETRA activation is mesangial cell contraction, extracellular matrix accumulation, and proliferation. The net effect of activation of ETRB tends to be vasorelaxant as well as causing autostimulation of ET-1 production. *AP-1*, activator protein-1; *MMP-2*, matrix metalloproteinase-2. (From Sorokin A, Kohan DE. Physiology and pathology of endothelin-1 in renal mesangium. *Am J Physiol Renal Physiol.* 2003;285:579, with permission.)

of chronic kidney disease, hypertension, and proteinuria. Declining renal function is associated with an increase in plasma ET levels.[858] Patients undergoing hemodialysis have higher ET levels than do patients undergoing peritoneal dialysis or uremic patients not yet on renal replacement therapy.[859] Erythropoietin administration, as well as acute volume contraction during hemodialysis, may contribute to the elevation of ET-1 level in patients undergoing hemodialysis.[860] Although the significance of elevated plasma levels of a primarily autocrine or paracrine hormone (ET) remains questionable at present, ET-secreting hemangioendotheliomas have been shown to produce marked hypertension.[861]

A pathophysiologic role for ET has been reported in several conditions affecting the kidney.[805,823,862] Urinary ET-1 excretion increases in patients with several forms of chronic progressive glomerulopathies.[862] MCs' ET-1 production is increased in rat models of immune complex GN, nephrotoxic serum nephritis, and mesangial proliferative GN. In addition, MC ET-1 production is augmented in human SLE and urinary ET-1 excretion is proportional to the severity of human mesangial proliferative GN.[832]

Although there is no direct evidence in vivo that MC ET-1 production is increased in diabetic nephropathy (DN), there are in vitro data suggesting that MC ET-1 synthesis is increased by hyperglycemia.[832] Studies suggest that MC ET-1 production is enhanced in DN and that excessive ET-1 action in the diabetic glomerulus can cause enhanced matrix accumulation, proteinuria, and reduced GFR.[832]

In animals subjected to surgical reduction of renal mass, ET-1 gene expression increases in parallel with proteinuria and glomerulosclerosis.[863] Proteinuria is currently known to be a renal and CV risk factor, and its reduction may confer CV protection.[838] Upregulation of the renal ET system exacerbates proteinuria. Implicated mechanisms include increased glomerular capillary hydrostatic pressure and permeability.[838] Chronic selective ET_A receptor blockade was associated with reduction of microalbuminuria in diabetic patients.[62] Renal ischemia, on the other hand, is a potent stimulus for ET-1 production.[63] ET-1 is upregulated after renal ischemia/reperfusion injury and may contribute to the injury by prolonging vasoconstriction. It is known that ET_A-selective, but not ET_B-selective, antagonist is protective against renal IR injury.[64] ET-1 aggravates cell damage through the activation of Na^+/Ca^{++} exchanger suggesting that Ca^{++} may play a critical role in hypoxia/reoxygenation-induced renal tubular injury.[65] Other renal diseases involving the ET system include: radiocontrast-induced renal injury,[66,823] cyclosporine-mediated nephrotoxicity,[67] immune nephritis, and the rat remnant kidney model.[68] However, data supporting a role for ET antagonists in the treatment of acute or chronic kidney disease in humans are currently lacking.[815]

In summary, ET is a potent vasoconstrictor peptide. In the kidney, it reduces RBF and GFR, contracts mesangial cells, and may function as a paracrine–autocrine factor in modulation of sodium and water balance. It is a potential mediator of growth and proliferative changes within the kidney. It is thought to play a pathophysiologic role in a number of kidney diseases. ET receptor antagonists may prove to be beneficial in certain conditions.

NITRIC OXIDE

NO is a paracrine mediator with a wide spectrum of physiologic actions, including the control of vascular tone, antithrombotic actions, cell cycle regulation, neurotransmission, signal transduction, and inflammation. NO is synthesized during conversion of its physiologic precursor L-arginine

(L-Arg) to L-citrulline.[864,865] This reaction is catalyzed by a family of enzymes known as NO synthases (NOS).[864,866–868] Three NOS isoforms (neuronal [nNOS, NOS1], inducible [iNOS, NOS2], and endothelial [eNOS, NOS3]) have been identified in mammalian tissues. Neuronal nitric oxide synthase (nNOS) was first found in the neuronal cells of the brain, but is also constitutively expressed in the kidney and may be of physiologic importance.[867] iNOS is expressed upon activation of macrophages and other cells. The third isozyme is found in endothelial cells (eNOS) and, like nNOS, is constitutive in nature.[868,869] NO produced from endothelial cells (eNOS-derived) is important as a tonic vasodilator and inhibitor of platelet aggregation and adhesion.

Renal Action of Nitric Oxide

All three NOS isoforms are expressed in the medulla and medullary NO production exceeds that in the cortex.[864] eNOS is found predominantly in renal vasculature, whereas iNOS immunoreactivity has been shown in the preglomerular portion of the afferent arteriole[871] and in glomerular mesangial cells. Isolated rat proximal tubule and inner medullary collecting duct cells express iNOS following stimulation with TNF-α and IFN-γ.[872]

Studies at the Single Nephron Level

NO has been shown to participate in the regulation of renal hemodynamics. The administration of a competitive inhibitor of NO production, L-NMMA, to normal rats causes dramatic glomerular hemodynamic changes, including reduced single nephron plasma flow, augmented afferent and efferent arteriolar resistances, decreased ultrafiltration coefficient, and increased glomerular capillary pressure.[873–876] Chronic oral supplementation with an L-arginine inhibitor in rats caused proteinuria, increased glomerular capillary pressure, and glomerular hemodynamic changes as described previously.[875] These observations suggest that NO might be an important regulator of glomerular capillary pressure and that its dysregulation might be involved in the development of glomerular sclerosis through increases in glomerular capillary pressure.

Dietary supplementation with L-arginine ameliorates the progression of renal disease in rats with subtotal nephrectomy,[876] at least in part because of its inhibitory effects on the development of glomerular hypertension.[877]

Role in Renal Injury

Increasing evidence implicates a role for decreased NO and increased peroxynitrite production in the pathophysiology of reactive oxygen species (ROS)-induced acute kidney injury.[878–880] Furthermore, Ang II promotes the production of O_2 and Ang II synthesis is increased in the kidney when there is a deficiency of NO. Once initiated, these events create a cycle that continues to increase Ang II levels in the glomerular circulation and to raise glomerular capillary pressure.[881] A locally activated renal RAS, in conjunction with increased TGF-1 expression, was shown to be a major feature of renal injury in rats with chronic inhibition of NO synthase.[881] Both exogenous and endogenous NO have protective effects against ischemia/reperfusion-induced renal dysfunction and tissue damage, probably through the suppression of endothelin-1 overproduction in postischemic kidneys.[882]

Therapeutic Implications

Not only do ACE inhibitors decrease Ang II synthesis, they also prevent the degradation of bradykinin, which is an important physiologic molecule involved in the release of NO. With ARBs, blocking the AT_1 receptor favors the synthesis of NO and stimulates factors that allow NO to be biologically more active.[881]

nNOS is abundantly expressed in MD cells, and its expression is stimulated in various high-renin states including salt restriction, administration of loop diuretics, or inhibition of the renin-angiotensin system—some of the same interventions that augment COX-2 expression. Direct evidence in support of MD NOS, presumably nNOS, acting as a positive regulator of renin secretion came from studies in the in vitro perfused juxtaglomerular apparatus (JGA). In this preparation, administration of L-arginine-stimulated renin secretion and NOS blockers almost completely abolished the stimulation of renin secretion by low levels of NaCl.[883] Studies have also demonstrated that tubuloglomerular feedback control of afferent arteriolar resistance is influenced by MD NO production, thereby enhancing autoregulation of RBF, GFR, and renin secretion.[884]

ERYTHROPOIETIN

Erythropoietin (EPO) is a 30.4-kDa glycoprotein hormone that acts on the bone marrow to stimulate red blood cell production.[885] The kidneys produce 85% to 90% of circulating erythropoietin in adults and the liver accounts for the remainder.[885,886] The liver is the major source of erythropoietin in the fetus.[886] In situ hybridization studies performed in anemic or hypoxic animals and erythropoietin-transgenic mice demonstrated that EPO is synthesized by peritubular cells of the renal cortex, particularly at the corticomedullary junction.[886–889] Other tissues were also found to produce EPO (peripheral endothelial cells, vascular smooth muscle cells, neurons, astrocytes, microglia, and cardiomyocytes). EPO receptors (EPO-R), members of the cytokine receptor superfamily, are localized in different parts of the kidney, as well as in the CNS, endothelial cells, solid tumors, liver, and uterus.[890] In erythroid progenitor cells, EPO binds to EPO-R, leading to activation of the JAK2 and downstream signal transduction pathways including STAT5, PI3 kinase, and MAPK.[891] The main stimulus for EPO production and secretion is decreased oxygen supply to renal tissue which most commonly results from anemia or hypoxemia. Decreased oxygen triggers a cascade of reactions mostly mediated by the so-called hypoxia-inducible factors (HIF), which activate a wide set of genes involved in protecting the

kidney from hypoxia including EPO gene.[892] This autocrine/paracrine secretion of EPO helps by decreasing apoptosis and cell necrosis in mesangial and tubular cells subjected to hypoxic (ischemia reflow injury) stress and toxic insult (cisplatinum), suggesting a potential therapeutic use of EPO or EPO analogs in tubular necrosis.[893–895] Extensive evidence indicates that EPO is a pleiotropic cytokine that confers broad tissue-protective properties as part of an innate response to stressors, promotes angiogenesis, and modulates wound healing responses.[896] It seems that SGK1 might contribute to the mediation of EPO effects under ischemic conditions.[897] Conversely, when the kidney is hyperoxygenated, as occurs after red cell transfusion, EPO production is reduced. In addition to modulation by oxygen availability, EPO production is influenced by several cytokines. IL-1 and TNF-α were shown to inhibit EPO mRNA levels and EPO formation in human hepatoma cell cultures and to reduce EPO production in isolated perfused rat kidneys.[898] Secretion of these cytokines by macrophages could contribute to defective EPO production and anemia in infectious or inflammatory diseases. Wang et al. showed that EPO has antioxidative properties in organs affected by diabetes and may prevent incipient microvascular damage in the diabetic retina.[899] Schiffer et al. studied the effects of different EPO molecules on podocyte signaling in vitro and on podocyte survival in an experimental model of diabetic kidney injury. EPO activates pro-survival intracellular pathways in podocytes in vitro, and ameliorates diabetes-induced podocyte loss in vivo.[900]

EPO has the potential to modulate oxygen delivery through regulation of endothelial NO production. In endothelial cell cultures, although short-term exposure to EPO decreases or leaves unchanged eNOS and endothelin-1 expression, the combination of EPO and hypoxia increases EPO-R and eNOS expression, and nitric oxide (NO) and cGMP production, demonstrating a direct effect of EPO on endothelial eNOS and NO production. EPO administration for 14 days in healthy rats increased hematocrit as well as eNOS expression and augmented NO-dependent vasodilatation.[901]

In healthy volunteers, Ang II injection increases EPO secretion in a dose-dependent manner. This effect is neutralized by a selective AT_1 receptor blocker, inferring that the stimulation of EPO by Ang II is probably via AT_1 receptors.[902] It has been postulated that EPO has antinatriuretic action, which could account for the worsening hypertension observed in some patients receiving recombinant EPO. In one study in isolated Wistar rat kidneys, EPO decreased Na excretion, possibly by increasing Ang II production.[904]

Cyanate, a compound that could spontaneously form from urea, reacts with EPO leading to carbamylated EPO, an EPO protein with reduced activity. In renal insufficiency, cyanate can reach levels high enough to reduce the activity of both endogenous and exogenous EPO. This might explain one mechanism of suboptimal responses to recombinant EPO in patients with ESRD on inadequate dialysis. It might also explain the superiority of continuous peritoneal dialysis over hemodialysis in terms of decreasing blood urea nitrogen (BUN) and, at the same time, improving anemia.[903]

INSULIN

Besides inhibiting gluconeogenesis in the proximal tubule, insulin exerts a significant vasodilatory effect on the kidney, thus increasing RPF; this effect appears to be mediated by PGs and partially counteracts the vasoconstrictor effect of Ang II.[905] In contrast, insulin appears to enhance the contractile effect of Ang II on mesangial cells.[906] It also potentiates AVP action in terms of water reabsorption at the collecting ducts by increasing the gene transcription of aquaporin-2 (AQP2).[907] In rats' renal proximal tubule cells, insulin and dopamine receptors interact to regulate renal sodium transport.[908] Studies using primarily cell culture have demonstrated that insulin can directly increase activity of the epithelial sodium channel, the sodium-phosphate cotransporter, the sodium-hydrogen exchanger type III, and Na-K-ATPase.[909]

C-peptide

C-peptide, or connecting peptide, for a long time thought of as an inert by-product of the conversion of proinsulin to insulin, has revealed multiple biologic roles by partially reversing or preventing complications of insulin-dependent diabetes.[910] Physiologic concentrations of C-peptide in diabetic rats activates pathways involved in cellular proliferation, and limits or prevents the glomerular hypertrophy and the mesangial matrix expansion seen in the posthyperfiltration phase of early diabetic nephropathy.[911] In the kidney, C-peptide reduces diabetes-induced glomerular hyperfiltration, albuminuria, and renal hypertrophy. C-peptide may induce constriction of afferent arterioles in diabetic mice thus reducing GFR—one of the renoprotective mechanisms of C-peptide in diabetes.[912] Following in vivo administration of C-peptide to patients with type 1 diabetes (T1DM), microvascular blood flow to tissues and organs, including muscle, skin, and kidney, is consistently augmented and likely relates to stimulatory effects on NO pathways.[913] One month treatment with C-peptide in T1DM patients results in a 6% decrease in GFR, and a 50% decrease in urinary albumin excretion.[914]

C-peptide reduces diabetes-induced hyperfiltration via a net dilation of the efferent arteriole and inhibition of tubular Na reabsorption, both potent regulators of the glomerular net filtration pressure.[915] In several studies, increased renal Na-K-ATPase activity has been linked to hyperfiltration and to the increase in oxygen consumption. Via inhibition of Na-K-ATPase, C-peptide may contribute to a normalization of the basal oxygen consumption of proximal tubules in diabetic animals. C-peptide may also influence oxygenation through effects on NO release. In addition, C-peptide can improve diabetes-reduced erythrocyte deformability. This has the potential to improve capillary blood flow, thus improving oxygen availability in the kidney, and

other affected tissues.[914] In vivo, C-peptide supplementation for 1 month improves body weight in STZ-induced diabetic rats and decreases urinary sodium wasting.[916] TGF-β1, overexpressed in renal cells in diabetes, has been suggested as a major malefactor in the development of morphologic changes in diabetes by stimulating the mesenchymal formation of collagen IV, and eliciting fibrosis in renal tissues. It has been reported that C-peptide has a protective effect on early diabetic glomerular changes in response to TGF-β1 in STZ-diabetic mice. In addition, an inhibitory effect of C-peptide on TGF-β1-induced gene expression has been demonstrated in a mouse podocyte cell line. C-peptide is reported to effectively reverse TGF-β1-induced structural changes in proximal tubular cells and to inhibit TGF-β1-induced gene expression in a mouse podocyte cell line. The mechanism by which this occurs is not fully understood. It has also been reported that C-peptide, via activation of NF-κB regulated survival genes, protects against TNF-α–mediated renal tubular injury.[914]

RENAL DEGRADATION OF HORMONES

Hormones fall in general into four structural groups: peptides and proteins, steroid hormones, amino acid derivatives, and fatty acid derivatives. Peptide hormones such as insulin have generally a short half-life and are extracted by the kidney, which, on average, removes between 16% and 40% of the hormone entering the renal circulation.[917] In exceptional cases, the kinins for example, more than 90% is removed during a single passage. The removal of biologically active and inactive peptides from the renal circulation occurs predominantly by glomerular filtration.

The rate of filtration is influenced by the size, shape, and charge of the molecule. After filtration, peptide hormones are degraded in the proximal tubule via two mechanisms. Larger peptides, such as insulin, require absorption by epithelial cells and degradation in lysosomes or endosomes. Growth hormone, for example, is also absorbed by tubules where it undergoes peritubular degradation.[918] Smaller peptides, such as bradykinin, angiotensin, and ANP, are degraded by hydrolysis on the brush border. Urinary hormone excretion accounts for less than 1% to 2% of the filtered load.[918] In most cases, the liver contributes significantly to peptide hormone metabolism. A few hormones, however, undergo negligible hepatic extraction and the kidneys are their predominant site of degradation.[917] Examples of these hormones are calcitonin, the amino-terminal fragment of PTH, and the C-peptide of proinsulin. Complete removal of insulin is also dependent on intact renal function.[919] Glomerular filtration accounts for approximately 60% of all the insulin removed by the kidney and the remaining is extracted from the peritubular circulation. Uptake and degradation occurs largely by receptor-mediated endocytosis of insulin on the basolateral membrane of tubule epithelial cells.[920,921] The kidneys contribute significantly to disposal of glycoprotein hormones, such as erythropoietin, follicle-stimulating hormone, and luteinizing hormone.[922,923]

Steroid hormones are derivatives of cholesterol and are typically eliminated by inactivating metabolic transformations and excretion in urine or bile. Examples include testosterone, cortisol, and vitamin D. Their half-lives vary considerably between a few minutes and a few hours. With the exception of vitamin D, the kidney plays only a minor role in the metabolsim of steroid hormones.[920]

Two major hormone groups are derivatives of amino acids: thyroid hormones and catecholamines. These were discussed in detail in previous sections of this chapter. Thyroid hormones are inactivated primarily by intracellular deiodinases and catecholamines are rapidly degraded by enzymes such as monoamine oxidase and catechol-O-methyl transferase.

Fatty acid derivatives include mainly eicosanoids and were discussed in other sections of this chapter. These hormones are rapidly inactivated by metabolism and have short half-lives.

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CHAPTER 9

Laboratory Evaluation of Kidney Disease

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Diseases of the kidney are often "silent" until late in the course of disease, when clinical signs and symptoms of uremia mark the onset of kidney failure. In contrast, laboratory evaluation for kidney disease reveals earlier manifestations and is an essential part of the clinical assessment of health and disease. In this chapter, we begin with a general approach to the laboratory evaluation of acute and chronic kidney disease (CKD). We then focus on glomerular filtration rate (GFR) as an index of overall kidney function, and proteinuria and other abnormalities in the urine sediment as markers of structural damage. In addition, we review all aspects of the routine urinalysis. Tubular functions, including concentration and dilution of the urine, urinary acidification, and reabsorption and secretion of electrolytes and other solutes are described in other chapters, as are production of hormones and metabolism by the kidney and novel biomarkers for specific diseases.

GENERAL APPROACH

Recent guidelines define kidney diseases according to alterations in kidney structure and function and their duration (Fig. 9.1 and Table 9.1).[1,2] Kidney diseases are further classified by severity of reduction in GFR and magnitude of albuminuria and by cause, reflecting the pathogenesis and pathologic abnormalities. The level of GFR is generally accepted as the best overall index of kidney function, and other kidney functions often decline in parallel to GFR in acute and chronic kidney diseases. Albuminuria generally reflects structural damage to the glomerular filtration barrier. Both measures appear to reflect kidney involvement in systemic vascular diseases as well as primary kidney diseases, and recent studies show that the severity of reduced GFR and magnitude of albuminuria are associated with a graded increase in risk for adverse outcomes across a wide variety of settings, including patients with acute and chronic kidney diseases, patients with increased risk from cardiovascular disease, and the general population (Figs. 9.2 and 9.3).[3] Abnormalities in the urine sediment, such as renal tubular cells and cellular casts, signify kidney damage and may provide a clue to the cause of kidney disease, but quantification is not well studied. Abnormalities on imaging studies and pathologic abnormalities are sufficient for diagnosis of acute or chronic kidney disease. A history of kidney transplantation is sufficient for a diagnosis of chronic kidney disease.

Recent guidelines also suggest simplification of initial diagnostic testing for detection and evaluation of acute and chronic kidney diseases. Although the importance of timed urine collections is acknowledged for gold standard measures of GFR and albumin excretion rate, they are impractical for routine general clinical practice. In this chapter, we emphasize initial testing using estimation of GFR from serum levels of endogenous filtration markers, estimation of albumin excretion rate from untimed "spot" urine albumin-to-creatinine ratio, and interpretation of reagent pads on the urine dipstick. Timed urine collections can be considered for more accurate assessment of GFR or albuminuria or further evaluation of abnormalities observed on the urine dipstick.

GLOMERULAR FILTRATION RATE

Glomerular Filtration: Determinants and Measurement

Normal Glomerular Filtration

The human kidney contains approximately 1 million glomeruli.[4,5] This number is determined at birth but is quite variable and a lower nephron number may be associated with development of hypertension and kidney disease in later life.[6,7] Each glomerulus attains an adult size of approximately 150 to 200 μm in diameter, providing a total surface area provided for filtration that approximates 1 square meter.[8] Approximately 180 L per day (or 125 mL per minute) of tubular fluid are produced from the rich renal plasma flow by the process of ultrafiltration. Glomerular filtration, driven by the high hydrostatic pressure across the glomerular capillaries, is facilitated by a hydraulic permeability of the glomerular capillary wall that is one to two orders of magnitude greater than other capillaries.[9]

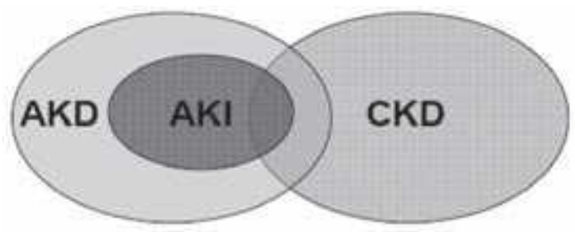


FIGURE 9.1 Conceptual model for integration of acute kidney injury (*AKI*), chronic kidney disease (*CKD*), and acute kidney diseases and disorders (*AKD*). Overlapping ovals show the relationships among AKI, AKD, and CKD. AKI is a subset of AKD. Both AKI and AKD without AKI can be superimposed upon CKD. Individuals without AKI, AKD, or CKD have no known kidney disease or disorder (NKD), not shown here. (Reproduced from Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int Suppl.* 2012;2(1):1–126.)

The glomerular filtration barrier is both size- and charge-dependent. Substances with molecular weights lower than 10,000 daltons cross the glomerular capillary wall as easily as water and electrolytes.[10–12] Micropuncture sampling of glomerular filtrate in amphibians and mammals shows the filtrate to be identical in nonprotein composition to plasma, with electrolyte concentrations conforming to the Gibbs-Donnan relationship.[11,13] As discussed later, plasma proteins are excluded from the filtrate as a consequence of the unique structure of the glomerular capillary wall.

Determinants of the Glomerular Filtration Rate

In principle, the GFR is dependent on the number of nephrons (N) and the single-nephron glomerular filtration rate (SNGFR), as described below:

$$GFR = N \times SNGFR \quad (1)$$

In normal individuals, regulation of GFR occurs via regulation of SNGFR. In patients with kidney disease, in whom the nephron number may be reduced, regulation of SNGFR remains important in modulating GFR. SNGFR is determined by two major factors. The first factor is the net ultrafiltration pressure (P_{UF}), determined by the difference between the net transcapillary hydraulic pressure (ΔP) favoring filtration and the net oncotic pressure ($\Delta\pi$)

9.1 Definitions of Kidney Disease

	Functional Criteria	Structural Criteria
Acute kidney injury (AKI)	Increase in serum creatinine by 50% within 7 days, OR Increase in serum creatinine by 0.3 mg/dl within 2 days, OR Oliguria	No criteria
Chronic kidney disease (CKD)	GFR $<$60 mL/min/1.73 m^2 for $\geq$3 months	Kidney damage for $\geq$3 months, including Albumin excretion rate $>$30 mg/d, OR, Urine sediment abnormalities, OR, Imaging abnormalities, OR Pathologic abnormalities, OR History of kidney transplantation
Acute kidney diseases and disorders (AKD)	AKI, OR GFR $<$60 mL/min/1.73 m^2 $<$3 months, OR Decrease in GFR by $>$35% or increase in serum creatinine by $>$50% for $<$3 months	Kidney damage for $<$3 months, as defined by above
No kidney disease or disorder (NKD)	GFR $>$60 mL/min/1.73 m^2, AND Stable serum creatinine	No kidney damage

Note: AKI and CKD have formal consensus definitions. The definition for AKD is proposed as an operational definition to classify individuals with alterations in kidney function and structure and function who do not meet the definitions for AKI and CKD. NKD indicates no functional or structural alterations that meet the definition for AKI, CKD, or AKD. Clinical judgement is required or individual decision-making regarding the extent of evaluation that is necessary to assess kidney function and structure. Glomerular filtration rate (GFR) may be assessed from estimated or measured GFR. Estimated GFR does not reflect measured GFR in AKI as accurately as in CKD. Albuminuria may be assessed from timed urine collections or "spot" urine albumin-to-creatinine ratio. Novel markers of kidney damage have been proposed, but none have been validated for inclusion in the definitions of AKI or CKD. A history of kidney transplantation is considered a marker of kidney damage for CKD but not AKD.

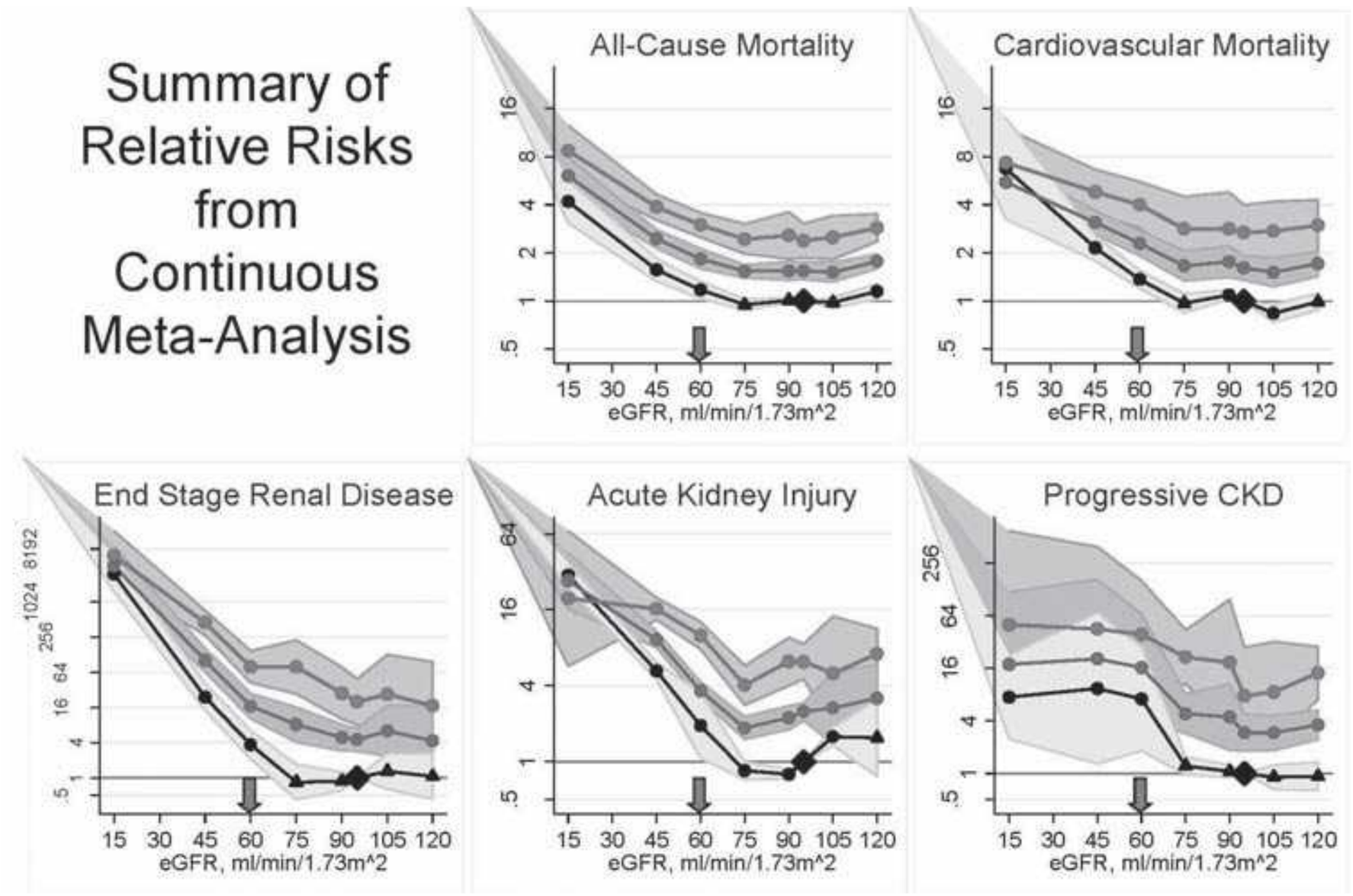


FIGURE 9.2 Summary of KDIGO Controversy Conference continuous meta-analysis (adjusted relative risk [RR]) for general population cohorts with albumin-to-creatinine ratio (ACR). Mortality is reported for general population cohorts assessing albuminuria as urine ACR. Kidney outcomes are reported for general population cohorts assessing albuminuria as either urine ACR or dipstick. Estimated glomerular filtration rate (eGFR) is expressed as a continuous variable. The three lines represent urine ACR of <30 mg per g or dipstick negative and trace (*blue*), urine ACR 30 to 299 mg per g or dipstick 1+ positive (*green*), and urine ACR >300 mg per g or dipstick ≥2+ positive (*red*). All results are adjusted for covariates and compared with reference point of eGFR of 95 mL/min/1.73m^2 and ACR of <30 mg per g or dipstick negative (*diamond*). Each point represents the pooled RR from a meta-analysis. *Solid circles* indicate statistical significance compared with the reference point ($P < 0.05$); *triangles* indicate nonsignificance. *Red arrows* indicate eGFR of 60 mL/min/1.73m^2, threshold value of eGFR for the current definition of chronic kidney disease (CKD). HR, hazards ratio; OR, odds ratio. (Reproduced from Levey AS, de Jong PE, Coresh J, et al. The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011;80:17–28.) (See Color Plate.)

opposing filtration. ΔP is determined by the difference between the glomerular capillary hydraulic pressure (P_{GC}) and that in the earliest proximal tubule (P_T). $\Delta\pi$ is determined by the glomerular oncotic pressure alone as the ultrafiltrate is virtually protein free. The second factor, K_f, describes the surface area and permeability characteristics of the glomerular ultrafiltration barrier. This relationship can be expressed by the equation:

$$\text{SNGFR} = K_f(\Delta P - \Delta\pi) \quad (2)$$

Absent from this equation is the renal plasma flow rate. Alterations in renal plasma flow affect SNGFR largely by affecting $\Delta\pi$. Changes in determinants of SNGFR as plasma traverses the glomerular capillary are demonstrated in Figure 9.4. For a detailed analysis of these determinants and the multiple factors that result in the regulation of glomerular filtration, the reader is directed to Chapter 2.

In acute and chronic kidney disease, decreased GFR can be due either to a decrease in nephron number or SNGFR. Interestingly, in a number of experimental chronic kidney diseases characterized by decreased nephron number, SNGFR is elevated, perhaps reflecting compensation in processes to maintain whole kidney GFR. Moreover, in some diseases, increased SNGFR precedes the decline in nephron number, thereby raising the hypothesis that hyperfiltration in single nephrons may give rise to the development or progression of chronic kidney disease.[14]

Normal Range and Variability of Glomerular Filtration Rate

The GFR cannot be measured directly. Instead, as discussed later, it is assessed from the urinary clearance of an ideal filtration marker, such as inulin. When measured repeatedly in a single individual, under constant conditions and according to a standard protocol, the GFR appears relatively constant. Homer Smith measured the inulin clearance in one "hospitalized but otherwise normal subject" 15 times during 1 year; the range was 113 to 137 mL per minute with a mean of 122 mL per minute.[15] However, variation among individuals is quite large, and normal values show considerable spread. As discussed later, the major causes of variability in healthy individuals are age, gender, and body size. Hence,

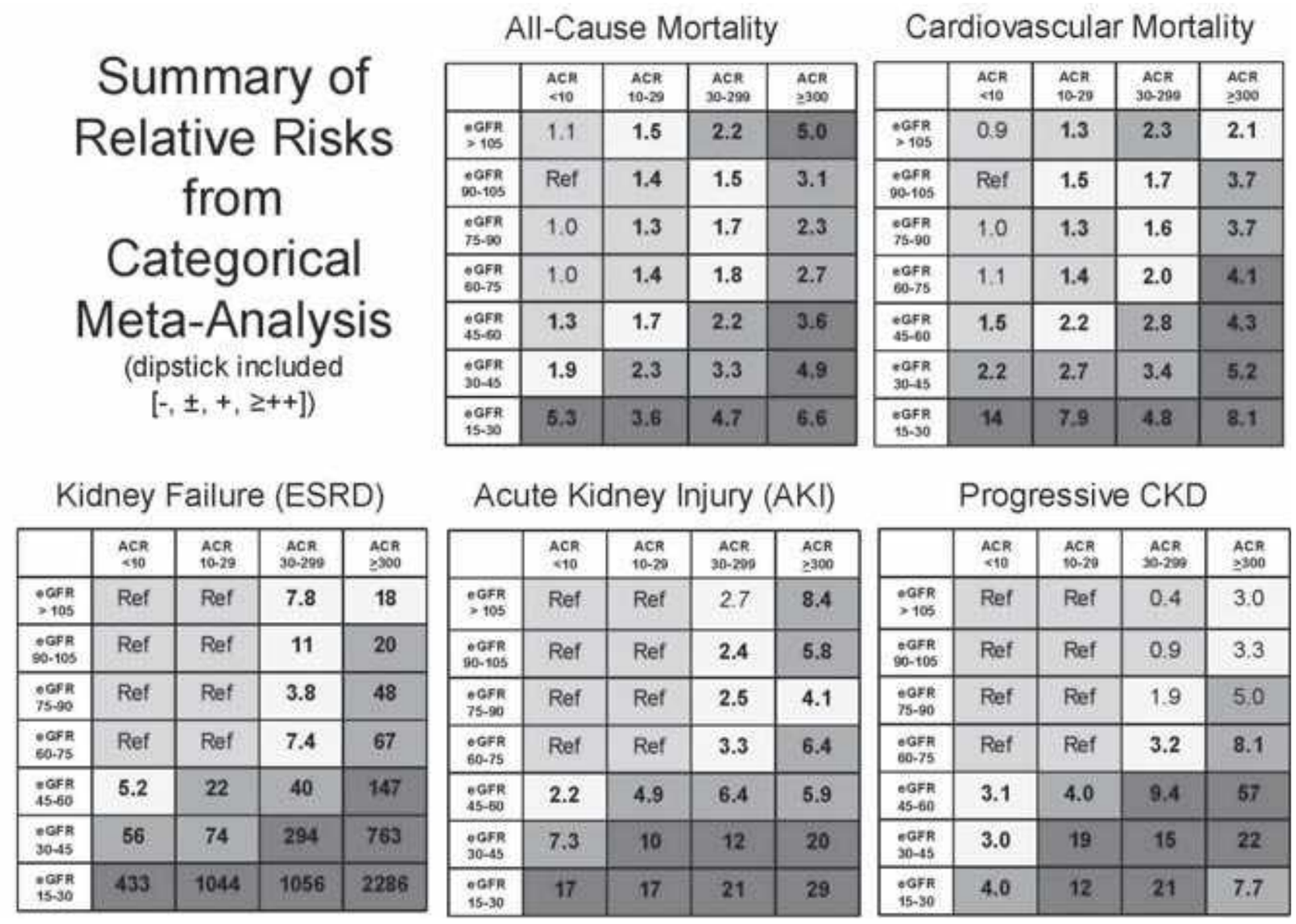


FIGURE 9.3 Summary of KDIGO Controversy Conference categorical meta-analysis (adjusted relative risk [RR]) for general population cohorts with albumin-to-creatinine ratio (ACR). Mortality is reported for general population cohorts assessing albuminuria as urine ACR. Kidney outcomes are reported for general population cohorts assessing albuminuria as either urine ACR or dipstick. Estimated glomerular filtration rate (eGFR) and albuminuria are expressed as categorical variables. All results are adjusted for covariates and compared with the reference cell (*Ref*). Each cell represents a pooled relative risk from a meta-analysis; *bold numbers* indicate statistical significance at $P < .05$. Incidence rates per 1,000 person-years for the reference cells are 7.0 for all-cause mortality, 4.5 for cardiovascular disease mortality, 0.04 for kidney failure, 0.98 for acute kidney injury (AKI), and 2.02 for kidney disease progression. Absolute risk can be computed by multiplying the RRs in each cell by the incidence rate in the reference cell. Colors reflect the ranking of adjusted relative risk. The point estimates for each cell were ranked from 1 to 28 (the lowest RR having rank number 1, and the highest number 28). The categories with rank numbers 1 to 8 are *green*, rank numbers 9 to 14 are *yellow*, the rank numbers 15 to 21 are *orange*, and the rank numbers 22 to 28 are colored *red*. (For the outcome of kidney disease progression, two cells with RR 1.0 are also green, leaving fewer cells as orange.) (Reproduced with permission from Levey AS, de Jong PE, Coresh J, et al. The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011;80:17–28.) (See Color Plate.)

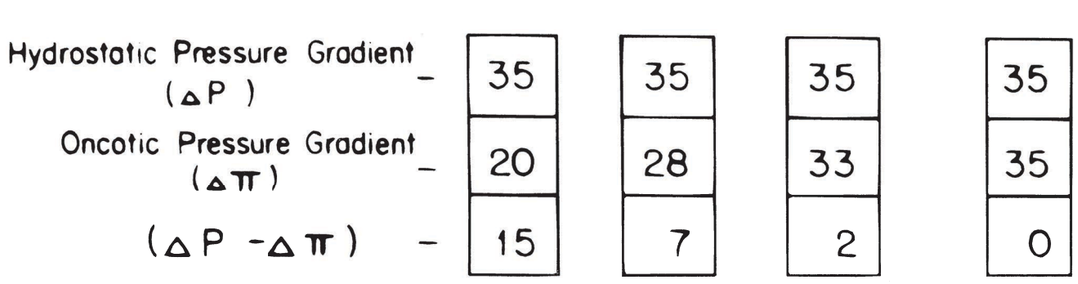


FIGURE 9.4 The changes in hydrostatic and oncotic pressures that occur as plasma traverses the glomerular capillary. As water is filtered without protein, the oncotic pressure gradually rises, thereby decreasing the net pressure favoring filtration. The pressure favoring filtration falls toward zero and filtration stops in this model before the plasma reaches the efferent arteriole. (From Deen WM, Robertson CR, Brenner BM. Glomerular ultrafiltration. *Fed Proc*. 1974;33:14, with permission.)

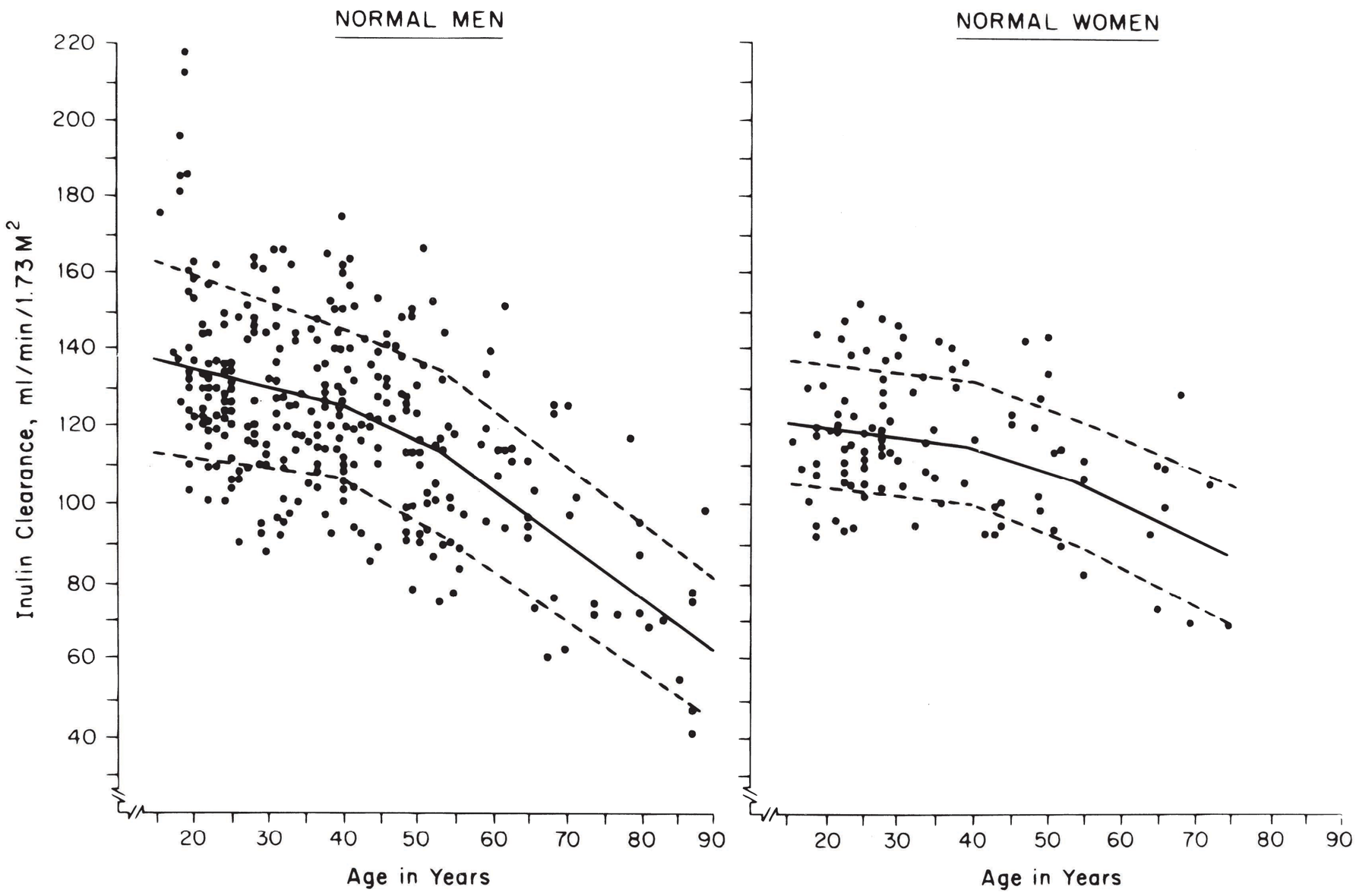


FIGURE 9.5 Normal values for glomerular filtration rate, adjusted for body surface area, in men and women of various ages. (From Wesson LG, ed. *Physiology of the Human Kidney*. New York: Grune & Stratton; 1969, with permission.)

measured values of GFR are typically adjusted for body size (surface area) and are traditionally compared to normative values for age and gender (Fig. 9.5).[16] Even after elimination of these sources of variation, important variability remains. A compilation of inulin clearance measurements in hydrated young adults (adjusted to a standard body surface area of 1.73 m^2) shows the mean value in men to be 131 mL per minute with a coefficient of variation (CV) (defined as the standard deviation divided by the mean) of 18%, and the mean value in women to be 120 mL per minute, with a CV of 14%.[15,16] The following sections discuss causes of normal variation. These same factors also contribute to variation in GFR in patients with kidney disease.

Age, Sex, Body Size, and Ethnicity. The surface area adjustment was first introduced to minimize variability in urea clearance results among normal adults and children.[17–19] Based on the relationship of GFR to glomerular surface area, it is not surprising that the level of GFR is related to kidney size, which in turn, is related to body surface area and metabolic activity.[20] Measured values for GFR are conventionally factored by 1.73 m^2, the mean surface area of men and women 25 years of age. Nonetheless, as described earlier, surface-area adjusted values for GFR are approximately 8% higher in young men than in women of the same age.

Glomerular tuft volume, renal size, and GFR increase during growth and development. The surface area adjustment is not appropriate for newborns, whose adjusted GFR is less than 50% of the value achieved at approximately 1 year of age.[21,22] More recent studies strongly suggest that in newborns, GFR should be expressed in mL/min/kg, with the normal value being 0.6 to 1.6 mL/min/kg. Such an approach reduces the apparent variation in measured GFR more than 10-fold.[23] Beyond age 1 to 2 years, however, GFR values in normal children, adjusted to 1.73 m^2, are the same as those for young adults.

The appropriateness of the surface area correction in obesity remains controversial.[24] Because adipose tissue is less metabolically active than lean body mass, the physiologic matching of GFR to body surface area may not be the same in obese as in lean individuals. There are few data to relate measured GFR to body size, metabolic activity, and risks for development of kidney disease in obesity, leaving many important questions unanswered.[25] Are estimates of body surface area from height and weight as accurate in obese as in lean individuals? Does GFR increase with weight gain in proportion to body surface area? If so, is the resulting hyperfiltration associated with increased risk for development of kidney disease, as hypothesized in other conditions with hyperfiltration, such as diabetes? If so, indexing GFR to body surface area in obesity may obscure detection of an important marker of disease. Cross-sectional and longitudinal studies of measured GFR in obesity, in association with measures of body size and metabolic activity, and markers of kidney damage are necessary to answer these questions.[24]

Most studies of measured GFR in populations without kidney disease have been conducted in North America or

Europe, so data on nonwhite races and other ethnicities is limited. Reports on small to moderate numbers of subjects have suggested a lower average value,[26,27] but these studies are somewhat limited by differences in GFR measurement methods and by incomplete ascertainment of protein intake (see below). A more recent report from a representative population in Pakistan suggests mean values of GFR in young adults only slightly below those in whites, with similar age-related decline.[28]

In older studies, both cross-sectional and longitudinal studies in normal men demonstrate an age-related decline in GFR of approximately 10 mL/min/1.73 m^2 per decade after the age of 30 years.[16,29–31] Recent studies in the general population have not been performed, but studies in kidney donors demonstrated a 4 mL/min/1.73m^2 lower measured GFR per decade up to the age of 45 years and a 7.5 mL/min/1.73m^2 lower measured GFR per decade thereafter.[32] Thus, using the data from the general population, during the 50 years from age 30 to 80, GFR declines by almost 40% from approximately 130 to 80 mL/min/1.73 m^2. Cross-sectional studies in normal women indicate roughly similar results, but comparable longitudinal studies have not been performed and there may be subtle differences related to effects of hormones, pregnancy, and propensity toward illnesses that impact the kidney. This age-related decline in GFR is consistent with the anatomic observation that the number of glomeruli in the normal human kidney declines with age; in the sixth and seventh decades, the number of glomeruli is less than one-half the number present in young adults.[4,33] The cause of age-related decline in GFR is not completely understood, but progressive glomerular sclerosis, independent of traditional kidney disease risk factors, likely contributes to the loss of glomeruli.[34,35] Recent epidemiologic studies demonstrate that this decline in GFR is associated with increased risk for all-cause and cardiovascular disease mortality as well as kidney disease, casting doubt on the traditional interpretation that it is normal.[36]

Pregnancy. Marked increases in GFR occur during pregnancy; elevations to an average as much as 50% occur during the first trimester, and these high levels persist until shortly after term.[37–40] These increments in GFR are associated with an increase in renal plasma flow and relatively constant filtration fraction throughout most of pregnancy, reflecting hemodynamic consequences of widespread vasodilatation. Late in pregnancy, it appears that hyperfiltration becomes dependent on reduced plasma oncotic pressure. This change persists in the very early postpartum period, but the GFR returns to normal in the first 4 to 8 weeks following the end of pregnancy.[40,41]

Interestingly, pregnancy-induced hyperfiltration also occurs in women with preexisting chronic kidney disease.[42] This observation suggests that the physiologic vasodilatation of pregnancy can further augment the single-nephron hyperperfusion and hyperfiltration associated with chronic kidney disease. However, this phenomenon may be restricted to women with only very mild reductions in GFR. Improvement of GFR was not observed in one study of 23 women with chronic kidney disease and pre-pregnancy serum creatinine levels greater than 1.4 mg/dL.[43]

Protein Intake. The effect of protein intake to modulate GFR in experimental animals was recognized 70 to 80 years ago.[44,45] It is now clear that these effects occur in humans, although the magnitude of the effect varies widely among studies.[46] Important causes of variation include the duration of protein feeding (habitual protein intake vs. meat meals or amino acid infusions), the type of protein (animal vs. vegetable or soya protein sources; essential vs. nonessential amino acids), and the filtration marker used to measure GFR (inulin vs. creatinine).

In a classic study, Pullman et al.[47] placed healthy humans on low (0.1 to 0.4 g/kg/day), medium (1.0 to 1.4 g/kg/day), and high (2.6 g/kg/day) protein diets for 2 weeks. Compared to the low protein diet, inulin clearance increased after ingestion of the medium and high protein diets by 9% and 22%, respectively. These changes were accompanied by parallel changes in renal plasma flow, indicating a hemodynamic basis for the changes in GFR. A longer period of habituation may have greater effects on GFR. Similarly, in patients with chronic malnutrition, inulin clearance was 27% to 64% lower than after repletion of nutritional status,[48–51] and returned to near normal values only after 1 month of refeeding. In addition, malnourished patients had smaller kidneys, suggesting that differences in kidney function were due to structural as well as hemodynamic alterations.[48] Increases in GFR and kidney size in association with increased protein intake have been noted in diverse clinical circumstances, such as in patients receiving total parenteral nutrition and in insulin-dependent diabetic patients with poor metabolic control.[52] Some studies suggest a greater response to animal than vegetable protein in habitual diets as well as in response to protein loads.[53–55] A recent study assessing the impact of sustained high protein feeding demonstrated an increase in GFR in young subjects (24 ± 1 years old), but actually a small decrease in GFR in older subjects (70 ± 2 years old).[56]

After a meat meal, GFR, renal plasma flow, and splanchnic blood flow rise within an hour and remain elevated for several hours.[57] In humans, the increment in inulin clearance is about 10%,[58,59] and appears to be less than the increment in creatinine clearance.[46] Nonessential amino acids are more potent than essential amino acids in inducing the postprandial rise in GFR, and branched-chain amino acids appear to have little or no effect.

It had been proposed that protein-induced hyperfiltration represents "renal reserve capacity," which is lost prior to the reduction in baseline GFR associated with kidney disease.[60] However, it has now been shown conclusively that changes in GFR occur in response to changes in habitual protein intake or meat meals in patients with kidney disease and reduced GFR.[59–63] This is consistent

with studies in animals with experimental kidney diseases, which show that changes in protein intake further modulate the determinants of single-nephron GFR. In particular, a high protein diet raises the already increased glomerular plasma flow and transcapillary hydrostatic pressure gradient.[64,65] Thus, protein-induced hyperfiltration augments the hyperperfusion and hyperfiltration of chronic kidney disease.

Diurnal Variation. A normal diurnal variation in filtration rate occurs, with 10% higher values occurring in the afternoon than in the middle of the night.[66] In large part, the diurnal variation is thought to be related to variation in protein intake during the day.[16,60] Possibly, diurnal variation may also be related to transient reductions in GFR associated with exercise. Indeed, a decrease of 40% or more is seen with severe exertion.[16,67,68] However, diurnal variation is also observed in quadriplegics,[69] arguing against physical activity as the sole cause of diurnal variation. Possibly, diurnal variation may also reflect variation in hydration. GFR increases with overhydration and decreases with water restriction. However, the changes are small except when gross disturbances in fluid balance occur.

Antihypertensive Therapy. As a result of powerful mechanisms for autoregulation of renal hemodynamics (Chapter 3), the level of GFR remains relatively constant throughout a wide range of blood pressure. Nonetheless, antihypertensive therapy can be associated with reductions in GFR, due, in part, to the effect of lowering blood pressure and, in part, to specific effects of classes of antihypertensive agents. Indeed, marked reduction in GFR can complicate treatment in patients with severe hypertension and acute or chronic kidney disease,[70] which is an effect thought to be due to the loss or reset of autoregulation due to sclerosis of the renal vasculature from hypertensive injury.[71] In normal individuals and in patients with kidney disease, GFR is transiently reduced by a variety of antihypertensive agents, including diuretics, beta-blockers, central alpha-2 agonists, and peripheral alpha blockers.[72] In contrast, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers, and directly acting vasodilators do not regularly lower GFR in healthy subjects. A large study in patients with chronic kidney disease and well-controlled hypertension showed persistent small (less than 5 mL per minute), but significant, reductions in GFR associated with the use of ACE inhibitors as well as diuretics and beta-blockers.[73] In addition, after controlling for the effect of these classes of antihypertensive agents, a small effect of lowering blood pressure remained. Because the effects of the various classes of medications and of lowering blood pressure appear to be independent, a clinically significant reduction in GFR could occur in patients with chronic kidney disease undergoing treatment with multiple antihypertensive agents.

Measurement of the Glomerular Filtration Rate

Clearance. As mentioned earlier, the GFR is assessed from the clearance of filtration markers, substances excreted by glomerular filtration that can be used to assess the GFR. The "gold standard" for the measurement of GFR is the urinary clearance of inulin. The term clearance was introduced into kidney physiology by Van Slyke and his colleagues in reference to studies of the excretion of urea in 1929.[18] Two years later, Jollife and Smith extended the use of the term to the excretion of creatinine and later to the excretion of many other substances.[74] In the many decades since these pioneering studies, the concept of clearance has maintained its primacy as the cornerstone of our understanding of the measurement of glomerular filtration.

The clearance of a substance is defined as the rate at which it is cleared from the plasma per unit concentration. The clearance of substance "x" (C_x) is given in the following equation:

$$C_x = A_x / P_x \tag{3}$$

where A_x is the amount of x eliminated from the plasma and P_x is the average plasma concentration. Hence, C_x is expressed in units of volume per time. The value for clearance does not represent an actual volume, but a virtual volume of plasma that is completely cleared of the substance per unit of time, without reference to the route of elimination. The value for clearance is related to the efficiency of elimination: the greater the rate of elimination, the higher the clearance.

Relationship of Glomerular Filtration Rate to Urinary Clearance. For a substance that is cleared by urinary excretion, the clearance formula may be rewritten as follows:

$$C_x = U_x \times V / P_x \tag{4}$$

where U_x is the urinary concentration of x and V is the urine flow rate. The term $U_x \times V$ is defined as the urinary excretion rate of x. If substance x is filtered freely across the glomerular capillary walls and excreted only by glomerular filtration, then the rate of filtration is equal to the rate of urinary excretion:

$$GFR \times P_x = U_x \times V \tag{5}$$

where the term $GFR \times P_x$ is defined as the filtered load of x. By substitution into Equation 9.2:

$$C_x = GFR \tag{6}$$

Hence, substance x would be defined as an "ideal filtration marker" whose urinary clearance could be used to measure GFR.

However, if substance x is also reabsorbed or secreted by the renal tubules, then the following equations apply:

$$U_x \times V = GFR \times P_x - TR_x + TS_x \quad (7)$$

$$GFR = (U_x \times V - TR_x + TS_x) / P_x \quad (8)$$

$$GFR = C_x - TR_x / P_x + TS_x / P_x \quad (9)$$

where TR_x and TS_x are the rates of tubular reabsorption and secretion of x, respectively, and TR_x/P_x and TS_x/P_x are the clearances of substance x due to reabsorption (C_{TRx}) and secretion (C_{TSx}), respectively. In this case, the rate of urinary excretion ($U_x \times V$) does not equal the filtered load ($GFR \times P_x$), and clearance does not equal GFR. Therefore, the value for urinary clearance of x (C_x) is determined not only by the rate of glomerular filtration, but also by the mechanism of excretion by the kidney. For substances that are filtered and secreted, clearance exceeds GFR, and for substances that are filtered and reabsorbed, clearance is less than GFR.

Inulin Clearance. The requirements for an ideal filtration marker, as outlined by Smith,[15] include the following:

1. It is freely filtered at the glomerulus. It passes from the glomerular capillary blood into Bowman's space unhindered by its size, charge, or binding to plasma proteins.
2. It is not altered during its passage through the nephron. It is not reabsorbed, secreted, synthesized, or metabolized by the tubules.
3. It is physiologically inert and does not alter the function of the kidney.

Inulin, a 5,200-dalton, inert, uncharged polymer of fructose, meets these criteria, and it remains the standard for experimental and clinical measurement of GFR.[15,75,76] The conclusion that inulin is freely filtered and is neither secreted nor reabsorbed in the normal kidney was originally based on indirect evidence, but a large body of direct micropuncture observations have verified this assumption.[11,77–80] Similar evidence is not available, however, in all experimental kidney diseases. For example, in several models of acute kidney failure with extensive tubular basement membrane damage, leakage of inulin across the tubules is readily demonstrated.[81,82] In such situations, of course, the urinary excretion of inulin is less than the filtered load, and inulin clearance is less than GFR.

Although the measurement of inulin clearance is a highly accurate and reproducible means of estimating GFR, there are several disadvantages that make it impractical for clinical use. First, the classical method includes measurement under fasting conditions in the morning, a continuous intravenous infusion, multiple clearance periods requiring repetitive blood and urine collections over 3 hours, oral water loading to stimulate diuresis, bladder catheterization to assure complete urine collection, and careful timing of blood sampling at the midpoint of the urine collection. Period-to-period variability in GFR (intratest variation; expressed as CV) is approximately 10%. Intratest variation may reflect incomplete bladder emptying and is often used to judge the quality of a urinary clearance study.[15] However, one recent study has shown that the precision of GFR determinations is only weakly affected by intratest variability,[83] probably because averaging over several clearance periods minimizes error due to incomplete bladder emptying. In a study in normal individuals using the classical method of inulin clearance, the CV for repeated measurements within an individual (intertest CV) was 7.5%.[84] These estimates of measurement error are probably lower than would be observed in most clinical settings. Second, inulin is difficult to dissolve in aqueous solutions, difficult to measure, and is in short supply. Because of these disadvantages, clinical assessment of GFR generally utilizes other filtration markers and clearance methods.

Urinary Clearance of Endogenous Filtration Markers. In principle, the simplest alternative to inulin clearance would be the urinary clearance of an endogenous filtration marker. The advantage of this method is that clearance can be computed from urine collections and blood sampling under usual clinical conditions without the need for administration of an exogenous marker. Indeed, this method is widely used for measuring creatinine clearance, as discussed later. The most common method is to collect a 24-hour urine collection and a single serum measurement, assuming a steady state. The urine collection is performed at home. At the onset of the collection period, the patient is instructed to empty the bladder and discard the urine. During the collection period, all subsequent urine is saved. At the end of the period, the patient is asked to void completely and to add this last specimen to the urine collection. Shortly thereafter, the blood sample is obtained.

Unfortunately, the accuracy of this method is limited because neither creatinine nor any other known endogenous filtration marker meets all the criteria for an ideal filtration marker and because timed urine collections under usual clinical conditions are notoriously inaccurate. Errors in timing or completeness can result from misunderstanding by the patient or personnel of the instructions, such as omitting urine specimens during the interval or incompletely emptying the bladder at the start or end of the collection period. At first glance, it might appear that the use of short urine collection intervals, such as 1-hour, carried out under close supervision by trained personnel might overcome these difficulties. However, using a shorter collection period, the small errors due to incomplete bladder emptying would have a greater impact on the estimate of the urine volume and hence the urine flow rate. Indeed, the 1-hour technique has been largely abandoned because the extra effort and personnel required do not significantly improve the accuracy as compared to the 24-hour

clearance.[85] However, averaging the results of three to four 30-minute collection periods does significantly improve the accuracy, probably due to cancellation of errors from incomplete bladder emptying.[86]

A similar method can be used to compute clearance for patients who are not in a steady state balance by obtaining additional blood samples during the urine collection to estimate the average serum concentration. The most common strategies are to collect blood at the mid-point of the urine collection, or at the beginning and end of the urine collection, and to average the serum concentrations.

Alternative Clearance Methods and Exogenous Filtration Markers. All alternative clearance methods have been designed to facilitate GFR measurement; however, all have limitations that should be understood for proper interpretation. Table 9.2 summarizes the strengths and limitations of these alternative clearance methods and markers, as well as the gold standard method.[75,87–89]

Changes to the clearance method include substitution of bolus intravenous or subcutaneous injection for a constant intravenous infusion and use of plasma clearance techniques to eliminate the need for urine collection. With a bolus injection, the pattern of decline in serum levels is more accurately modeled as an exponential rather than linear of decline.[83] In the bolus subcutaneous technique, the marker substance (e.g., ^{125}I-iothalamate, ^{51}Cr-EDTA) can be given with a small dose of aqueous epinephrine to slow its release into the circulation, providing fairly constant plasma levels.[90,91] More recently subcutaneous continuous infusions have been used.[92]

Plasma clearance is computed from Equation 9.3 using either the entire area or a one-compartment or two-compartment model of the plasma disappearance plot.[93–95] There are several caveats. First, a relatively long time (3 to 5 hours) is required to accurately determine the declining plasma concentration of the marker, with longer times for people with reduced GFR. Second, filtration markers utilized for this method must meet an additional criterion of rapid equilibration with the extracellular volume, and inulin is therefore not appropriate for use.[96] Third, for some markers, simultaneous assessment of plasma and urinary clearance of a filtration marker typically yields a higher level for plasma clearance, presumably due to extrarenal excretion of the marker.[97,98] This underestimation is more apparent at a lower GFR. Fourth, plasma clearance overestimates GFR in patients with moderate to severe edema probably because of the larger than expected volume of distribution and lower than expected plasma levels of the marker.[99]

Alternative exogenous markers include radioisotope-linked markers ^{125}I-iothalamate, ^{51}Cr-ethylene diamine tetraacetic acid (EDTA), and its analogue, ^{99m}Tc-diethylene triamine pentaacetic acid (DTPA), that can be readily and inexpensively measured using radioactive counters; and nonradioactive markers iohexol and iothalamate that can be measured by X-ray fluorescence and high performance liquid chromatography (HPLC) methods. The advantage of the latter two is the avoidance of radiation exposure; however, the assay methods are more expensive and generally performed in specialized laboratories. All other filtration markers deviate from ideal behavior. Overall, there is suggestion by some but not all studies that iothalamate clearance results in a higher GFR than inulin clearance, presumably due to secretion of iothalamate by the tubules. Other studies suggest that iohexol clearance may underestimate inulin clearance. DTPA readily dissociates from its radioactive tracer, allowing binding of the tracer to plasma proteins leading to retention of the tracer and underestimation of GFR.

GFR can also by measured by counting of a radioactive exogenous filtration marker over the kidneys and bladder. This technique can be combined with renal imaging, usually using ^{99m}Tc-DTPA, and is useful for determination of split kidney function.[88,100] Several studies indicate poor correlation of ^{99m}Tc-DTPA dynamic renal imaging with simultaneous urinary or plasma clearance, reflecting both bias and imprecision, and lesser accuracy than estimated GFR.[101–103] Currently, magnetic resonance imaging (MRI) is being investigated for measurement of GFR. Many protocols are in use which will require consolidation before introduction into clinical practice.[104,105]

Because of these limitations, all values for measured GFR contain an element of error, which differentiates them from true GFR. As such there is variability in the literature as to how each of these markers and methods compare to the gold standard method.

Estimation of the Glomerular Filtration Rate

Relationship of Glomerular Filtration Rate to the Plasma Solute Concentration

The plasma level of a solute (P_x) is determined by its generation (G_x) from cells and diet, extrarenal elimination (E_x) by gut and liver, and urinary excretion ($U_x \times V$) by the kidney (Fig. 9.6).[106] Physiologic processes other than GFR that affect the plasma level of a solute (P_x) are termed "non-GFR determinants." The following discussion relates concepts of plasma levels of filtration markers, their non-GFR determinants, and the physiologic basis for GFR estimating equations.

An important concept for this discussion is the steady state of solute balance. A steady state with regard to substance x is achieved when the rate of generation in body fluids (either from endogenous production or exogenous intake) is constant and equal to its rate of elimination from body fluids (either from excretion or metabolism). Therefore, in the steady state, the plasma concentration of substance x is constant:

$$G_x - E_x = U_x \times V \quad (10)$$

where G_x and E_x are the rates of generation and extra-renal elimination of x. If the substance is excreted only in the

9.2 Strengths and Limitations of Glomerular Filtration Rate Measurement Methods and Markers

Approach	Strengths	Limitations
Methods		
Urinary Clearance		
Bladder catheter and continuous intravenous infusion of marker	■ Gold standard method	■ Invasive
Spontaneous bladder emptying	■ Patient comfort ■ Less invasive	■ Possibility of incomplete bladder emptying ■ Low flow rates in people with low levels of GFR
Bolus administration of marker	■ Shorter duration	■ Rapidly declining plasma levels at high levels of GFR ■ Longer equilibration time in extracellular volume expansion
24-hour urinary collection		■ Cumbersome ■ Prone to error
Plasma clearance	■ No urine collection required ■ Potential for increased precision	■ Overestimation of GFR in extracellular volume expansion ■ Inaccurate values with one-sample technique, particularly at lower GFR levels ■ Longer duration of plasma sampling required for low GFR
Nuclear imaging	■ No urine collection or repeated blood samples required ■ Relatively short duration	■ Less accurate
Markers*		
Inulin	■ Gold standard ■ No side effects	■ Expensive ■ Difficult to dissolve and maintain into solution ■ Short supply
Creatinine	■ Endogenous marker, no need for administration ■ Assay available in all clinical laboratories	■ Secretion which can vary among and within individuals
Iothalamate	■ Inexpensive ■ Long half-life	■ Probable tubular secretion ■ Requirement for storage, administration, and disposal of radioactive substances when iothalamate-125 used as tracer ■ Use of nonradioactive iothalamate requires expensive assay ■ Cannot be used in patients with allergies to iodine
Iohexol	■ Not radioactive ■ Inexpensive ■ Sensitive assay allows for low dose	■ Possible tubular reabsorption or protein binding ■ Use of low doses requires expensive assay ■ Cannot be used in patients with allergies to iodine ■ Nephrotoxicity and risk for allergic reactions at high doses

(continued)

9.2 Strengths and Limitations of Glomerular Filtration Rate Measurement Methods and Markers (continued)

Approach	Strengths	Limitations
EDTA	■ Widely available in Europe	■ Probable tubular reabsorption ■ Requirement for storage, administration, and disposal of radioactive substances when ^{51}Cr is used as tracer
DTPA	■ Widely available in the United States ■ New sensitive and easy to use assay for gadolinium	■ Requirement for storage, administration, and disposal of radioactive substances when ^{99m}Tc used as tracer ■ Requires standardization for ^{99m}Tc ■ Dissociation and protein binding of ^{99m}Tc ■ Concern for NSF when gadolinium is used as the tracer

^{51}Cr, chromium-51; ^{99m}Tc, technetium-99m; DTPA, diethylene triamine pentaacetic acid; EDTA, Ethylenediaminetetraacetic acid; GFR, glomerular filtration rate; NSF, nephrogenic systemic fibrosis.

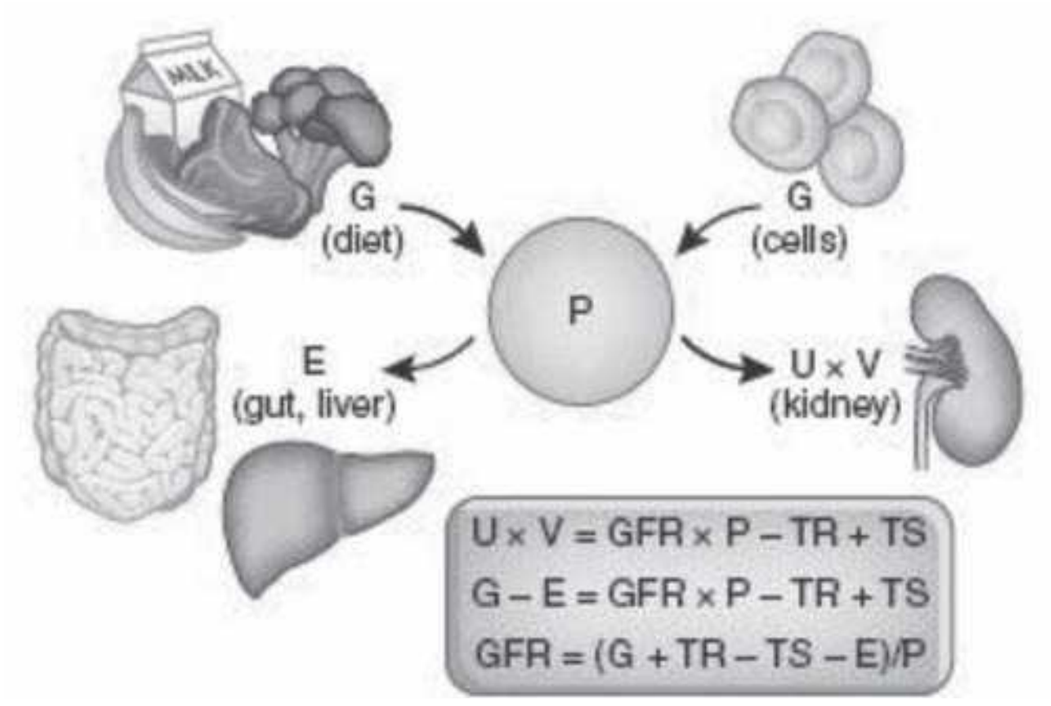


FIGURE 9.6 Determinants of the serum level of endogenous filtration markers. The plasma level (P) of an endogenous filtration marker is determined by its generation (G) from cells and diet, extrarenal elimination (E) by gut and liver, and urinary excretion (UV) by the kidney. Urinary excretion is the sum of filtered load (GFR × P), tubular secretion (TS), and reabsorption (TR). In the steady state, urinary excretion equals generation and extrarenal elimination. By substitution and rearrangement, GFR can be expressed as the ratio of the non-GFR determinants (G, TS, TR, and E) to the plasma level. (Reproduced from Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J Am Soc Nephrol.* 2009;20(11):2305–2313.)

urine, in the steady state, the rate of generation can be assessed from the urinary excretion rate.

$$G_x = U_x \times V \quad (11)$$

By rearrangement of Equations 9.7 and 9.10 and solving for P_x, we obtain the following:

$$P_x = (G_x - TR_x + TS_x - E_x) / GFR \quad (12)$$

Hence, P_x is inversely related to GFR, and directly related to its non-GFR determinants.

$$GFR = (G_x - TR_x + TS_x - E_x) / P_x \quad (13)$$

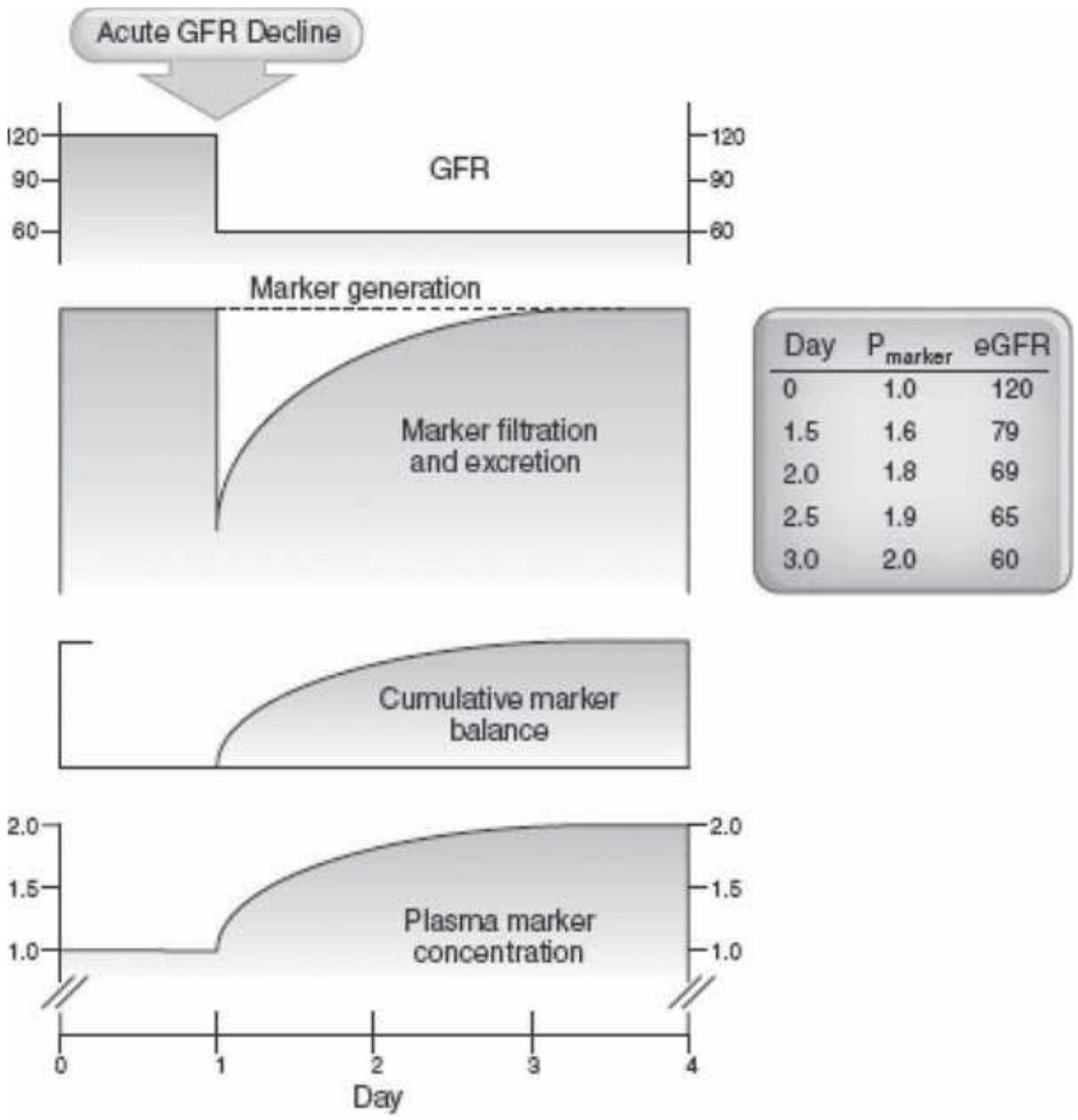
For a substance that is eliminated entirely by glomerular filtration, this relationship simplifies to the following.

$$GFR = G_x / P_x \quad (14)$$

If the rate of generation is constant across individuals and over time, the level of GFR can be estimated by the plasma level and proportionality constant.

$$GFR = k / P_x \quad (15)$$

Figure 9.7 shows the hypothetical change in levels of a filtration marker GFR after an acute change in GFR.[106,107] After an acute GFR decline, generation of the marker is unchanged, but filtration and excretion are reduced, resulting in retention of the marker (a rising positive balance) and a rising plasma level (non–steady state). Although GFR remains reduced, the rise in plasma level leads to an increase



Day	P_{marker}	eGFR
0	1.0	120
1.5	1.6	79
2.0	1.8	69
2.5	1.9	65
3.0	2.0	60

FIGURE 9.7 Effect of an acute glomerular filtration rate (GFR) decline on generation, filtration, excretion, balance, and serum level of endogenous filtration markers. GFR is expressed in units of milliliter per minute per 1.73 m^2. Tubular secretion and reabsorption and extrarenal elimination are assumed to be zero. (Reproduced with permission from Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J Am Soc Nephrol.* 2009;20(11):2305–2313. Modified from Kassirer JP. Clinical evaluation of kidney function—glomerular function. *N Engl J Med.* 1971;285:385–389.)

in filtered load ($GFR \times P_x$) until it equals generation (G_x). At that time, cumulative balance and the plasma level plateau at a new steady state. In this example, a halving of GFR is associated with a doubling of the plasma concentration of the marker.

Physiologic Basis of Glomerular Filtration Rate Estimating Equations

This section discusses general principles of GFR estimating equations. Specific estimating equations for GFR are discussed in more detail later in the chapter. Estimating GFR from the plasma level of endogenous filtration markers has the advantages of eliminating the need for infusion of an exogenous filtration marker and urine collections. Unfortunately, the plasma levels of all endogenous filtration markers are influenced by physiologic processes other than GFR, these processes are generally not measured in clinical practice, and clinical conditions affecting these physiologic processes are not known for all filtration markers.

Estimating equations for GFR are regression equations that estimate measured GFR from plasma levels of endogenous filtration markers and demographic and clinical variables as observed surrogates for the unmeasured physiologic processes (non-GFR determinants).[108] By definition, an estimating equation provides a more accurate estimate of measured GFR than the plasma concentration alone. For example, the equation below shows the hypothetical relationship of numerical values for demographic and clinical variables X, Y, and Z to generation of the filtration marker (G_x).

$$G_x \sim X + Y + Z \tag{16}$$

Therefore, by substitution into Equation 9.14

$$eGFR = (bX + cY + dZ) / aP_x + \varepsilon \tag{17}$$

where eGFR is estimated GFR and a, b, c, and d are regression coefficients relating P_x and other variables to measured GFR, and ε is the error based on uncertainty due to measurement, biologic variability, and statistical techniques used to derive the coefficients. Estimating equations for GFR are often expressed on the logarithmic scale and, therefore, have the appearance of

$$\log eGFR = aP_x + bX + cY + dZ + \varepsilon \tag{18}$$

$$eGFR = [P_x]^a \times X^b \times Y^c \times Z^d \times \varepsilon \tag{19}$$

where a is a negative coefficient to account for the inverse relationship between GFR and the plasma level of the filtration marker.

GFR estimating equations are derived in the steady state; hence, GFR estimates are more accurate in the steady state than in the non–steady state. In the non–steady state (Fig. 9.7), the rate and direction of change in the level of the

filtration marker and eGFR are affected by the magnitude of change in GFR, but also by the non-GFR determinants and the volume of distribution of the filtration marker.[109] Hence, the plasma level of the filtration marker reflects the magnitude and direction of the change in GFR but does not accurately reflect the level of GFR. After a fall in GFR, the decline in eGFR is less than the decline in GFR, and eGFR thus exceeds GFR. Conversely, after a rise in GFR, the rise in eGFR is less than the rise in GFR, and eGFR is thus less than GFR. As the plasma level approaches the new steady state, eGFR approaches GFR and the level of the filtration marker varies inversely with GFR.

Development and Validation of Glomerular Filtration Rate Estimating Equations

Development and validation of GFR estimating equations should be undertaken with appropriate attention to epidemiologic and statistical techniques. In general, a large sample size (n > 500 subjects) with a wide range of GFR is required for developing a GFR estimating equation. It is important to include both men and women across a wide age range and from a variety of racial and ethnic groups for international use. Validation should be undertaken in a separate population, selected according to similar criteria and with similar clinical and demographic characteristics to the development population. GFR should be measured in both populations using either inulin or an exogenous filtration marker and clearance method validated against inulin clearance. Plasma or serum concentrations of the endogenous filtration markers should be measured using assays calibrated to reference standard. The development process should proceed according to a protocol for introduction and selection of important covariates that are hypothesized to reflect non-GFR determinants of the filtration markers. The validation process should systematically evaluate bias, precision, and accuracy in the overall validation population and in clinically relevant subgroups (Table 9.3).[110] Bias reflects a systematic difference in performance, generally due to differences between the development and validation population in measurement methods for GFR, assays for filtration markers, or selection of study subjects. Imprecision reflects random error, and is generally greater at higher GFR values, due to greater GFR measurement error and greater variation in non-GFR determinants, than at lower GFR. In principle, the use of multiple filtration markers can improve precision by cancelling errors due to variation in non-GFR determinants.

Creatinine as a Filtration Marker

Creatinine is the most frequently measured endogenous filtration marker in routine clinical practice. It has been estimated that serum creatinine is measured more than 280 million times per year in the United States.[111] The classical assay was first introduced more than 125 years ago by Jaffé.[112] The normal level of GFR is sufficient to maintain a low concentration of creatinine in serum, approximately 0.7 to 0.9 mg per dl in healthy young people. Reference ranges cited by clinical laboratories vary because of variation in serum creatinine assays. More importantly, reference ranges are difficult to interpret because of variation among individuals in non-GFR determinants (Table 9.4)[113]; serum creatinine may not rise above the upper limit of the reference range unless GFR is less than 60 mL/min/1.73 m^2. Recent interest in more accurate GFR estimation has led to worldwide standardization of serum creatinine assays and reporting of estimated GFR when serum creatinine is measured.[114] Using eGFR overcomes some of these limitations, but imprecision remains, especially at higher GFR.

9.3 Metrics for Evaluation of Glomerular Filtration Rate Estimating Equations

Criteria	Metric	Definition
Bias	Median difference	mGFR − eGFR
	Median percent difference	(mGFR − eGFR)/mGFR * 100
Precision	IQR difference	Interquartile range of (mGFR − eGFR)
	IQR % difference	Interquartile range of [(mGFR − eGFR)/m GFR] * 100
Accuracy	Median absolute difference	Median of the absolute value of eGFR − mGFR
	P_{30}	Percent of estimates within 30% of measured GFR
	RMSE	Square root of mean $(\log mGFR - \log eGFR)^2$

*Measures of accuracy assess precision when bias is 0 (development datasets).
IQR, interquartile range; eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate; RMSE, root-mean-square deviation.
From Stevens LA, Zhang Y, Schmid CH. Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol. 2008;21(6):797–807.

9.4 Clinical Conditions that Cause Errors in the Estimation of GFR from Measurement of Creatinine Clearance or Serum Creatinine

Condition	Effect on C_{cr}	Effect on P_{cr}	Comment
Plasma Ketosis	None	Increase	Interference with the picric acid assay for creatinine
Medications			
Certain cephalosporins or flucytosine	None	Increase	Interference with the picric acid and iminohydrolase assays for creatinine, respectively
Cimetidine or trimethoprim	Decrease	Increase	Inhibition of tubular secretion of creatinine
Dietary Protein			
Ingesting cooked meat	Increase	Increase	Transient increase in GFR and creatinine generation
Restriction of dietary protein	Decrease	Decrease	Sustained decrease in GFR and creatinine generation
Muscle Change			
Vigorous prolonged exercise	Decrease	Increase	Transient decrease in GFR and increase in muscle creatinine generation
Muscle wasting	None	Decrease	Decrease in muscle creatinine generation
Muscle growth	None	Decrease	Increase in muscle creatinine generation
Kidney Disease[a]	Increase	Decrease	Decrease in GFR, but stimulation of tubular secretion of creatinine, and possible decrease in creatinine generation

[a]Effects on C_{cr} and P_{cr} relative to effects on GFR (i.e., C_{cr} is higher than expected and P_{cr} is lower than expected for the reduction in GFR; see text).
C_{cr}, creatinine clearance; P_{cr}, serum creatinine; GFR, glomerular filtration rate.
From Levey AS. Clinical evaluation of renal function. In: Greenberg A, ed. Primer of Kidney Diseases. San Diego: Academic Press; 1998:23.

Kidney Handling of Creatinine

Creatinine is small (molecular weight 113 daltons, molecular radius 0.3 nm) and not bound to plasma proteins; hence, it passes freely through the glomerular capillary wall into the Bowman's space. However, it is also secreted by the tubules, probably by the same pathway used for other organic cations.[115] Therefore, creatinine is excreted not only by glomerular filtration, but also by tubular secretion.

$$U_{cr} \times V = GFR \times S_{cr} + TS_{cr} \quad (20)$$

where Scr is serum creatinine concentration (virtually identical to plasma concentration) and TScr is the rate of tubular secretion. Consequently, it is not an ideal filtration marker. The true relationship between creatinine clearance and GFR is as follows

$$C_{cr} = GFR + TS_{cr} / S_{cr} \quad (21)$$

where TS_{cr} / P_{cr} is the clearance of creatinine due to tubular secretion (C_{TScr}). Thus, at all levels of GFR, creatinine clearance exceeds GFR by an amount equal to the clearance of creatinine due to tubular secretion.

Tubular Secretion of Creatinine. Creatinine secretion was recognized long ago,[116] and has been reemphasized in the modern era.[117] It was not initially recognized as a limitation to the estimation of GFR from creatinine clearance; the major reason was related to the method of measurement of serum creatinine used in the past. As discussed later, the classical method, the Jaffé reaction, used a colorimetric reaction that detects both creatinine and a number of noncreatinine chromogens in serum, but not in urine. Thus, the serum "chromogen creatinine" exceeded the true serum creatinine measured by more accurate methods, and using the "chromogen creatinine" to calculate creatinine clearance led to a systematic underestimation of the true value. On the other hand, because of tubular secretion, the true creatinine clearance exceeded GFR. The net result was that estimated creatinine clearance deviated little from GFR in normal individuals. With the introduction of more accurate methods to measure serum creatinine, the discrepancy between creatinine clearance and GFR became more apparent.

Using older assays, the level of serum creatinine in the low range is overestimated, and average creatinine secretion in normal individuals accounted for 5% to 10% of the excreted creatinine. Hence, creatinine clearance exceeded GFR by approximately 10 mL/min/1.73 m^2. However, with the newer assays, normal serum levels are lower, so creatinine secretion can exceed GFR by much larger amounts. The magnitude of this overestimation has not been well quantified. Most studies find proportionately greater creatinine secretion in patients with reduced GFR, which leads to a clear disparity between creatinine clearance and GFR.[118] Moreover, the magnitude of creatinine secretion is variable among individuals and over time. Only some of the factors responsible for this variability are known. The level of GFR appears to be a major determinant.[117] The mean difference between C_{cr} and GFR (the clearance due to tubular secretion) within the range of GFR from 40 to 80 mL/min/1.73 m^2 is approximately 35 mL/min/1.73 m^2 and lower at lower GFR.

Other factors determining the magnitude of creatinine secretion are the type of kidney disease and the quantity of dietary protein intake. Patients with polycystic kidney disease and tubulointerstitial diseases have lower mean values for creatinine clearance due to secretion than patients with glomerular diseases and other diseases,[61] perhaps reflecting more serious tubular injury and limitation of tubular secretion. On the other hand, higher protein intake is associated with higher mean values for creatinine clearance due to secretion,[61] perhaps due to stimulation of secretion due to protein ingestion. This finding may account for the greater effect of protein loads on creatinine clearance compared to GFR.[46]

Several commonly used medications, including cimetidine and trimethoprim,[119] competitively inhibit creatinine secretion, thereby reducing creatinine clearance and raising the serum creatinine concentration, despite no effect on GFR. Clinically, it can be difficult to distinguish a rise in serum creatinine due to drug-induced inhibition of creatinine secretion from a decline in GFR. A clue to inhibition of creatinine secretion is that urea clearance and blood urea nitrogen concentration are unchanged.

Some investigators have proposed using cimetidine to inhibit creatinine secretion during creatinine clearance measurements, thereby permitting a more accurate assessment of GFR.[120,121] However, complete inhibition of creatinine secretion may require prolonged high dose cimetidine therapy.[122] Variable inhibition of tubular secretion by cimetidine makes interpretation of the test difficult.

Tubular Reabsorption of Creatinine. To a limited extent, creatinine may also be reabsorbed by the tubules. Studies in normal animals and humans with very low urine flow rates,[123–125] and in patients with decompensated congestive heart failure or uncontrolled diabetes mellitus[126–130] have demonstrated a ratio of clearances of creatinine and inulin <1.0. Reabsorption of creatinine may be due to its passive back-diffusion from the lumen to blood because of the high tubular creatinine concentration that occurs during low urine flow. Based on the clearance ratios observed in these studies, the maximum effect of creatinine reabsorption probably would be a 5% to 10% decrease in creatinine clearance.

Creatinine Metabolism

Generation. Creatinine is distributed throughout total body water. It is generated in muscle from the nonenzymatic conversion of creatine and phosphocreatine (Fig. 9.8).[131] Approximately 98% of the total creatine pool is contained in muscle and about 1.6% to 1.7% per day is converted to creatinine.[131] For example, in an individual with a total creatine pool of 100 g, creatinine generation would be 1.6 to 1.7 g per day. Thus, creatinine generation is proportional to muscle mass, which can be estimated from age, gender, and body size (Fig. 9.9).[132] Based on five reports containing data on 1,100 healthy individuals and patients without renal or hepatic disease, Walser derived the following equations to estimate urine creatinine excretion[133]:

$$eU_{cr} \times V = 28.2 - 0.172 \times \text{age (men)} \quad (22)$$

$$eU_{cr} \times V = 21.9 - 0.115 \times \text{age (women)} \quad (23)$$

where creatinine excretion (given in mg/kg/day) is assumed to equal creatinine generation and age is given in years. These equations do not take into account racial and ethnic differences in muscle mass. African American men and women have higher muscle mass and, consequently, higher creatinine excretion than their European American counterparts.[134–138]

Recently, Ix and colleagues derived equations in a pooled dataset of six studies of 2,466 black and white subjects with and without kidney disease and diabetes.[139] These equations were more accurate than those proposed by Walser and may be more generalizable.

$$\begin{aligned} eU_{cr} \times V = {} & 879.89 + 12.51 \times \text{weight (kg)} \\ & - 6.19 \times \text{age} + 34.51 \text{ (if black)} \\ & - 379.42 \text{ (if female)} \end{aligned} \quad (24)$$

$$\begin{aligned} eU_{cr} \times V = {} & 1115.89 + 11.97 \times \text{weight (kg)} \\ & - 5.83 \times \text{age} - 60.18 \\ & \times \text{phosphorus (mg/dl)} + 52.82 \\ & \text{(if black)} - 368.75 \text{ (if female)} \end{aligned} \quad (25)$$

The relationship of creatinine generation to age, gender, and body weight is affected by muscle mass and diet. Muscle wasting is associated with a decreased creatine pool, which leads to decreased creatinine generation and excretion.[140–143] However, some muscle diseases are associated with increased creatine turnover,[141] which in principle could transiently

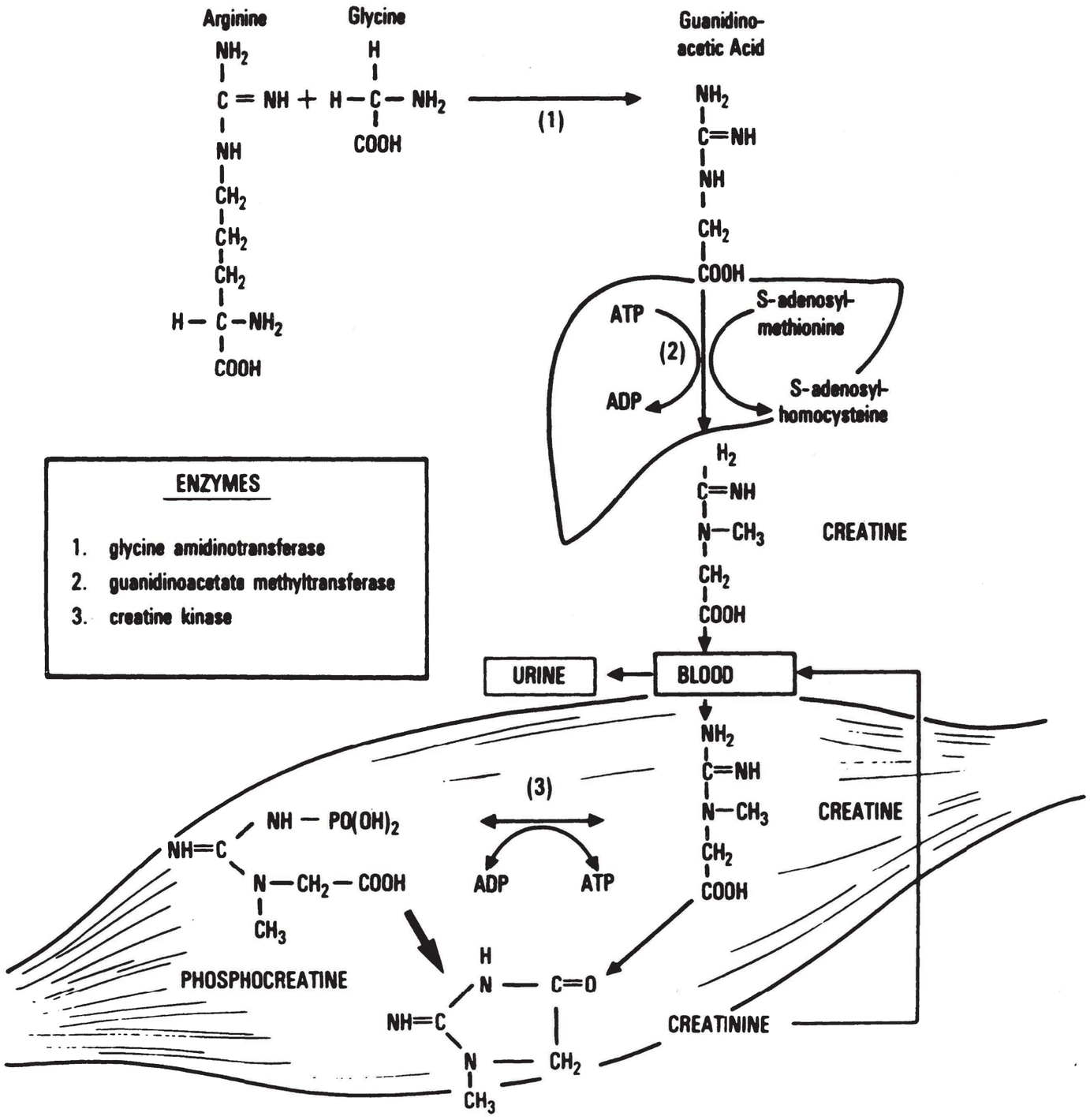


FIGURE 9.8 Pathways of creatinine metabolism. (From Heymsfield SB, Arteaga C, McManus C, et al. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr.* 1983;37:478, with permission.)

increase creatinine generation and excretion. Reduction in dietary protein causes a decrease in the creatine pool by 5% to 15%, which is probably due to the reduction of the availability of creatine precursors, arginine, and glycine.[131,144] Of greater importance is the effect of creatine in the diet. Creatine is contained largely in meat; uncooked lean beef contains about 3.5 to 5 mg of creatine per g.[145,146] Elimination of creatine from the diet decreases urinary creatinine excretion by as much as 30%.[144,147,148] Conversely, ingesting a creatine supplement increases the size of the creatine pool and increases creatinine excretion.[144,149–151] Meat intake also affects creatinine generation and excretion independent of its effect on the creatine pool. During cooking, a variable amount (18% to 65%) of the creatine in meat is converted to creatinine, which is absorbed from the gastrointestinal tract. Therefore, following ingestion of cooked meat, there is a sudden transient increase in the serum creatinine concentration and urinary creatinine excretion. These findings are not observed when a similar quantity of uncooked meat is ingested.[152,153]

Extrarenal Elimination. Extrarenal loss of creatinine is not detectable in normal individuals, but may account for up to 68% of daily creatinine generation in patients with severe decrease in GFR. One likely, but still not established, mechanism is degradation of creatinine within the intestinal lumen by microorganisms due to induction of the enzyme creatininase.[154–158]

Thus, in patients with kidney disease, creatinine excretion underestimates creatinine generation:

$$U_{cr} \times V = G_{cr} - E_{cr} \quad (26)$$

where E_{cr} is the rate of elimination of creatinine by extrarenal routes.

Measurement of Creatinine

Creatinine can be measured easily in serum, plasma, and urine and a variety of methods are used by clinical laboratories. The National Kidney Disease Education Program (NKDEP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recently completed standardization of serum creatinine assays to minimize differences in clinical laboratories and facilitate more accurate reporting of estimated GFR.[114,159] The reference standard for creatinine assay is isotope dilution mass spectrometry (IDMS) using either gas or liquid chromatography.[114,160,161] All instruments can now be calibrated to standardized serum creatinine using secondary reference materials and proficiency testing programs.[162] Calibration does not eliminate the problem of interference by specific substances in serum with specific assays.[161]

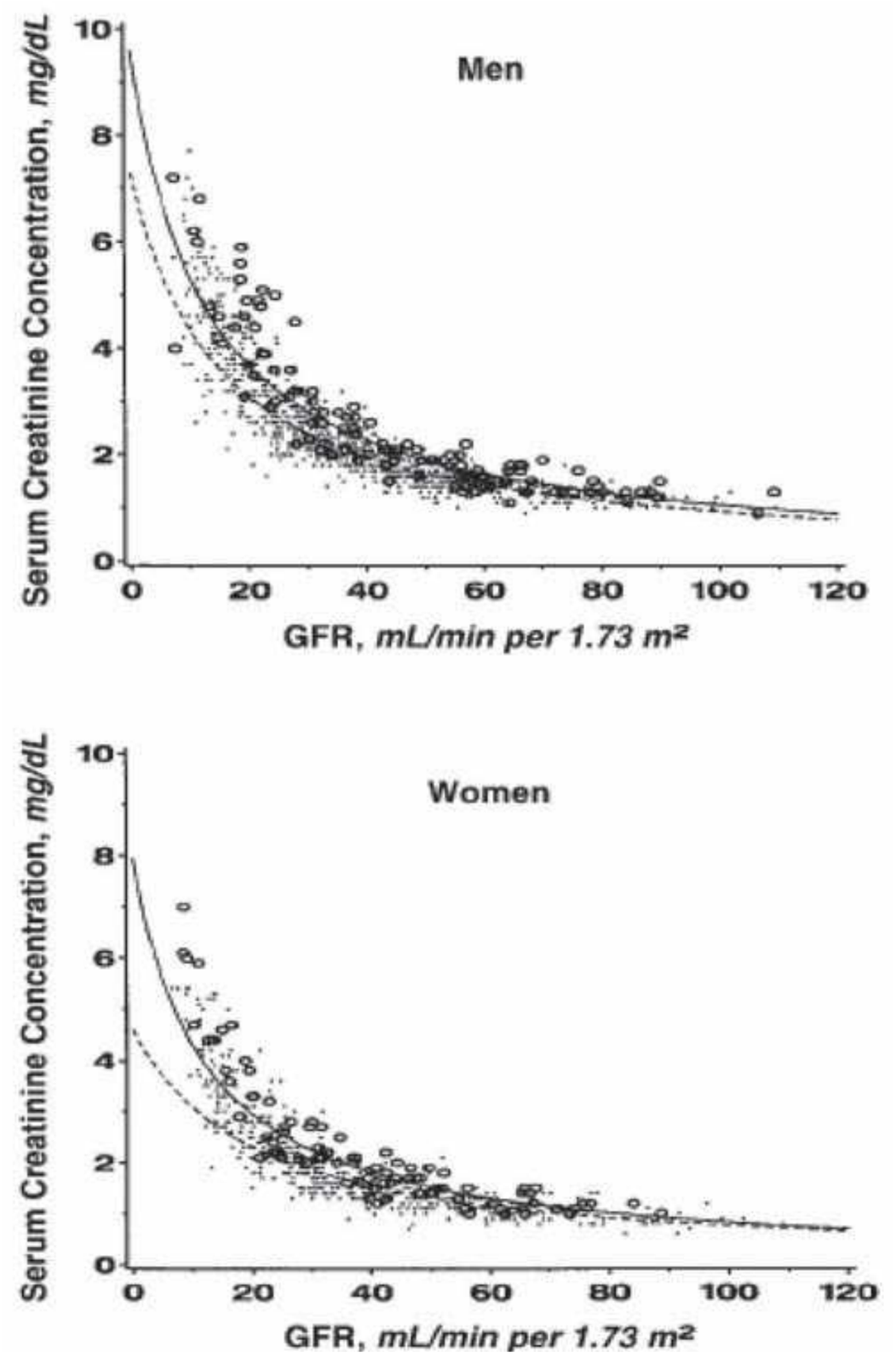


FIGURE 9.9 Relationship of serum creatinine concentration to measured glomerular filtration rate (GFR) in the Modification of Diet in Renal Disease Study. GFR was measured as the urinary clearance of ^{125}I-iothalamate. Serum creatinine concentration was measured using a Beckman Astra CX3 analyzer and a kinetic alkaline picrate assay.[33,47] Regression lines were computed from the relationship of reciprocal of serum creatinine versus GFR. When GFR is 60 mL/min/1.73 m^2, the 95% confidence interval for the serum creatinine concentration is 1.4 to 1.8 mg per dl for white men (n = 802) and 1.3 to 1.5 for African American men (n = 113) (*left panel*), and 1.1 to 1.4 mg per dl (97.2 and 123.8 μmol per L) for white women (n = 502) and 1.0 to 1.2 mg per dl (88.4 and 106.1 μmol per L) for African American women (n = 84) (*right panel*). These levels are close to the upper limit of the reference range. Confidence intervals for serum creatinine levels are wider at lower levels of GFR. (Reproduced with permission from Stevens LA, Coresh J, Greene T, et al. Assessing kidney function—measured and estimated glomerular filtration rate. *N Engl J Med*. 2006;354(23):2473–2483.)

The classic method uses the Jaffé reaction in which creatinine reacts directly with picrate ion under alkaline conditions to form a red-orange complex that is easily detected and quantified.[163] However, in normal subjects, up to 20% of the color reaction in serum or plasma is due to substances other than creatinine. Two classes of positive interferences have been described: substances such as glucose, ascorbate, and uric acid, which slowly reduce the alkaline picrate, and substances such as acetoacetate, pyruvate, other ketoacids, fluorescein, furosemide, hemoglobin, paraquat and diquat, and serum proteins which react with alkaline picrate to form colored complexes. The error in measurement can be greater, however, in diabetic ketoacidosis due to the increased concentration of acetoacetate, and in patients taking certain cephalosporins which can contribute to the colorimetric reaction. Very high serum bilirubin levels can cause falsely lower creatinine levels. In patients with kidney disease, noncreatinine chromogens are not retained to the same degree as creatinine. Consequently, the overestimation of serum creatinine and the corresponding underestimation of creatinine clearance are reduced. In general, noncreatinine chromogens are not present in sufficient concentration in urine to interfere with creatinine measurement. Hence, measurement of creatinine clearance in normal individuals using the Jaffé reaction results in values that are approximately 20% lower than the true value.

The kinetic alkaline picrate method takes advantage of the differential rate of color development for noncreatinine chromogens compared to creatinine. It significantly reduces, but does not eliminate, both types of positive interferences described earlier. A survey by the College of American Pathologists (CAP) in 2004 found that assays based on the alkaline picrate method were the most widely used in clinical laboratories in the United States.[162]

To circumvent interferences in the alkaline picrate reaction, other methods have been developed which are increasingly used by clinical laboratories. Enzymatic methods include the creatinine iminohydrolase and creatininase-creatinase-sarcosine oxidase methods. The antifungal agent, flucytosine, interferes with the creatinine iminohydrolase method, whereas bilirubin, dopamine, dobutamine, ascorbic acid, and sarcosine may interfere with the creatinase-creatininase methods. HPLC is a fairly sensitive and analytically specific method for measuring serum creatinine, but technically more difficult than enzymatic methods. Enzymatic and HPLC methods usually provide values that are 10% to 20% lower than kinetic alkaline picrate methods and are closer to the reference standard.

Serum Creatinine as an Index of Kidney Function

Based on substitutions and rearrangements of Equations 9.20 and 9.24, the relationship between GFR and serum creatinine is as follows:

$$GFR = (G_{cr} - E_{cr} - TS_{cr}) / S_{cr} \quad (27)$$

Estimating equations have been developed to estimate creatinine clearance[164–170] and GFR.[171–174] Most use age, sex, and body size as surrogates for creatinine generation. According to the June 2008 Chemistry Survey of the College of American Pathologists (CAP), 77% of clinical laboratories report eGFR when serum creatinine is measured.[114]

Due to its relative ease of use, one of the first estimating equations to be widely used is the Cockcroft and Gault formula.[164]

$$eC_{cr} = [140 - \text{age} \times \text{body weight}] \times 0.85 \text{ (if female)} / [S_{cr} \times 72] \quad (28)$$

where C_{cr} is expressed in mL per minute, age is expressed in years, body weight is expressed in kg, and S_{cr} is expressed in mg per dl. The formula was derived in 236 men (mean measured creatinine clearance of 73 mL per minute) in 1973. The formula for women was based on the assumption that creatinine generation is 15% less in women than in men. The Cockcroft and Gault formula was extensively validated before standardization of creatinine assays, but cannot be re-expressed for use with standardized creatinine assays. Use of standardized serum creatinine values in the Cockcroft and Gault equation leads to overestimates of creatinine clearance. Because measured creatinine clearance exceeds measured GFR, these overestimations may be particularly misleading.

Recent studies have developed equations to estimate GFR rather than creatinine clearance. The most commonly used equation is the Modification of Diet in Renal Disease (MDRD) Study.[132,175]

$$\text{eGFR} = 186 \times S_{cr} \text{ (mg per dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times 0.742 \text{ (if female)} \times 1.210 \text{ (if black)} \quad (29)$$

where eGFR is expressed in mL/min/1.73 m^2, S_{cr} is expressed in mg per dl, and age in years. The MDRD Study equation has now been re-expressed for standardized serum creatinine as

$$\text{eGFR} = 175 \times \text{standardized } S_{cr} \text{ (mg per dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times 0.742 \text{ (if female)} \times 1.210 \text{ (if black)} \quad (30)$$

The MDRD Study equation was developed in 1,628 patients with chronic kidney disease (mean GFR of 40 mL/min/1.73 m^2) who were predominantly white and had predominantly nondiabetic kidney disease. The equation was reported in 1999 and has been validated in African Americans with hypertensive nephrosclerosis, diabetic kidney disease, and kidney transplant recipients.[176] Inclusion of the race term significantly improved the prediction, which is likely because of the larger muscle mass in African Americans compared to whites. The MDRD Study equation is more accurate than the Cockcroft-Gault equation as well as measured urinary creatinine clearance. Its main disadvantage is a systemic underestimation of measured GFR and imprecision at higher values. Because of this, NKDEP recommends that eGFR >60 mL/min/1.73 m^2 using this equation not be reported as a numeric value.

In 2009, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) reported a more accurate equation using a large diverse dataset pooled from multiple studies. The development dataset included 8,254 individuals from 10 studies with a mean measured GFR of 68 mL/min/1.73 m^2. The validation dataset included 3,859 individuals from 16 additional studies with measured GFR.[177]

$$\text{eGFR} = 141 \times \min(\text{standardized } S_{cr}/\kappa, 1)^{\alpha} \times \max(\text{standardized } S_{cr}/\kappa, 1)^{1.209} \times 0.993^{\text{age}} \times 1.1018 \text{ (if female)} \times 1.159 \text{ (if black)} \quad (31)$$

where eGFR is expressed in mL/min/1.73 m^2, standardized serum creatinine is expressed as mg per dl, age is expressed in years, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and max indicates maximum of S_{cr}/κ or 1. The CKD-EPI equation uses the same variables as the MDRD Study equation, but includes a nonlinear term for serum creatinine that substantially reduces bias at higher GFR, enabling numeric eGFR reports throughout the range (Fig. 9.10). The main disadvantage is imprecision in the high range for eGFR. Most but not all studies confirm the greater accuracy of the CKD-EPI equation compared to the MDRD Study equation.[178–184] In addition, because of lesser bias, use of the CKD-EPI equation leads to lower prevalence estimates of decreased GFR in cross-sectional studies and more steep risk relationships of eGFR to adverse outcomes in longitudinal studies.[111]

Modifications to the MDRD Study and CKD-EPI equations have been proposed to account for racial, ethnic, and regional differences in diet and muscle mass.[185–187] Where these modifications lead to more accurate GFR estimations, it may be reasonable to substitute them for the MDRD Study and CKD-EPI equations, but it is not clear from the current literature whether these modifications truly reflect population differences in non-GFR determinants or methodologic differences, such as GFR measurement, serum creatinine assay, or subject selection.

Currently, most clinical laboratories report eGFR using the MDRD Study. In April 2011, large commercial clinical laboratories in the United States began to use the CKD-EPI equation and it is likely that it will be used more widely in the future. Only a small number of clinical laboratories in the United States report estimated creatinine clearance using the Cockcroft and Gault equation. However, since 1979, the U.S. Food and Drug Administration (FDA) has recommended the Cockcroft and Gault equation for pharmacokinetic studies used for drug development and labeling. For these reasons, drug dosing recommendations by pharmacists are generally based on estimated creatinine clearance computed using the Cockcroft and Gault rather than the MDRD Study or CKD-EPI

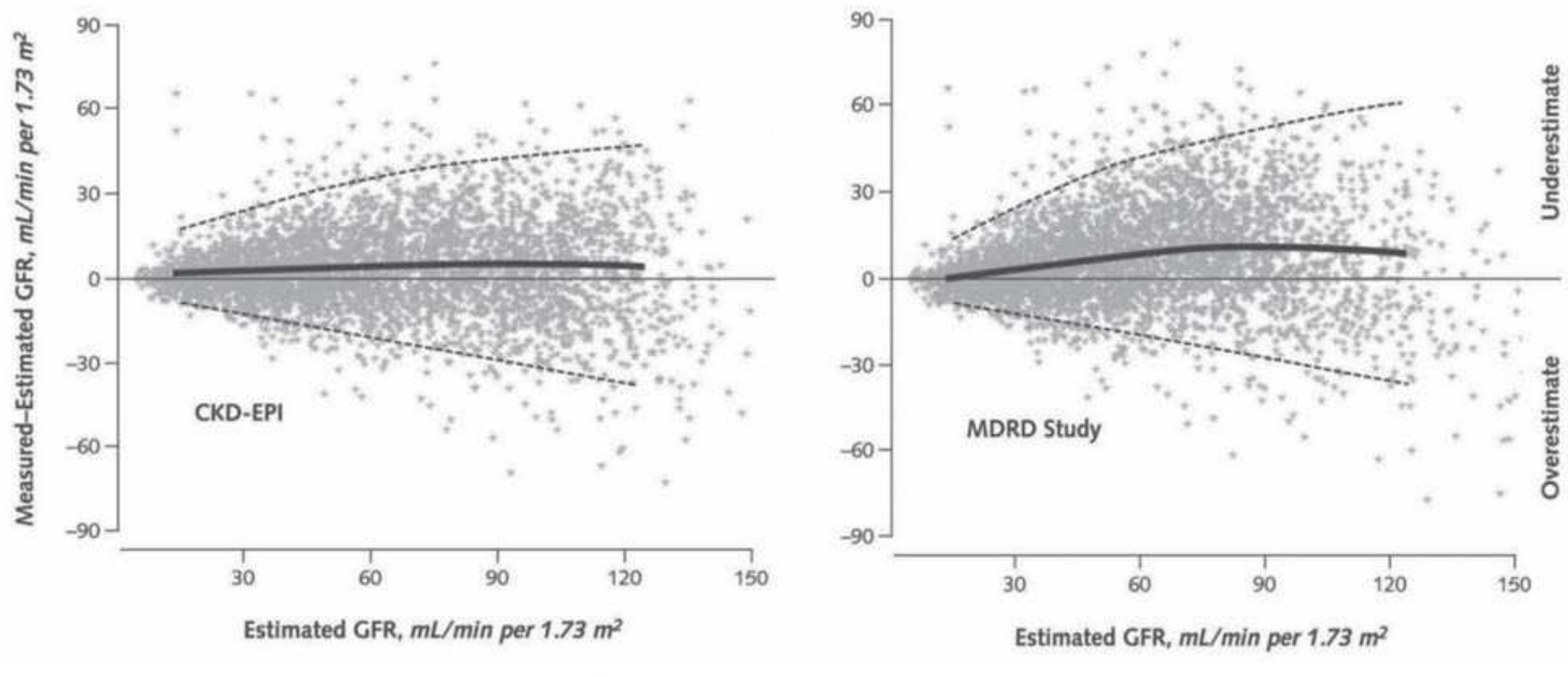


FIGURE 9.10 Comparison of performance of Modification of Diet in Renal Disease (MDRD) Study and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations by estimated glomerular filtration rate (GFR) in the external validation dataset. *Left*. Measured versus estimated GFR. *Right*. Difference between measured and estimated versus estimated GFR. Shown are smoothed regression line and 95% confidence interval (computed using the lowest smoothing function in R), using quantile regression, excluding lowest and highest 2.5% of estimated GFR values. To convert GFR from mL/min/1.73 m^2 to mL/s/m^2, multiply by 0.0167. (Reproduced with permission from Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604–612.)

equations.[188] One study shows a relatively high concordance in drug dosing recommendation using all three of the equations compared to measured GFR, and the NKDEP suggests using GFR estimates reported by clinical laboratories for drug dosing.[189,190] Further guidance by the FDA is needed.

In summary, there are limitations to the use of estimating equations based on the physiologic, analytical, and statistical principles described earlier.[106,108] Tables 9.4 and 9.5 list clinical situations in which estimating equations may not be accurate and clearance measurements may be indicated as a confirmatory test.[106]

9.5 Clinical Situations in Which Estimating Equations for Creatinine Clearance or Glomerular Filtration Rate Measurements May Not be Accurate and Clearance Measurements May be Recommended

- Extremes of age and body size
- Severe malnutrition or obesity
- Diseases of skeletal muscle
- Paraplegia or quadriplegia
- Vegetarian diet
- Rapidly changing kidney function
- Pregnancy
- Prior to dosing drugs with significant toxicity that are excreted by the kidneys

Urea as a Filtration Marker

A relationship between serum urea and kidney function was recognized long before the development of the concept of clearance of or techniques to assess GFR.[191] The factors influencing both the production of urea and its renal excretion, however, are considerably more complex and variable than those for creatinine (Table 9.6).[113] In the United States, urea is traditionally assayed as urea nitrogen. The usual concentration of serum urea nitrogen (for historical reasons, often referred to as the blood urea nitrogen, or BUN) in healthy young people is in the range of 8 to 12 mg per dl, but the reference ranges in clinical laboratories are wider to take into account variation among individuals. The urea clearance is rarely used today as a measure of kidney function, and the serum urea nitrogen concentration has been replaced largely by the serum creatinine concentration as an index of GFR in routine clinical practice. Nonetheless, measurement of the BUN remains useful both as a diagnostic aid in distinguishing among the various causes of acute decline in GFR and as a rough correlate of uremic symptoms in kidney failure. To understand the utility and shortcomings of BUN measurements, a brief summary of the kidney handling and metabolism of urea is presented subsequently.

Kidney Handling of Urea

Urea (molecular weight 60 daltons) is filtered freely by the glomerulus and reabsorbed in both the proximal and distal

9.6 Clinical Conditions that Cause Errors in the Estimation of Glomerular Filtration Rate from Measurement of Urea Clearance or Blood Urea Nitrogen

Condition	Effect on C_{urea}	Effect on BUN	Comment
Extracellular Volume			
Dehydration	Decrease	Increase	Increased urea reabsorption
Reduced renal perfusion (volume depletion, congestive heart failure)	Decrease	Increase	Reduced GFR, increased urea reabsorption, increased urea generation
Overhydration	Increase	Decrease	Reduced urea reabsorption
Increased renal perfusion (volume expansion, pregnancy, syndrome of inappropriate ADH secretion)	Increase	Decrease	Increased GFR, reduced urea reabsorption
Dietary Protein or Catabolism			
Restriction of dietary protein	Decrease	Decrease	Sustained decrease in GFR and reduced urea generation
Increased dietary protein	Increase	Increase	Sustained increase in GFR and increased urea generation
Accelerated catabolism (fever, trauma, GI bleeding, cell lysis, therapy with tetracycline or corticosteroids)	None	Increase	Increased urea generation
Liver Disease	Decrease[a]	Decrease[a]	Decreased GFR, decreased urea reabsorption, decreased urea generation
Kidney Disease	None[a]	Decrease[a]	Decreased GFR, no change in urea reabsorption, decreased urea generation (if dietary protein is restricted)

[a]Effects on C_{urea} and BUN relative to effects on GFR (i.e., C_{urea} is lower than expected for the reduction in GFR).
ADH, antidiuretic hormone; BUN, blood (serum) urea nitrogen; C_{urea}, urea clearance; GFR, glomerular filtration rate; GI, gastrointestinal.
From Levey AS. Clinical evaluation of renal function. In: Greenberg A, ed. Primer of Kidney Diseases. San Diego: Academic Press; 1998.

nephron. Hence, urea excretion ($U_{UN} \times V$) is determined by both the filtered load and tubular reabsorption (TR_{UN})

$$U_{UN} \times V = GFR \times BUN - TR_{UN} \quad (32)$$

Consequently, clearance of urea (or urea nitrogen, C_{UN}) is less than GFR

$$C_{UN} = GFR - TR_{UN} / BUN \quad (33)$$

A large fraction of the filtered load of urea is reabsorbed in the proximal convoluted tubule. In the medullary collecting duct, urea reabsorption is linked closely to water reabsorption. In the absence of antidiuretic hormone (diuresis), the medullary collecting duct is relatively impermeable to urea; thus, urea reabsorption is minimal. Conversely, in the presence of antidiuretic hormone (antidiuresis), permeability rises and urea reabsorption increases. In normal individuals, the ratio of urea clearance to GFR varies from as high as 0.65 during diuresis to as low as 0.35 during antidiuresis.

In patients with GFR less than 20 mL/min/1.73 m^2, the ratio of urea clearance to GFR is higher (0.7 to 0.9) and is not influenced greatly by the state of diuresis. Thus, urea clearance is approximately 5 mL per minute less than GFR. By coincidence, at this level of GFR, the difference between the values of GFR and urea clearance is similar to the difference between the values of creatinine clearance and GFR. Hence, the average of the clearances of urea and creatinine approximates the level of GFR.[172,173] This coincidence provides a relatively simple method to assess GFR in advanced renal disease. A single blood sample and 24-hour urine collection may be analyzed for creatinine and urea nitrogen

and the values for clearance may be averaged. However, the kidney handling of urea and creatinine is influenced by different physiologic and pathologic processes and may vary independently, causing deviations from this approximation.

Urea Metabolism

The metabolism of urea, its relationship to dietary protein intake, and the effect of renal insufficiency on protein metabolism are discussed in detail in Chapter 72. Briefly, urea is the end product of protein catabolism and is synthesized primarily by the liver. Approximately one quarter of synthesized urea is metabolized in the intestine to carbon dioxide and ammonia; thus, the ammonia that is generated returns to the liver and is reconverted to urea.

Dietary protein intake is the principal determinant of urea generation and may be estimated as follows:

$$EPI = 6.25 \times G_{UN} \quad (34)$$

where EPI is estimated protein intake, G_{UN} is urea generation, and both are measured in g per day.[192] Usual protein intake in the United States is approximately 100 g per day,[193–195] corresponding to a usual value for urea nitrogen generation of approximately 15 g per day.

In the steady state, urea generation can be estimated from the measurements of urea excretion, as shown below:

$$G_{UN} = U_{UN} \times V + 0.031 \times \text{weight} \quad (35)$$

where G_{UN} and $U_{UN} \times V$ are measured in g per day, weight is measured in kg, and 0.031 g/kg/day is a predicted value for nitrogen losses other than urine urea nitrogen.[196] For a 70-kg individual with a dietary protein intake of 100 g per day, urea excretion and other nitrogen losses would be approximately 13 and 2 g per day, respectively.

Measurement of Urea

The urease method assays the release of ammonia in serum or urine after reaction with the enzyme urease.[197] The presence of ammonium in reagents or use of ammonium heparin as an anticoagulant may falsely elevate the BUN, as can the drugs chloral hydrate, chlorbutanol, and guanethidine.[198] Urea is also subject to degradation by bacterial urease. Bacterial growth in urine samples can be inhibited by refrigerating the sample until measurement or by adding an acid to the collection container to maintain urine pH <4.0.

Blood Urea Nitrogen as an Index of Kidney Function and Protein Intake

In the steady state, the BUN level reflects the levels of urea clearance and generation.

$$BUN = G_{UN} / C_{UN} = U_{UN} \times V / C_{UN} \quad (36)$$

Consequently, many factors influence the level of BUN (Table 9.6). Nonetheless, the BUN can be a useful tool in some clinical circumstances.

As mentioned earlier, the state of diuresis has a large effect on urea reabsorption and a small effect on GFR, but does not affect creatinine secretion. Hence, the state of diuresis affects urea clearance more than creatinine clearance, and is reflected in the ratio of BUN to serum creatinine. The normal ratio of BUN to serum creatinine is approximately 10:1. In principle, a reduction in GFR without a change in the state of diuresis would not alter the ratio. However, conditions causing antidiuresis (dehydration or decreased kidney perfusion) would decrease GFR and increase urea reabsorption, thus raising the BUN-to-creatinine ratio. Consequently, the BUN-to-creatinine ratio is a useful aid in the differential diagnosis of acute GFR decline. Conversely, overhydration or increased renal perfusion would raise GFR and decrease urea reabsorption, thus lowering the serum creatinine and the BUN-to-creatinine ratio.

Also important is the well-recognized relationship of the level of renal function, the BUN level, and clinical features of uremia. A useful "rule" is that a BUN level greater than 100 mg per dl is associated with a higher risk of complications in both acute and chronic kidney failure and may indicate the need to initiate dialysis.[199,200] In both acute and chronic kidney disease, restriction of dietary protein intake to 40 to 50 g per day would reduce urea nitrogen excretion to approximately 4.5 g per day. Consequently, the BUN level might rise to only 40 to 60 mg per dl, despite severe reduction in GFR. Although protein restriction may temporarily ameliorate some of the uremic symptoms, severe reduction in GFR is associated with development of uremic symptoms despite only moderate elevation in BUN.

Urea generation and the BUN are also influenced by factors other than protein intake.[192] An increase is observed after the administration of corticosteroids, diuretics, or tetracyclines; after the absorption of blood from the gut; and in infection, renal failure, trauma, congestive heart failure, and sodium depletion. Decreases in urea generation and BUN may occur in severe malnutrition and liver disease. These conditions may also affect the BUN and the BUN-to-creatinine ratio.

Cystatin C as a Filtration Marker

Cystatin C has been proposed as an endogenous filtration marker. Assays for cystatin C are available in some countries in Europe but are not yet available in the United States. Research studies show that serum levels in healthy young adults are approximately 0.8 mg per L.[201] Studies in human subjects demonstrate a good correlation of serum cystatin C levels with GFR; typically better than that of serum creatinine levels alone, but equivalent or worse than serum creatinine adjusted for age, sex, and race.[202,203] A summary of issues related to its kidney handling, metabolism, measurement, and use as an index of GFR is presented subsequently. Table 9.7 lists the factors that influence the level of cystatin C.

9.7 Clinical Conditions that Cause Errors in the Estimation of Glomerular Filteation Rate from Measurement of Cystatin C

Condition	Effect on Cystatin C	Comment
Demographics		
Age	Decrease	
Male sex	Increase	
Race	No change	When tested in blacks vs. whites; finding has not been validated and requires testing in other racial groups
Cell turnover		
Inflammation	Increase	Seen in inflammatory conditions as indicated by WBC, CRP
Corticosteroids	Increase	
Hyperthyroid	Increase	
Hypothyroid	Decrease	
Diabetes	Increase	
Fat Mass	Increase	
Kidney Disease	Increase	Decreased GFR, suspect may be decrease in extrarenal elimination of cystatin C at low levels of GFR

CRP, C-reactive protein; GFR, glomerular filtration rate; WBC, white blood cell.

Kidney Handling of Cystatin C

Based on its small size (13 kD) and limited direct measurements in the rat, it appears that cystatin C is freely filtered.[204–211] It is then reabsorbed and catabolized by the renal tubules.[204–206] Urinary cystatin C is a marker of kidney damage; it is found in the urine of patients with tubulointerstitial kidney disease,[212] in particular in patients with acute kidney disease, and some glomerular diseases,[213] presumably due to impaired catabolism.[207,208] There is no evidence for tubular secretion,[210] whereas there is indirect evidence for extra-renal elimination of cystatin C in animal studies.[204,211] Direct evaluation of kidney handling in humans has not yet been performed.

Cystatin C Metabolism

Cystatin C is a nonglycosylated basic protein—its mRNA is found in every human tissue.[214] Molecular analysis of its promoter suggests that cystatin C is encoded on a "housekeeping" gene.[214,215] Indirect evidence suggests that there is variability in the generation rate, in particular with states associated with higher or lower cell turnover, such as hyperthyroid or hypothyroid states,[216] or steroid use.[217–220] Epidemiologic studies have suggested that non-GFR determinants of cystatin C—age, sex, body mass index, diabetes, white blood cell count, albumin, and C-reactive protein—were significantly related to higher levels of cystatin C,[202,221] whereas other studies have not shown a relationship to inflammation[222] or diet.[223]

Measurement of Cystatin C

There are several commercially available autoanalyzers to assay cystatin C. At present, methods use nephelometric, turbidimetric, or enzyme-linked immunosorbent assay (ELISA) methods. Despite high precision and reproducibility of the assays, there are large differences among them.[224–226] One study has compared two turbidimetric and one nephelometric cystatin C assay and showed large variation when the assays are used with patient samples, but not when control samples were used, suggesting interference of the assays with substances found in patient samples.[225] Other studies have shown large within and between laboratory variations even for the same assay.[225,227] Variations in the assay would lead to inaccurate GFR estimates. Recently, the International Federation of Clinical Chemists (IFCC) made available a reference material for cystatin C that will allow for standardization of the assays across platforms.[228] At present, the reference materials are not yet FDA approved, and so likely standardization of the commercial platforms will not be uniform in the United States until at least 2015.

Cystatin C as an Index of Kidney Function

Based on these considerations the relationship of GFR to serum levels of cystatin would be as follows:

$$GFR = G_{cys} / S_{cys} \quad (37)$$

Several factors could influence the level of cystatin C independent from the GFR, leading to errors in estimation of GFR (Table 9.7).

Multiple studies have compared serum cystatin C and creatinine as filtration markers in the general population,[182] in those with CKD,[202,229,230] and in special populations with reduced muscle mass[203,231–234] where cystatin C is hypothesized to have a particular advantage and results are mixed. In general, when the two analytes are compared alone, cystatin C appears to be a better filtration marker. When compared

to GFR estimates based on serum creatinine adjusted for age, sex, and race, there is no clear advantage of cystatin C. These results do not suggest that cystatin C should replace creatinine or that there are specific populations in which cystatin C should be used. In combination, creatinine and cystatin C result in a more precise estimate of GFR than either marker alone.[202,235–240] In acute GFR decline, studies in animals and in humans demonstrate that cystatin C increases prior to serum creatinine, and has been interpreted as a more sensitive marker; however, few studies have compared changes in cystatin C to changes in measured GFR.[241]

In contrast to the data on cystatin C as a marker of GFR, the data on cystatin C as a prognostic marker show that it provides consistently better information than creatinine or creatinine-based estimating equations.[241] It is not known whether this improvement is because cystatin C is indeed a better marker of kidney function in these study populations or because non-GFR determinants of cystatin C are also associated with adverse outcomes, as described previously.

PROTEINURIA

The plasma filtered by the kidneys each day contains approximately 11,000 to 14,000 g of protein, yet the final urine is virtually protein-free due to selectivity of glomerular filtration. This conservation of essential proteins is necessary for oncotic regulation, for immune protection, for normal coagulation, and for a host of other vital processes.

An increased protein excretion rate (proteinuria) is usually due to kidney disease, and most kidney diseases are associated with some degree of proteinuria. Proteinuria does not generally cause clinical signs or symptoms. An exception is the nephrotic syndrome, characterized by loss of proteinuria sufficient to cause hypoalbuminemia, edema, and hypercholesterolemia (usually >3.5 g per day). The detection and evaluation of lesser quantities of proteinuria has gained additional significance years as multiple studies have demonstrated its diagnostic and prognostic importance. It has long been known that the degree of proteinuria is a risk factor for kidney disease progression. It has now been shown that the presence of even mildly increased amounts of protein in the urine serves as an independent risk marker for cardiovascular disease and death, independent of other risk factors such as diabetes, hypertension, or advancing age. Recent experimental and clinical studies also suggest an important role for proteinuria in the pathogenesis of the progression of kidney disease.[242] The physiology of protein handling by the kidney and the pathophysiology of proteinuria are extensively covered elsewhere in this book.

This section considers (1) mechanisms by which the kidney handles proteins, (2) methods to measure urine protein, (3) patterns of proteinuria, and (4) clinical interpretation of proteinuria. For several reasons, clinical terminology is slowly changing to focus on albuminuria rather than proteinuria. Albumin is the principal component of urinary protein in most kidney diseases. Recent recommendations for measurement of urine proteins emphasize quantification of albuminuria rather than total protein[1,243,244]; recent epidemiologic data demonstrate a strong graded relationship of the quantity of urine albumin with both kidney and cardiovascular disease risk[36,245–247]; and a recent international conference suggested classification of kidney disease by albuminuria in addition to GFR.[3] In this chapter we will refer to proteinuria when discussing general concepts and will refer either to total protein, albumin, or other specific proteins when discussing measurements, patterns, and interpretation of proteinuria.

Protein Handling by the Kidney

In healthy individuals, the daily urinary protein excretion averages 40 to 80 mg, and the upper limit of normal ranges from 75 to 150 mg. Urine protein is a mixture of plasma proteins that cross the filtration barrier and other proteins that originate in the tubules and lower urinary tract. Of the total, albumin constitutes 30% to 40%, immunoglobulin G (IgG) 5% to 10%, light chains 5%, and IgA 3%. Tamm-Horsfall protein (THP), also known as uromodulin, is a glycoprotein not found in plasma[248,249] and is the most abundant protein in normal human urine and constitutes the remainder.[250] Large molecules, such as IgD and IgM, normally are not detected in the urine.[248,251]

The handling of plasma proteins by the kidney is complex, but consists of two major components: the permeability of the glomerular filter to plasma proteins and the tubular metabolism of filtered proteins. For a detailed review of these mechanisms, the reader is referred to Chapters 72 and 73.

Urine Proteins of Plasma Origin

Low Molecular Weight Proteins. Low molecular weight proteins (less than 25,000 daltons or radius less than 2.3 nm) are extensively filtered by the glomeruli, taken up by the tubules, and subsequently handled by proximal tubular degradation.[252] Biologically important low molecular weight proteins handled by the kidney include enzymes (lysozyme and ribonuclease), immunoglobulins (light chains and beta-2 microglobulin), fibrin-fibrinogen degradation products, and hormones (insulin, growth hormone, and parathyroid hormone). The tubular concentration of these proteins ranges from 50% to 90% of their plasma concentrations (Table 9.8). Low molecular weight proteins are small enough that their charge plays only a minor role in their filtration.

Despite the significant amount of low molecular weight protein that is filtered, only minimal quantities appear in the urine. The proteins are taken up in the proximal tubule and hydrolyzed into amino acids by the vacuolar-lysosomal system. Small amounts of these proteins are actually reabsorbed intact.[253–255] The tubular capacity for some of these low molecular weight proteins is significantly greater than the filtered load. For example, when purified lysozyme was given in an isolated perfused rat kidney, lysozyme did not appear in the urine until the filtered load was increased

9.8 Handling of Plasma Proteins by the Kidney

Protein	Molecular weight (daltons)	Approximate Stokes-Einstein radius (nm)	Approximate ratio of glomerular filtrate to plasma concentrations
Inulin (for reference)	5,200	1.4	1.0
Insulin	6,000	1.6	0.9
Lysozyme	14,600	1.9	0.75
Myoglobulin	16,900	1.9	0.75
Parathyroid hormone (cow)	9,000	2.1	0.65
Growth hormone (rat)	20,000	2.1	0.6–0.7
Light chains	44,000	2.8	0.09[a]
Amylase	48,000	2.9	0.02
Albumin	69,000	3.6	0.02
Gamma globulin	160,000	5.5	0.0
Ferritin	480,000	6.1	0.02

[a]Can be as high as 0.45 if light-chain monomers predominate over dimers in plasma.
From Kanwar YS. Biology of disease: biophysiology of glomerular filtration and proteinuria. Lab Invest. 1984;51:7. Maack T, Johnson B, Kau ST, et al. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. Kidney Int. 1979;16:251.

nearly threefold.[253] Through the process of filtration, tubular absorption, and excretion, the kidney accounts for between 30% and 80% of the metabolic clearance of low molecular weight proteins.[253]

Immunoglobulin light chains are handled in a similar manner. The monomer (molecular weight 22,000 daltons) is filtered freely and then degraded by the tubules with small amounts appearing in the urine. In contrast, the dimer (molecular weight 44,000 daltons, radius 2.8 nm) is restricted with only approximately 10% filtered. Horseradish peroxidase, a neutral tracer molecule of similar weight and size to light chains, is handled in a similar manner.[256,257]

Albumin is the principal plasma protein and has a molecular weight of 69,000 and radius of 3.6 nm. Under normal situations, any significant amount of albumin is prevented from entering the urine space by the glomerular permselectivity barrier. However, under certain conditions such as reduced glomerular plasma flow, albumin passes into the urine, demonstrating that size selectivity alone is not sufficient to restrict the filtration of albumin. Rather, it appears that the negative charge on the various structures of the glomerular barrier contributes significantly to restricting the filtration of albumin. Of these structures, the negative charge on the basement membrane has been considered the major obstacle to albumin crossing the glomerular capillary wall.[257–263]

Changes in filtered albumin sufficient to account for heavy proteinuria have been documented by micropuncture studies in experimental nephrotic syndrome and by indirect studies in humans. In rats with aminonucleoside nephrosis, the increase in filtered albumin accounts entirely for the increase in protein excretion.[264,265] In fact, in animals with either aminonucleoside nephritis or nephrotoxic nephritis, the proximal tubular albumin concentration is increased 8- to 12-fold.[265,266] These animals may excrete 100 to 400 times as much albumin as controls.[265,266] In humans, clearance studies have provided indirect evidence of increased filtration of protein; in patients with nephrotic syndrome, the minimal protein concentration in the glomerular filtrate (calculated by correcting the urine protein concentration by the fraction of water reabsorbed) far exceeds the concentration of filtered protein observed in the proximal tubular fluid in normal animals.[267] Furthermore, urine albumin excretion is linearly related to plasma albumin concentration when the latter is increased by infusion.[267,268] Such a relationship is characteristic of substances excreted mainly by glomerular filtration.[15]

Although increased filtration of protein appears to be the major factor leading to proteinuria, the specific defects in the capillary wall that are responsible for the protein loss are not completely defined. New information regarding the

major role of the podocyte and its slit diaphragm in many proteinuric diseases has become available from multiple studies.[269–272]

Filtered albumin is also absorbed and catabolized by the proximal tubule with little or no reabsorption of intact albumin. As in the case of low molecular weight proteins, there is excess capacity above normal, but this pathway is saturable.[273] This explanation of albumin handling by the kidney has been challenged lately, with the suggestion that albumin is, in fact, highly filterable and not impeded by charge, but handled largely by tubular reabsorption.[274] Rebuttals against this theory have been effective, including new insights regarding the importance of the podocyte slit diaphragm in genetic causes of proteinuria and the molecular mechanisms of albumin transport in the proximal tubule. Presently glomerular size and charge selectivity are still considered the main barriers to albuminuria.[275,276] For a detailed discussion regarding the pathophysiology of glomerular proteinuria, the reader is directed to Chapter 45.

Large Plasma Proteins. Large molecular weight plasma proteins are restrained from crossing the glomerular barrier. Proteins such as globulins (molecular weight 160,000 daltons, radius, 5.5 nm) are undoubtedly restricted by the basement membrane, but the contribution of the endothelial fenestrae is uncertain. A tiny fractional clearance of large plasma weight proteins has been established and animal studies suggest that this is due to the presence of rare, very large pores in the glomerular ultrafiltration barrier.[277] Changes in glomerular plasma flow do not alter the restriction of these molecules from the urine space.

Proteins in Urine Not of Plasma Origin

The major protein in normal human urine that has no counterpart in plasma is THP, a glycoprotein with a molecular weight of 7 million.[250,278,279] It is excreted in amounts of 20 to 100 mg per day.[280,281] Immunofluorescent staining techniques in human kidneys have demonstrated that THP is confined to the cells lining the thick ascending limb of Henle's loop and the most proximal part of the distal convoluted tubule, which strongly suggests that these cells are the source of the THP in the urine.[282,283] THP is the major protein component of urinary casts.[282,284] Excretion of this protein increases only slightly in patients with nephrotic syndrome, and its excretion rate does not appear to be related quantitatively either to the number of casts or to the degree of proteinuria.[284] In vitro studies indicate that the addition of albumin to THP-containing solutions leads to precipitation of THP,[284] which suggests that increased albumin excretion may lead to precipitation of THP in the tubules causing cast formation. The structure and function of this unusual glycoprotein has been extensively reviewed.[250] Mutations in the gene coding for THP are associated with rare hereditary tubulointerstitial kidney diseases, and in large populations, genetic variation is associated with CKD.[285,286]

In addition to THP, many other discrete proteins unrelated to plasma proteins have been identified in trace amounts in urine.[278] These proteins presumably originate in the lower urinary tract and prostate gland. Endothelin, the potent endogenous vasoconstrictor peptide, is produced by renal epithelial cell lines in vitro, appears in human urine, and may serve as a nonspecific marker of kidney damage.[287] In fact, intense efforts are underway to identify urinary proteins that may signal acute or chronic kidney damage.[288]

Measurement of Urine Protein

Urine protein excretion is routinely measured to detect, evaluate, and manage kidney disease. Historically, total urine protein was considered the preferred measure of proteinuria because of the simplicity of the assays. However, due to lack of a gold standard for total urine protein, and due to the evidence that albumin excretion rises substantially before total urine protein becomes abnormal, there has been a shift of emphasis to measurement of albuminuria. Nonetheless, total urine protein is still widely measured using a variety of methods (Table 9.9).[289]

The simplest and most widely used methods are semiquantitative tests done on random urine samples. Although these tests are extremely useful in screening for proteinuria, they detect an abnormal concentration of total urine protein, not an abnormal excretion rate. Therefore, they might be positive in patients with low urine volume even if the excretion rate is normal, and they may be negative in patients with high urine volume even if the excretion rate is elevated. For more definitive evaluation and management of patients with proteinuria, quantitative protein analysis must be undertaken in timed urine collections. A number of different methods are available for assay of specific proteins.[290] Semiautomated, two-dimensional, electrophoretic systems, which employ ultrathin gels, combined with silver staining, allow the detection of a host of specific urinary proteins on a routine basis.[291,292] These techniques also improve the characterization of urinary proteins with molecular weights less than 70,000.[293] Specific immunoassays are available for detection of individual proteins within the urine, as described subsequently for albumin. Additionally, broad descriptions of patterns of urinary protein excretion have become possible by proteomic techniques.[294]

Semiquantitative Tests for Total Urine Protein

Semiquantitative tests for urinary protein involve either precipitation of protein or protein-induced color changes of an indicator dye on a dipstick. The precipitation tests may be performed by adding either 5% sulfosalicylic acid or concentrated nitric acid to an aliquot of urine or by heating the urine and adding glacial acetic acid.[289] With these methods, the quantity of precipitate is graded from 0 (no precipitate) to 4+ (heavy gelatinous precipitate). Urine samples with a protein concentration as low as 5 to 10 mg per dl will give a positive reaction with the acetic acid precipitation test,

9.9 Definitions of Proteinuria and Albuminuria

	Urine Collection Method	Name (units)	Interpretation of Results: Normal to High Normal	High	Very High
Albumin	24-hour collection	AER (mg/day)	<30	30–300	>300
	Spot urine albumin-to-creatinine ratio	ACR (mg/g)	<30	30–300	>300
Total Protein	24-hour collection	PER (mg/day)	<150	150–499	>500
	Spot urine	PCR (mg/mmol, mg/g)	<150	150–499	>500
	Spot urine	Protein dipstick	negative to trace	trace to 1+	>1+

To convert from mg/g creatinine to mg/mmol of creatinine multiply by 0.113.
ACR; albumin/creatinine ratio; AER, albumin excretion rate; PCR, protein/creatinine ratio; PER, protein excretion rate.

but radiopaque contrast materials, tolbutamide, or large amounts of penicillin, nafcillin, or oxacillin may produce a false-positive reaction.[289,295,296]

The dipstick test for protein (also see Urinalysis section) utilizes a paper strip impregnated with a pH indicator dye (tetrabromophenol blue) buffered to maintain the pH in the paper at 3.0. The test is based on the capacity of proteins to change the color of tetrabromophenol, and is more sensitive to albumin than other proteins.[289] The degree of color change is roughly proportional to the amount of protein present, with the color varying from yellow, with low protein concentrations, to blue, with high protein concentrations. A color comparison chart is provided with the dipstick that contains a scale of protein concentrations as well as a 0 to 3 or 4+ rating. It should be noted that the correlation between color change and actual protein concentration is only approximate. In one study, for example, comparison with quantitative methods indicated agreement only 60% to 70% of the time.[297] The use of the dipstick test is further restricted by the finding of substantial interobserver variation between technicians in interpretation of the results,[298,299] which can be improved by semiautomated and automated reading devices.[300] Additionally, different brands of dipsticks may have different performance characteristics.

The dipstick method has the advantage that it is not affected by urine turbidity, radiopaque material, or drugs.[289,296] It can give a false-positive value in highly buffered alkaline urine, but such samples are encountered rarely. The major fault of the dipstick test is its insensitivity. Although dipstick tests can detect protein concentrations as low as 6 to 15 mg per dl,[298] it is only protein concentrations of 30 mg per dl and above that are detected with certainty. Below this level, the test is negative or trace positive in over half the samples tested.[297,301] In a patient excreting 300 mg of protein per day in a total volume of 1,500 mL, the protein concentration is only 20 mg per dl, and this concentration may not be detected using the dipstick method. Also, the test is insensitive to light chains and can give a negative reaction even when the excretion of this protein is moderately high.[302–304] In selected populations, dipstick screening for proteinuria carries a high risk for false-positive and -negative results with a sensitivity of less than 67% and specificity of 74%.[305]

Quantitative Tests for Total Urine Protein

As discussed previously, the major limitation to tests for total urine protein is the absence of an absolute gold standard due to the presence of many different types of protein in the urine in health and disease. Thus, it is not possible to entirely standardize measurements across laboratories or to determine precise cut-off values for the definition of normal or various diseases. Despite this limitation, a large number of tests are available.

Excretion in Timed Collection. Quantitative methods for measuring protein excretion have been traditionally based on precipitation of protein, usually accomplished using trichloroacetic acid or sulfosalicylic acid. Presently, these methods have been largely replaced by precipitation with other agents, such as benzethonium chloride or benzalkonium chloride, or by colorimetric methods employing automated dye binding assays, using pyrogallol red or pyrocatechol violet dyes.[306,307]

In the precipitation methods, the denaturing substance is added to an aliquot of urine, and the turbidity, measured with a photometer or nephelometer, is compared to standards prepared by the addition of known amounts of

protein to urine. The dye binding assays use a photometer to measure absorbance at a given wavelength of color.[308] These methods remain only roughly quantitative, however, because they have a CV as large as 20%.[289,309] Light chains (Bence Jones protein), however, are effectively measured by these methods, although the precision of measurement is poor compared to pheresis and ELISA. Relative insensitivity to globulins has been reduced by the use of TCA or other precipitating agents, but still is an issue.[309] Iodinated contrast material can falsely elevate the turbidity regardless of agent, and it is best to wait 24 hours after contrast to determine protein excretion rates.[310] With all of these tests, the protein concentration is multiplied by the total volume of the sample and result reported in milligrams or grams per unit of time (usually 24 hours).

Excretion in an Untimed Collection ("Spot Urine Sample"). Twenty-four-hour protein excretion can be easily approximated by measurement of both protein and creatinine in a random urine specimen. Because the excretion of both creatinine and protein is fairly stable throughout the day, if the daily creatinine excretion is known, the ratio of the concentrations of protein and creatinine in a random urine specimen provides an estimate of the daily protein excretion (Fig. 9.11).[311–313] In most circumstances, however, urine creatinine excretion is not known, but is assumed to be 1.0 g per day. However, as discussed before, in the steady state, creatinine excretion is a reflection of creatinine generation, which is affected by age, sex, race, and body size and changes in GFR. Thus, the protein-to-creatinine ratio may differ substantially from protein excretion rate, especially in the non–steady state. Nonetheless, within populations, correlations of protein-creatinine ratio with protein excretion rate are moderate to high and associations of protein-creatinine ratio with disease outcomes are strong. Consequently, this test is now widely used as a first quantitative test, with confirmation using a timed urine collection if necessary.

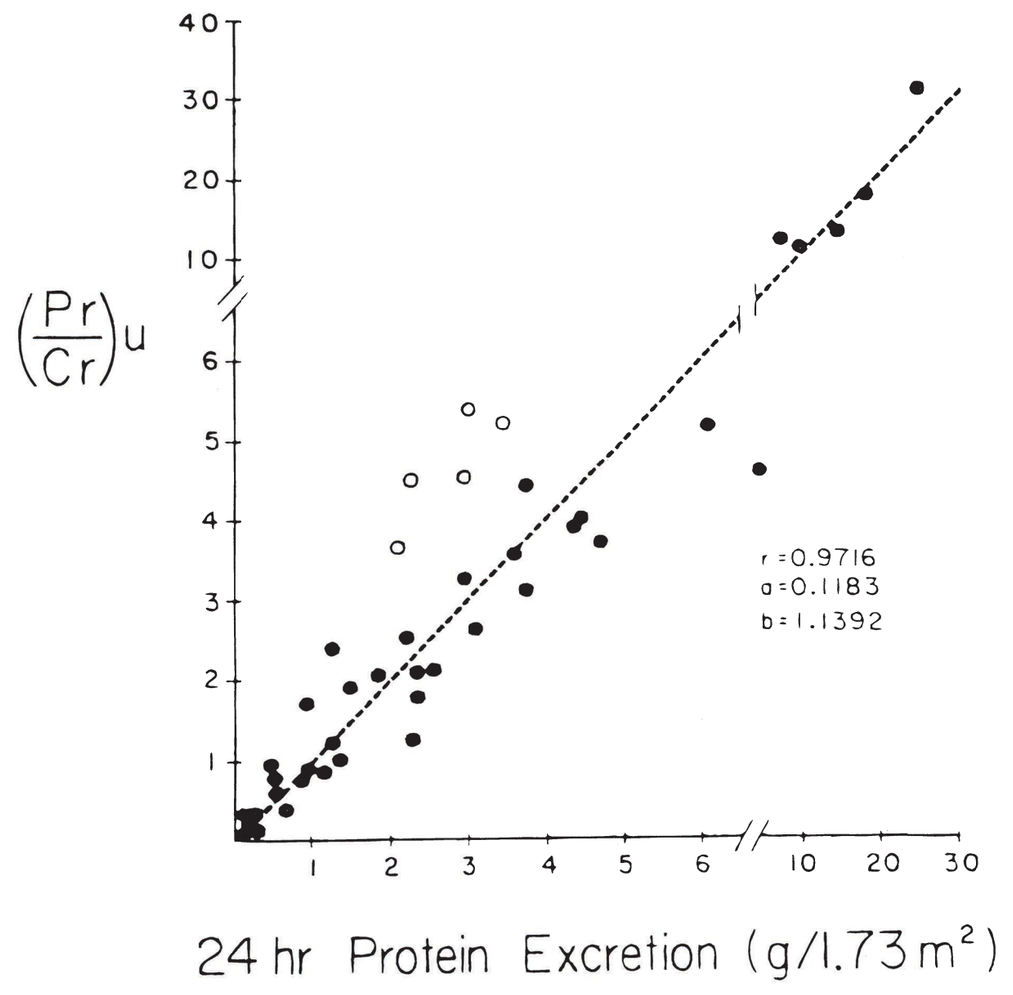


FIGURE 9.11 Ratio of urinary protein to creatinine concentration (Pr/Cr) of random single voided urine samples expressed as a function of protein excretion per 24 hours per 1.73 m^2. (From Ginsberg JM, Chang BS, Matarese RA, et al. Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med.* 1983;309:1543, with permission.)

Recently, dipsticks have become more widely available as an alternative method to measure the urine protein-to-creatinine ratio with initial results suggesting excellent reasonable correlation with standard measures for screening.[314,315]

It has also been demonstrated that the protein-osmolality ratio in a random urine sample may reliably predict protein excretion rates. This ratio was successful in screening for abnormal proteinuria in normal and proteinuric populations with a sensitivity of 96% and a specificity of 93%, superior to routine dipstick performance and equal to the protein-creatinine ratio.[316] Adequate validation studies have not been performed to date, and this test cannot yet be recommended in place of the protein-creatinine ratio.

Clinical practice guidelines by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) recommend measurement of total urine protein in adults using spot urine protein-to-creatinine ratios and expressing the results as total protein in milligrams per creatinine in gram. The normal value varies with the laboratory, but is approximately <200 mg per g (Table 9.9).

Tests for Specific Proteins

Albumin. Specific radioimmunoassay is the standard test for detecting and quantitating albumin concentrations, although turbidimetric assays can be used with similar precision.[317] HPLC techniques allow for even more accurate and early detection of abnormal albumin excretion rates but are not widely available and also have limitations in reproducibility.[318] Many different screening tests are also available as qualitative screens with varying ability to detect albuminuria in the normal and above normal range.[319–322]

Albumin excretion in timed urine samples is considered the gold standard for classification of albuminuria. Given the wide variability in urinary albumin excretion at the low range, several urine samples should be tested to classify albumin as high.[323] The albumin-to-creatinine ratio in a spot urine sample is widely used as a first test for albuminuria, with high correlation with albumin excretion rate,[324,325] but is subject to the same limitations as the protein-to-creatinine ratio. Moreover, spot samples for albumin concentration or for albumin-to-creatinine ratio are associated with a strong graded relationship with adverse outcomes in the general population and populations at increased risk for cardiovascular and kidney diseases, with increased risk detectable at levels greater than 10 mg per g.[3,247,326] Some but not all

recent studies suggest optimal prediction of outcome with albumin-to-creatinine ratio (tested in first morning samples) rather than other measures of proteinuria.[327,328] NKF KDOQI, Kidney Disease: Improving Global Outcomes (KDIGO), and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) National Kidney Disease Education Program (NKDEP) all recommend spot urine albumin-to-creatinine ratio for detecting and following chronic kidney disease in adults (Table 9.9).[1,243,329–331]

Dipsticks are also available to measure the albumin-to-creatinine ratio[332] but validation studies remain mixed in terms of their sensitivity and specificity,[333] although performance of these dipsticks are improved by adjustment for specific gravity.[334] Presently, they may be most useful for screening high-risk populations with quantification better served by laboratory testing.

Immunoglobulin Light Chains. Identification of immunoglobulin fragments in the urine is useful in establishing the diagnosis of multiple myeloma and other monoclonal gammopathies. Light chains are often sought by the traditional Bence Jones test, a method that depends on the unusual solubility characteristics of these proteins. When the urine is heated to 45°C to 55°C, light chains precipitate, particularly when the pH is brought to 4.9 by the addition of an acetate buffer.[335] When the urine is then brought to a boil, the precipitated light chains redissolve partially or completely. This test is difficult to carry out properly and can be rather insensitive. It is positive only when the concentration of light chains exceeds 800 to 1,600 mg per L,[336,337] and even in the presence of such concentrations, it may still be falsely negative.[304,336,338] As noted earlier, the semiquantitative dipstick test also may be negative when light chains are present in the urine due to the insensitivity of the indicator dye to globulins.[302,303] By far the most sensitive tests for detection of light chains and other immunoglobulin fragments are routine electrophoresis and immunoelectrophoresis. In the presence of light chains, routine electrophoresis discloses a monoclonal peak, and immunoelectrophoresis (of concentrated urine) makes it possible to accurately identify the specific protein, even at low concentrations.

Patterns of Proteinuria

Proteinuria can be classified according to its pathophysiology into three major groups: glomerular proteinuria, tubular proteinuria, and overproduction proteinuria.

Glomerular Proteinuria

Glomerular proteinuria is defined as proteinuria due to increased permselectivity of the glomerular filtration barrier to plasma proteins. Therefore, the hallmark of glomerular proteinuria is albuminuria (Fig. 9.12). Albuminuria may be a transient phenomenon in normal individuals without kidney disease. However, persistent albuminuria (albumin-creatinine ratio >30 mg per g for 3 or more months)

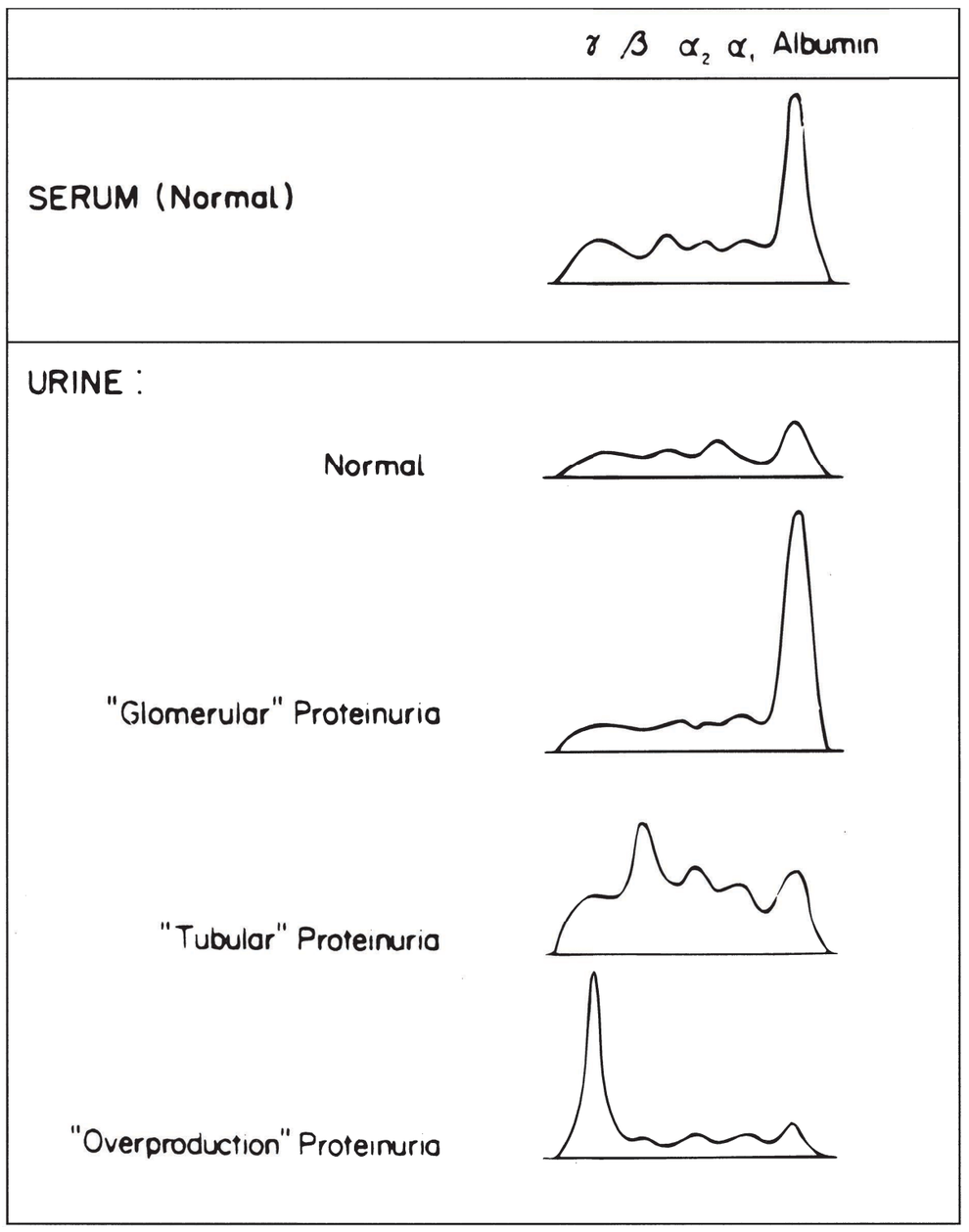


FIGURE 9.12 Electrophoretic patterns of normal serum, normal urine, and urine with three types of abnormal protein excretion. The electrophoresis pattern shown for overproduction proteinuria is taken from a patient with multiple myeloma.

indicates the presence of kidney disease, even when kidney function is normal, when the urine sediment contains no abnormalities, and when the patient has no signs or symptoms of kidney disease.[339–341] Indeed, albuminuria occurs in the great majority of kidney diseases. Whether the primary site of injury is the glomerulus or the tubulointerstitial compartment, albumin makes up 60% to 90% of the urinary protein (Fig. 9.12). The excretion of low molecular weight proteins usually remains minimal.[342,343]

The persistent excretion of small amounts of albumin has been termed microalbuminuria. The term has been defined variably, but usually refers to an albumin excretion rate of 30 to 300 mg per day, which is above the normal values of 5 to 20 mg per day, but less than that detected by tests for total urine protein (i.e., 200–300 mg per day). Albumin excretion greater than 300 mg per day is sometimes referred to as macroalbuminuria or clinical proteinuria, because it can sometimes be detected by the usual dipstick. Despite their widespread usage, these terms should probably be abandoned, because they are technically incorrect or imprecise. Neither microalbuminuria nor macroalbuminuria refer to the size of urinary albumin fragments, and clinical proteinuria does not convey a specific meaning. It has now been proposed to classify albuminuria using the albumin-to-creatinine ratio as normal (<10 mg per g), high normal (10–29 mg per g),

high (30–299 mg per g), very high (300–1,999 mg per g), or nephrotic (>2,200 mg per g).[344]

An albumin excretion rate between 300 and 2200 mg per day, corresponding to a total protein excretion rate between 500 mg and 3,500 mg per day can be seen in many types of CKD, whereas excretion rates greater than this amount are almost invariably the result of glomerular disease. Persistent excretion of ≥2.2 g per day of albumin or ≥3.5 g per day of total protein usually leads to the nephrotic syndrome.[345] Clinical and laboratory evaluation can identify the cause of CKD in many patients with the nephrotic syndrome, but the various histologic subtypes can be definitively identified only by kidney biopsy.

Functional Albuminuria. A transient increase in albumin excretion occurs in a variety of physiologic and experimental settings in the absence of kidney disease. Protein excretion is increased twofold to threefold during and immediately following heavy exercise,[346–348] and the increase is accounted for largely by plasma protein components.[249,349,350] Minor abnormalities in the urine sediment can accompany the proteinuria, but both the proteinuria and the sediment abnormalities usually disappear within hours after the completion of exercise. Similar increases in protein excretion can be induced by fever,[351] severe emotional stress,[352] infusions of norepinephrine or angiotensin,[353,354] and prolonged assumption of the lordotic position.[355] In addition, mild to moderate proteinuria often is observed in patients with congestive heart failure.[356]

Orthostatic Albuminuria. In patients with CKD, proteinuria typically increases in the upright position to levels above those present in the recumbent position. This orthostatic change in excretion appears to have no special diagnostic or prognostic importance. The finding of proteinuria (mainly albumin) only in the upright position is known as orthostatic or postural proteinuria.[357,358] In this condition, total daily excretion usually does not exceed 1 g. Postural proteinuria occurs in the healing phase of many glomerular diseases and also in the absence of kidney disease. In the latter group, minor histologic abnormalities are found on kidney biopsy in approximately one half of the patients.[359] Kidney biopsy in patients with postural proteinuria typically discloses few morphologic abnormalities either on light or electron microscopy,[359–361] although in two reports both immunoglobulins and complement were identified in a substantial fraction of such patients on immunofluorescence microscopy.[362,363] The significance of this finding is uncertain.

To test for postural proteinuria, the patient is instructed to collect a urine sample in the upright position, while carrying out his other usual daily activities. A 16-hour collection can begin in the morning and end just before the patient goes to bed. On retiring, the patient begins an 8-hour recumbent urine collection, including voiding at the time of arising. The amount of protein in both samples is extrapolated to 24 hours. Patients with postural proteinuria have an increased excretion in the specimen collected in the upright position and a normal excretion in the specimen collected when recumbent. If protein excretion is increased in both specimens, the patient has persistent rather than postural proteinuria.

Long-term follow-up studies strongly suggest that postural proteinuria is a benign condition.[358,364] After 10 years of follow-up in one study, it was found that over half of the patients no longer had proteinuria, somewhat less than half continued to have postural proteinuria, and only a small minority developed persistent proteinuria.[365] Decreased GFR was not observed, and hypertension was a rare occurrence. After 20 years of follow-up of many of the same patients, all those examined had normal kidney function, the prevalence of hypertension was no different from that in the general population, and only one third had proteinuria. In half of the proteinuric group, the pattern of protein excretion was still the postural variety.[366] Thus, the prognosis of patients with postural proteinuria appears to be excellent, and patients with this condition should be reassured about the benign nature of the disorder.

Protein Selectivity. Proteinuria can be classified into either a selective or a nonselective pattern, based on a comparison of the clearance of larger molecular weight proteins, such as globulins, with the clearance of albumin.[290,291,367] Investigators have sought to gauge the severity of the glomerular leak by measuring the relative clearance rates of proteins of various sizes.[268,368,369] In patients with proteinuria secondary to a wide variety of CKDs, the clearance rates of large molecules, such as IgG, range from less than 10% to greater than 60% of the clearance rate of albumin or transferrin, a protein similar in size to albumin. Patients with a clearance ratio of IgG/albumin (or transferrin) of less than 0.10 are considered to have only a modest increase in glomerular permeability and are defined as having a "highly selective" pattern of protein excretion. Conversely, patients in whom the clearance ratio is 0.5 or greater are considered to have a relatively porous filter and are defined as having a poorly selective pattern.

Studies of the pattern of protein excretion have shown that the majority of patients with proteinuria have a nonselective pattern.[368,369] Among patients with the idiopathic nephrotic syndrome, however, two populations emerge. One group has selective proteinuria and, in most cases, has minimal change in the disease. The second group has nonselective proteinuria and usually has one of the more severe histologic varieties, such as membranous nephropathy or membranoproliferative glomerulonephritis. Because of this correlation and the frequency of the minimal change lesion among patients with the idiopathic nephrotic syndrome, selectivity studies could have some value in predicting the presence of the minimal change lesion. However, the technical difficulties involved in carrying out the protein analyses, the failure of the test to distinguish among the many subgroups of nephrotic syndrome, and the low risk of kidney biopsy relative to its diagnostic yield have made measurements of selectivity superfluous in the study of the nephrotic patient.

Tubular Proteinuria

A pattern of abnormal protein excretion in which low molecular weight proteins predominate is found in patients with a diverse group of kidney diseases characterized by primary tubular injury. This includes hereditary tubular disorders, such as Fanconi syndrome and Wilson disease,[370] chronic potassium depletion, acute renal failure due to acute tubular necrosis, Balkan nephropathy,[371] and cadmium poisoning.[372,373] The low molecular weight proteins excreted by these patients are the plasma constituents described earlier that are present in only minute amounts in the urine of normal individuals.[374] As many as 20 of these proteins have been identified. A typical pattern seen in these patients is shown in Figure 9.12. The magnitude of tubular proteinuria exceeds 150 mg per day and rarely is greater than 2 g per day.[375–377]

As described previously, the urinary clearance rates of these low molecular weight proteins in normal individuals and in patients with glomerular disease are very low despite the fact that these proteins are filtered readily, suggesting that when the tubules are intact, extensive tubular reabsorption and degradation of these substances occurs.[254,370] By contrast, in patients with primary tubular diseases, the clearance rates of these proteins are markedly increased. In fact, in these patients, the clearance rate correlates closely with the predicted filtration rates of these proteins (estimated from molecular size) if the assumption is made that no tubular uptake occurs.[370] On the basis of these observations, it appears that tubular proteinuria is due to impaired tubular reabsorption of low molecular weight proteins rather than to increased glomerular permeability.[343,370]

Among the low molecular weight proteins excreted in excess in tubular and interstitial diseases are N-acetyl-beta-D-glucosaminidase (NAG), beta-2 microglobulin (B2M), neutral endopeptidase, and lysozyme (muramidase), an enzyme with a molecular weight of 14,600. The finding of increased amounts of these proteins has received attention as a diagnostic aid in identifying tubular and interstitial disease as well as serving as an early marker of acute kidney injury (AKI).[378–381] The cause for increased excretion of these low molecular weight proteins is thought to be ineffective reabsorption and catabolism by the injured, dysfunctional tubules.[382] Lysozyme excretion is increased in patients with tubular damage secondary to infection, transplant rejection, nephrotoxic agents, and Fanconi syndrome. Unfortunately, the diagnostic utility of this determination is limited because many patients with interstitial and tubular disease do not have lysozymuria and because increased excretion of lysozyme occurs in some patients with glomerular diseases.[379] The largest increase in lysozyme excretion occurs in patients with leukemia, presumably secondary to increased production of this protein (see Overproduction Proteinuria).

The interpretation of increased excretion of light chains poses a problem similar to that encountered in patients with lysozymuria—namely, to distinguish between increased excretion secondary to tubular disease on the one hand or to overproduction of the protein on the other. In the case of light chains, a slight increase in excretion and a finding of a mixture of both kappa and lambda fragments points to a primary tubular defect, whereas high levels of excretion (greater than 500 mg per day) and the presence of only a single type of light chain points to accelerated synthesis.[383,384]

Overproduction Proteinuria

When the plasma concentration of a filterable protein is increased beyond the capacity of the tubules to reabsorb it, it then appears in the urine. Enhanced excretion of light chains, heavy chains, and other fragments of immunoglobulins occurs predominantly in the monoclonal gammopathies, including multiple myeloma, macroglobulinemia, heavy-chain disease, and idiopathic light-chain proteinuria (Fig. 9.12). Overproduction with increased filtration rather than a primary tubular defect appears to account for the increased excretion of these substances. Both light-chain and heavy-chain fragments of immunoglobulins are excreted in minute amounts in the urine of normal individuals.[352,385] Normally, only 3 mg or so of light chains are excreted daily and the ratio of kappa to lambda light chains is approximately 3 to 1.[386–388] Approximately 25% are present as monomers and the remainder as dimers, even though light chains are normally synthesized as monomers.[386,389,390]

Because light chains are small in size and a substantial amount is filtered, it follows that an increase in their delivery into the glomerular filtrate will result in increased excretion unless tubular reabsorption is concomitantly increased. In fact, the clearance of light chains in patients with multiple myeloma is quite high and is inversely related to molecular size—a finding consistent with the view that tubular reabsorption is readily saturated.[391] In these patients, light chains have clearances ranging from one tenth to one half that of creatinine, depending on the size of the specific protein destined for excretion (Table 9.8). Light-chain proteinuria is most often found in patients with multiple myeloma and, in this disease, some patients have a daily excretion greater than 15 g. Although a mild increase in albumin excretion is common in patients with monoclonal gammopathies, the excretion of light chains usually predominates, unless the glomerular lesion of renal amyloidosis (or light-chain deposition disease) supervenes. Increased excretion of lysozyme in acute leukemia,[392] amylase in pancreatitis, myoglobin in muscle injury, and hemoglobin following hemolysis are other examples of overproduction proteinuria. The quantity of urine proteins can serve as an index of clinical disease. In the case of light chains, the quantity of light-chain excretion is a reflection of tumor burden and is used clinically as a biomarker of remission and relapse after treatment.

Overproduction proteinuria can have important clinical consequences. Patients with light-chain proteinuria can develop acute or chronic kidney failure, and others manifest the Fanconi syndrome,[393,394] distal renal tubular acidosis,[384] nephrogenic diabetes insipidus, or various combinations of these disorders.[383,384] The association between light-chain

proteinuria and tubular nephropathies has led to the speculation that light chains are toxic to renal tubular cells.[384,393,394] Because some patients with increased excretion of light chains have no abnormalities of tubular function, the specific factors producing tubular dysfunction remain to be defined.[383] Similarly, the lysozymuria associated with leukemia has been implicated as a cause of renal potassium wasting seen in some patients.[395] However, in view of studies demonstrating potassium wasting in some leukemic patients in the absence of lysozymuria, this thesis must be considered unproven.[396,397] It should be noted that lysozymuria also occurs in experimental glomerulonephritis and that its excretion is in direct proportion to the magnitude of the albuminuria.[398]

The Interpretation of Proteinuria

Proteinuria is central to the detection, evaluation, and management of CKD (Table 9.10). The pattern of proteinuria can be assessed by first determining whether the urine protein contains albumin. A positive dipstick test is strongly suggestive of albuminuria, which, when substantial, most likely indicates glomerular proteinuria. Quantification of albumin excretion provides a clue to diagnosis, prognosis, and response to therapy. A negative dipstick test in the presence of elevated total urine protein excretion suggests nonalbumin protein due to tubular proteinuria or overload proteinuria. Electrophoresis or other tests should be performed to detect light chains or other low molecular weight proteins when suspected.

NKF KDOQI guidelines on CKD define persistent proteinuria for 3 months as a marker of kidney damage, which is sufficient for the detection of CKD, even in the absence of other markers of kidney damage or decreased GFR. Persistent albumin excretion (>30 mg per day, roughly equivalent to an albumin-creatinine ratio >30 mg per g) is widely acknowledged to be the earliest sign of diabetic nephropathy. Persistent albuminuria in this range is generally required for the diagnosis of diabetic nephropathy, and precedes the decline in GFR in most patients.[399] The literature indicates that albuminuria in this range is one of the earliest markers of kidney damage in hypertension, but does not occur in all patients prior to the reduction in GFR.[400,401] However, there is a substantial heterogeneity in the presentation of these diseases, likely due to the substantial coexistence of type 2 diabetes and hypertension. In these diseases and others, it seems likely that albuminuria is associated with systemic endothelial dysfunction in addition to altered glomerular permselectivity, which may relate to its increased risk for cardiovascular disease. Using this definition of albuminuria as a marker of kidney damage has enabled studies of the prevalence of earlier stages of CKD, regardless of specific cause, in large populations.[402]

A large number of clinical practice guidelines now suggest routine testing for urine albumin-to-creatinine ratio. The National Kidney Foundation recommends testing for albuminuria in all individuals at increased risk for CKD, including those with hypertension, diabetes, a family history of kidney disease, or advancing age.[1] The American Diabetes Association also endorses routine testing for albuminuria in all diabetic subjects as part of their evidence-based guidelines.[403] Furthermore, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) recommended routine urinalysis in the evaluation of all patients with hypertension with the option of measuring urine albumin excretion as well.[404] Increasingly, guidelines focus on the urine albumin-to-creatinine ratio as the screening test of choice, and the optimal way to quantitate proteinuria in CKD.

9.10 Importance of Proteinuria in Chronic Kidney Disease

Interpretation	Explanation
Marker of kidney damage	Spot urine albumin-to-creatinine ratio >30 mg/g or spot urine total protein-to-creatinine ratio >150 mg/g for ≥3 months defines CKD
Clue to the type (diagnosis) of CKD	Spot urine total protein-to-creatinine ratio >500–1000 mg/g suggests diabetic kidney disease, glomerular diseases, or transplant glomerulopathy
Risk factor for adverse outcomes	Higher proteinuria predicts faster progression of kidney disease and increased risk of CVD
Effect modifier for interventions	Strict blood pressure control and ACE inhibitors are more effective in slowing kidney disease progression in patients with higher baseline proteinuria
Hypothesized surrogate outcomes and target for interventions	If validated, then lowering proteinuria would be a goal of therapy

ACE, angiotensin-converting enzyme; CKD, chronic kidney disease; CVD, cardiovascular disease.

Initial and repeated measurement of protein excretion is a valuable guide in following the course of patients with kidney disease. Several studies have established that the magnitude of proteinuria is directly correlated with the risk of progressive decline in kidney function, regardless of the cause or type of kidney disease.[405–407] This is also true, independent of the level of GFR.[408]

Secondary analysis of recent clinical trials demonstrates that the beneficial effects of lowering blood pressure and inhibition of the renin-angiotensin system are greater in patients with higher levels of proteinuria at the beginning of therapy.[409–413] Consequently, the magnitude of proteinuria is central to the new guidelines for the management of hypertension in chronic kidney disease.[400] For patients with persistent albumin-to-creatinine ratio >30 mg per g, the guidelines recommend a lower target blood pressure (<130/80 mm Hg) and initial antihypertensive therapy using an ACE inhibitor or ARB. Furthermore, evidence has mounted to demonstrate that the degree of reduction in proteinuria serves as an important guide to the prognosis of patients treated with ACE inhibitor or ARB therapy. Studies demonstrate that the better the antiproteinuric response to therapy, the better the outcome.[409,414,415] This may lead to the selection of agents and dosing based on the antiproteinuric response. This strategy would need to be tested in a prospective fashion, in various kidney diseases, before being broadly adopted, as adverse effects of higher dose or combination agents may outweigh their benefits.[416]

URINALYSIS

Urine examination has been used in medicine for more than 6,000 years.[417] Ancient Hindu, Babylonian, and Egyptian physicians are known to have used urine for diagnosing illnesses, and the connection between sweet urine and diabetes was made as early as 600 BC.[417] Early western physicians like Hippocrates (460–355 BC) and Galen (129–200 AD) have written about the associations between different urinary characteristics and disease states.[417] During medieval times (500–1500 AD), urinalysis established its place as an unrivalled diagnostic tool and was also widely used by unscrupulous profiteers to prognosticate future events.[418]

The modern urinalysis techniques took a foothold in the 19th century with the use of microscopy; description of casts; discovery of chemical analysis methods for urine, glucose, bile acid, protein, and blood; and the development of urinary test strips.[419,420] Dipsticks became commercially available in 1956 and, since the early 21st century, the manual reading of dipsticks is increasingly being replaced by sophisticated semiautomatic or automatic strip readers with both chemical and sediment analysis capabilities.[300] In spite of advances in microscopy, chemical analysis, and automation, direct examination of urine by physicians provides invaluable diagnostic information and remains indispensable for evaluating patients who are suspected of having acute or CKD.

Urinalysis entails macroscopic, dipstick, and microscopic examinations of the urine. The standards for urinalysis have not yet been uniformly applied and there is a wide inter-operator variability in the interpretation of both macroscopic and microscopic findings.[298,299,421–423] A number of organizations have recently developed guidelines to make laboratory urinalysis more uniform, but most nephrologists perform this procedure in their office outside of laboratory purview.[424,425]

Urine Collection

Urine specimens can be obtained by voiding into a container, urethral catheterization, or suprapubic needle aspiration of the bladder. Voiding into a container is the most commonly used method. Urethral catheterization is usually reserved for patients who are unable to void due to urinary obstruction, incontinence, or impaired consciousness. Suprapubic needle aspiration of the bladder is mostly done in infants and is only used in older children or adults if urine cannot be obtained by any other means. Regardless of the method used, every effort should be made to avoid specimen contamination with the contents of skin, urethra, or vagina. Urethral catheterization and suprapubic aspiration should be done using a sterile technique to prevent introduction of infection into the bladder.

A clean catch sample is essential for accurate assessment. Patients should be instructed to wash their hands before sample collection. Men should retract the foreskin and wash or use sterile wipes to clean the external genitalia. For uncircumcised men, the foreskin should be held back during the entire collection time. Women should separate the labia and clean the area around the urethral meatus with sterile wipes going from front to back. The same procedures are used for children. For infants and very young children, external genitalia are cleaned and dried using wipes and towels, and a sterile urine collection bag is placed over the area using adhesives to adhere to the skin. A mid-stream clean catch sample is preferred as this is least likely to be contaminated. The specimen should be examined as soon as possible after the collection as the chemical composition of urine changes and formed elements degenerate over time. Highly concentrated and acidic urine favor cellular preservation. Refrigeration is acceptable but leukocytes break down rapidly and cell counts performed after 2 to 4 hours may be questionable even with refrigeration.[426] Microbiologic investigations should be done within 2 hours and if more than 2 hours of delay is expected then the sample should be refrigerated at 4°C.[424]

The first morning urine is traditionally considered to be the standard specimen for urinalysis as it best correlates with a 24-hour urine sample.[1] The first morning specimen is least likely to be affected by prior food or fluid intake, movement, and also allows for preservation of formed elements as it is usually most concentrated and acidic.[424] A random urine sample is an acceptable alternative to the first morning urine in an acute setting, and when the collection, storage, and timely transportation of the first morning specimen is difficult.

Macroscopic Examination

Normal urine color is determined by the concentration of the pigment urochrome. Urochrome is the product of hemoglobin metabolism in the liver. Urine is pale yellow when it is dilute and dark yellow or amber colored when it is concentrated. Occasionally, the precipitation of phosphate crystals in alkaline urine and urate crystals in acidic urine may give rise to cloudy urine in the absence of any disease. The appearance of urine changes in certain disease states, and with the ingestion of certain foods or drugs.[300] Cloudy urine may be seen in urinary tract infection or in the presence of significant pyuria. Foamy urine suggests moderate to heavy proteinuria. Reddish urine indicates hematuria, hemoglobinuria, myoglobinuria, or the intake of rifampin, phenytoin, phenazopyridine, or beet-root. Yellow-brown urine may be seen in hyperbilirubinemia, or following the ingestion of chloroquine, nitrofurantoin, senna, or rhubarb. White milky urine suggests chyluria, and dark or black urine may be seen in alkaptonuria, porphyria, or malignant melanoma.

Urine odor usually does not have much clinical significance. A pungent odor may indicate bacterial ammonia production. Sweet or fruity odor suggests ketonuria. Certain rare hereditary metabolic diseases may give rise to strong unusual urine odor: maple-syrup urine disease, maple syrup odor; phenylketonuria, musty or mousy odor; isovaleric academia, sweaty feet odor; hypermethioninemia, rancid butter or fishy smell; Oasthouse urine disease, brewery odor; tyrosinemia, cabbagelike or fishy odor; trimethylaminuria, stale fish odor; and hawkinsinuria, swimming pool odor.[427]

Dipstick Examination

Use of a single- or multiple-test reagent strip, commonly called a dipstick, allows for a rapid and convenient chemical screening of urine specimens. The dipstick method uses a paper or plastic strip embedded with pads that contain reagents for different chemical reactions. Reactions in these pads result in color change when a particular analyte is present in the urine. The degree of color change in each pad is then compared against the range of colors on brand-specific color charts to get a semiquantitative result for the analyte in question.

Although the dipstick method is simple to perform, certain precautions need to be taken to obtain accurate and reliable results.[300] Reagent strips should not be exposed to the extremes of temperature and must be stored in a dry place away from direct sunlight. Only the container provided by the manufacturer should be used for storage as these containers are light-sealed and have desiccants to prevent moisture. While performing the test, the reagent strip should be dipped in urine in one continuous motion and the excess urine needs to be removed by touching the edge of the strip to the urine container as mixing or dilution of reagents gives rise to false results. The color change in reagent pads takes time and reading should be done only after the manufacturer specified wait time. Strips usually have a control pad and the color change in that pad must always be compared with the standard before interpreting reactions in other pads.

Dipsticks commonly include tests for specific gravity, pH, blood, protein, glucose, ketones, bilirubin, urobilinogen, nitrites, and leukocytes. The composition of reagents and detection limits vary with different brands and manufacturers. Table 9.11 lists chemical reactions, detection limits, and conditions associated with false-positive and false-negative results for a typical dipstick. Dipstick results provide important diagnostic clues and abnormal dipstick findings usually lead to further evaluation with confirmatory tests (Table 9.12).

Dipstick reactions perform variably when compared to confirmatory tests. The dipstick specific gravity reaction detects ion concentration rather than particle mass, thus the dipstick will show a lower value for urine specific gravity than hygrometry or refractometry when nonionized molecules like glucose and radiocontrast dye are present.[428,429] The dipstick specific gravity, however, correlates fairly well with urine osmolality in most situations. Specific gravity is the relative mass of the urine compared to water, and thus reflects the total number of particles in solution and their size and density, whereas osmolality reflects only the number of particles. Therefore, a solution of glucose (molecular weight = 180 daltons) equal in osmolality to a solution of urea (molecular weight = 60 daltons) has a higher specific gravity (Fig. 9.13). For total protein, dipsticks have low sensitivity and variable specificity and positively charged proteins like immunoglobulin light chains may escape detection even when concentrations are high.[300] Dipsticks perform poorly as a screening tool for diabetes as fasting urine glucose testing has sensitivity of only 17% even though specificity can be as high as 98%.[430] Similarly, dipsticks cannot be used solely to estimate the level of ketosis as beta-hydroxybutyrate, the most abundant serum ketone during ketosis, is not detected by dipstick. Dipsticks perform well for white blood cells (WBCs) and are usually positive when more than five WBCs are present per high power field (HPF).[431,432] Dipsticks are also reliable for detecting red blood cells (RBCs) with the sensitivity and specificity of 80% to 95% and 95% to 99% for more than three RBCs per HPF, respectively.[433–435] For the detection of urinary infection, negative dipstick results for leukocytes and nitrites are likely sufficient to exclude microscopic and culture abnormalities.[432,436–438] It has been shown that 95% of urines with negative dipstick results for protein, glucose, ketones, blood, leukocytes, and nitrites have normal microscopic examination and, in most cases, microscopy can be reserved for only those with abnormal dipstick results.[432,439] Discolored urines and urine samples of patients with urinary tract symptoms or kidney disease, however, are best examined microscopically as the dipstick examination alone may not pick up all potentially relevant abnormalities.[436]

Microscopic Examination

Microscopic examination of the urine is primarily performed to identify cells, casts, crystals, and microorganisms. The examination can be a qualitative or semiquantitative procedure.

9.11 Urine Dipstick Testing

Test	Reaction in Dipstick Pad	Detection Limit[a]	Associated with False-positive Results	Conditions Associated with False-negative Results
Specific gravity	Urinary cations compete with H+ bound to polyionic polymer causing a release of free H+ that alters pH of a pH-sensitive dye	1.000–1.030	Heavy proteinuria Acidic urine	Alkaline urine
pH	H+ reacts with methyl red and bromthymol blue	pH 5.0–9.0	Prolonged storage (falsely alkaline) Formaldehyde (falsely acidic)	—
Protein	Proteins (primarily albumin) alter pH of a pH-sensitive dye (commonly tetrabromophenol blue)	≥18–32 mg/dl (albumin)	Concentrated urine Alkaline urine Phenazopyridine Polyvinylpyrrolidone (blood substitute) Chlorhexidine	Dilute urine Acidic urine
Blood	Hemoglobin or myoglobin oxidizes ortho-toluidine and organic peroxidase	≥5–20 RBC/μl	Oxidizing detergents Dilute urine Alkaline urine Hemoglobinuria Myoglobinuria Bacteria with pseudo-peroxidase activity (Enterobacteriaceae, staphylococci, streptococci)	Ascorbic acid Formalin preservative Acidic urine
Glucose	Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. Hydrogen peroxide then reacts with chromogen.	≥30–40 mg/dl	Oxidizing detergents	Ascorbic acid Keto-acids Aspirin Bacteria Concentrated urine
Ketone	Acetoacetate and acetone reacts with nitroprusside reagent	≥5–15 mg/dl (acetoacetic acid) ≥70 mg/dl (acetone)	Ascorbic acid Phenazopyridine Levodopa Mesna Free sulfhydryl group (N-acetylcysteine, captopril)	Improper storage
Bilirubin	Conjugated bilirubin reacts with aniline dye	≥0.5–1.0 mg/dl	Fecal contamination Chlorpromazine Phenazopyridine	Ascorbic acid

(continued)

9.11 Urine Dipstick Testing (continued)

Test	Reaction in Dipstick Pad	Detection Limit[a]	Associated with False-positive Results	Conditions Associated with False-negative Results
Urobilinogen	Urobilinogen reacts with dimethylaminobenz-aldehyde (Ehrlich's reaction)	≥0.4–2.0 mg/dl	Sulfonamides Phenazopyridine Procaine Alkaline urine	Prolonged storage
Nitrite	Bacteria with nitrate reductase activity reduces urinary nitrates to nitrites. Nitrites react with p-arsanilic acid, forming a diazonium compound.	≥0.05–0.10 mg/dl (typically >10 organisms/mL)	Phenazopyridine	Prolonged storage Short bladder incubation (<4 hours) Ascorbic acid Low vegetable diet Bacteria without nitrate reductase activity (Enterococcus, Neisseria, Mycobacterium)
Leukocyte esterase	Pyrrole amino acid esters are cleaved forming free pyrrole that reacts with diazonium compound.	>25–35 WBC/μl	Vaginal contamination Beets Formaldehyde Imipenem Meropenem Clavulanic acid	Concentrated urine Ascorbic acid Glycosuria Heavy proteinuria Cephalexin Gentamicin Tetracycline Nitrofurantoin

[a]Based on Chemstrip® 10 MD COBAS® of Roche Diagnostics, Indianapolis, IN 46256, USA.
RBC, red blood cell; WBC, white blood cell.

Data from references: Lam MH. False 'hematuria' due to bacteriuria. Arch Pathol Lab Med. 1995;119(8):717–721; Brigden ML, Edgell D, McPherson M, et al. High incidence of significant urinary ascorbic acid concentrations in a west coast population—implications for routine urinalysis. Clin Chem. 1992;38(3):426–431; Mundt LA, Shanahan K, eds. Graff's Textbook of Routine Urinalysis and Body Fluids, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2011; Wallace J, ed. Interpretation of Diagnostic Tests, 8th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2007.

Preparation of the urine sediment and examination methods are of the utmost importance. About 10 mL of fresh or properly stored urine is centrifuged in a conical tube at approximately 2,000 revolutions per minute for at least 5 minutes. The supernatant is carefully decanted and the pellet is resuspended in a small amount of urine that remains in the tube by gentle agitation. A pipette is then used to transfer a drop of this resuspended pellet onto the microscope slide. A coverslip is gently placed on top of the urine before transferring the slide to the microscope. The sediment is usually examined unstained. Papanicolaou stain may be used to enhance details, and Wright's or Hansel's stain is used in special circumstances to identify eosinophils.

Microscopic examination of the urine is most commonly performed using a bright field microscope. Polarized light is used to identify some crystals and fat droplets, and phase-contrast microscopy is occasionally used when detailed examination of cell membranes is required.[439,440] The use of interference contrast microscopy, scanning electron microscopy, and transmission electron microscopy have also been reported but there use is mostly limited to research settings.[441–443] The examination is first done under low magnification (×100) to identify formed elements. Higher magnification (×400) is important to differentiate the types of casts, cells, crystals, or other abnormalities. Laboratories provide quantification by counting and averaging the

9.12 Clinical Significance and Confirmatory Tests for Urine Dipstick Results

Test	Clinical Significance	Confirmatory Test
Specific gravity	1.000–1.005: Excess water intake, diabetes insipidus 1.010 (Isosthenuric urine): Acute tubular necrosis, severe CKD >1.030: Volume depletion, glycosuria, extrinsic osmotic agent	Refractometry or hygrometry Urine osmolality
pH	<5.0: Metabolic acidosis of nonrenal cause, volume depletion, hyperaldosteronism, high protein diet >6.0–6.5: Type I renal tubular acidosis, low protein diet, infection with urea-splitting organisms (e.g., >Proteus)	pH-electrode under oil emulsification
Protein	Persistently positive in CKD with elevated albuminuria	Further quantification and qualification using a timed or spot urine sample (see Measurement of Urine Protein)
Blood	Spot staining: Hematuria Diffuse staining: Marked hematuria, hemoglobinuria, myoglobinuria	Urine microscopy
Glucose	Positive in proximal tubular dysfunction, or when serum glucose concentration is more than renal glucose threshold (180 mg/dl)	Serum glucose (diabetes); Urine amino-acid, phosphorus and uric acid (proximal tubular dysfunction)
Ketone	Positive in diabetic ketoacidosis, alcoholic ketoacidosis, starvation, and severe volume depletion	Serum keto acids
Bilirubin	Positive in conjugated hyperbilirubinemia Positive bilirubin and negative urobilinogen may indicate intestinal obstruction with conjugated hyperbilirubinemia	Serum bilirubin and liver enzymes, abdominal imaging if obstruction is suspected
Urobilinogen	Positive in conjugated hyperbilirubinemia	Serum bilirubin and liver enzymes
Nitrite	Positive in urinary infection with nitrate reducing bacteria	Urine culture
Leukocyte esterase	Positive in interstitial nephritis and urinary infection	Urine microscopy and urine culture

CKD, chronic kidney disease.

number of elements seen in at least 10 fields in different areas of the sample.[424] The number of casts is usually reported as a number of each type seen per low power field (LPF). The number of cells, crystals, or bacteria is usually reported as a number of each type seen per high power field (HPF). In the office setting, however, physicians use a variety of nonspecific terms like occasional, few, rare, frequent, many, and numerous to quantify formed elements.

Cells

Red Blood Cells. RBCs in the urine originate either from the kidney parenchyma or the urinary tract. Occasionally they may be seen in the absence of kidney or urologic diseases, especially when specimens are obtained during fever, menstruation, or following exercise.[444–446] The upper limit of normal for the number of RBCs in the urine is unclear. Addis was the first to report that healthy people may excrete

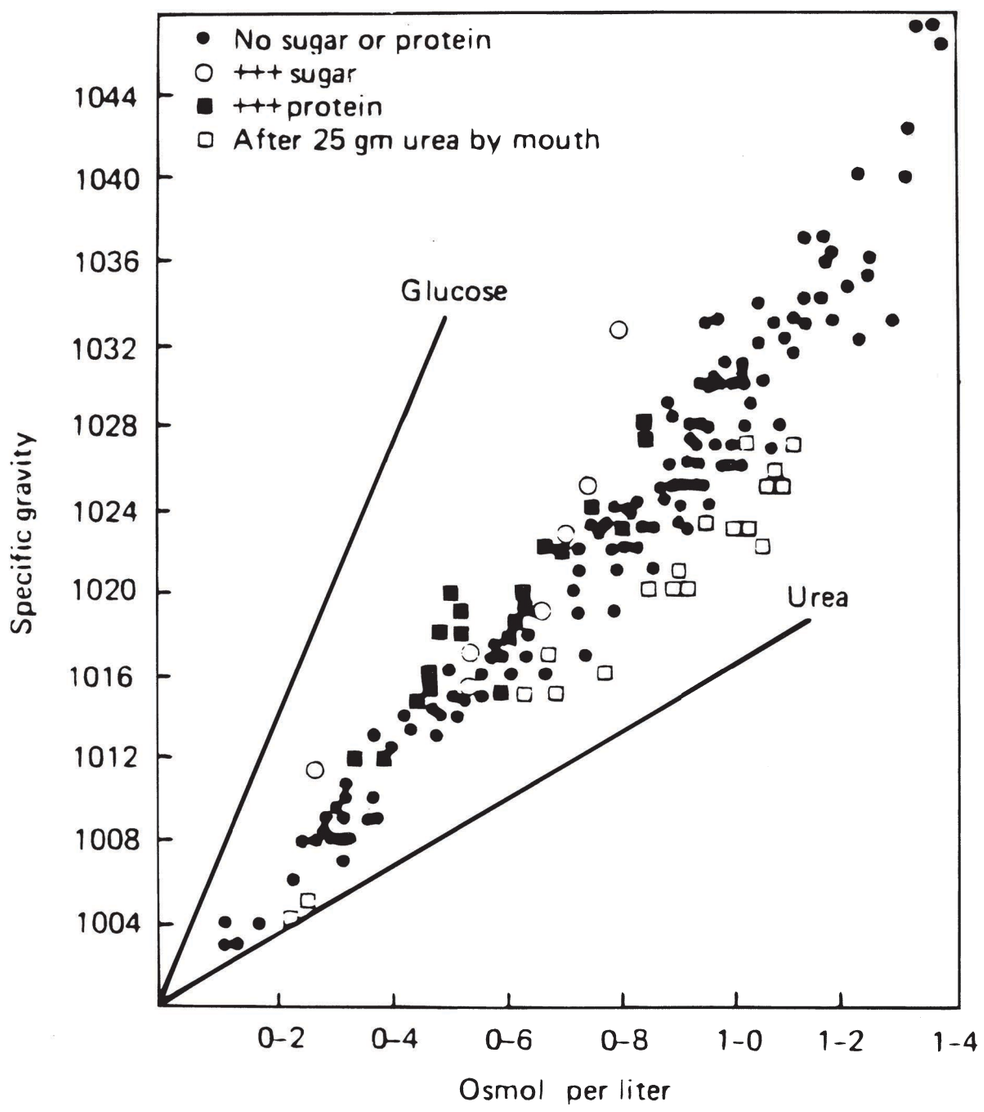


FIGURE 9.13 Relationship between specific gravity and osmolality of the urine. Different urine samples are shown as follows: *small filled circles*, with no sugar or protein; *large open circles*, 3+ sugar; *small filled squares*, 3+ protein; *large open squares*, after 25 g of urea by mouth. The lines show the relation between specific gravity and osmolality for glucose and urea solution. (From Miles BE, Paton A, de Wardener HE. Maximum urine concentration. *Br Med J.* 1954;2:901, with permission.)

up to 425,000 RBCs in urine in a 12-hour period.[444] Subsequent investigators have reported excretion rates in healthy individuals that range from 5,000 to 8,000 RBCs per mL of urine.[447,448] One study revealed that RBCs in healthy individuals typically exhibit a dysmorphic pattern (speculated, crenated, or with cell membrane blebs or folding), suggesting that RBCs enter the urine through the glomeruli.[449] It is believed that RBCs lose their typical biconcave structures as they pass through glomerular basement membrane and get exposed to osmolality changes in renal tubules.[450,451] The presence of more than two to five RBCs per HPF in the urine on two or more occasions is considered by most to warrant further evaluation.[447,452]

The differential diagnosis of hematuria is broad and it is useful to differentiate between hematuria of glomerular origin and nonglomerular origin. The presence of RBC casts or heavy proteinuria points toward glomerular causes for hematuria. Similarly, the presence of a high number of dysmorphic RBCs (particularly acanthocytes) suggests glomerular hematuria (Figs. 9.14 and 9.15).[453,454] Dysmorphic RBCs are best visualized using a phase-contrast microscope.[455,456] The number of dysmorphic RBCs depends on the type of glomerular disease and is considerably higher in proliferative than in nonproliferative glomerular processes.[457]

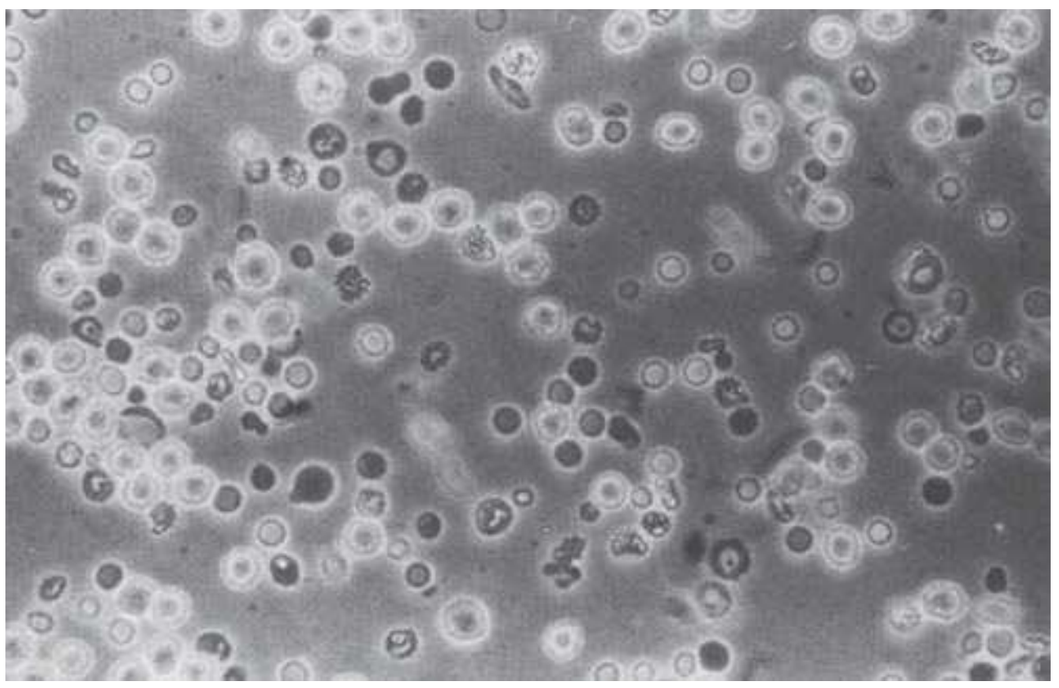


FIGURE 9.14 Urine from a patient with immunoglobulin A glomerulonephritis. Monomorphic and dysmorphic red blood cells are seen. The coexistence of a nonglomerular source of bleeding needs to be considered in such a case, but this "mixed" pattern may be seen in glomerulonephritis in a setting of marked hematuria.

Manual detection of dysmorphic RBCs has a high interobserver variability,[458] and a meta-analysis of 21 studies reported that the average sensitivity and specificity of this technique in detecting glomerular disease in referral centers is between 86% and 90% and 93% and 97%, respectively.[459] Automated red cell volume analysis and urinary flow cytometry have been evaluated as an alternative to the manual examination. The sensitivity and specificity of these techniques in distinguishing between glomerular and nonglomerular hematuria ranges from 98% to 100% and 80% to 91%, respectively, for red cell volume analysis, and 90% to 100% and 87% to 93%, respectively, for urinary flow cytometry.[459,460]

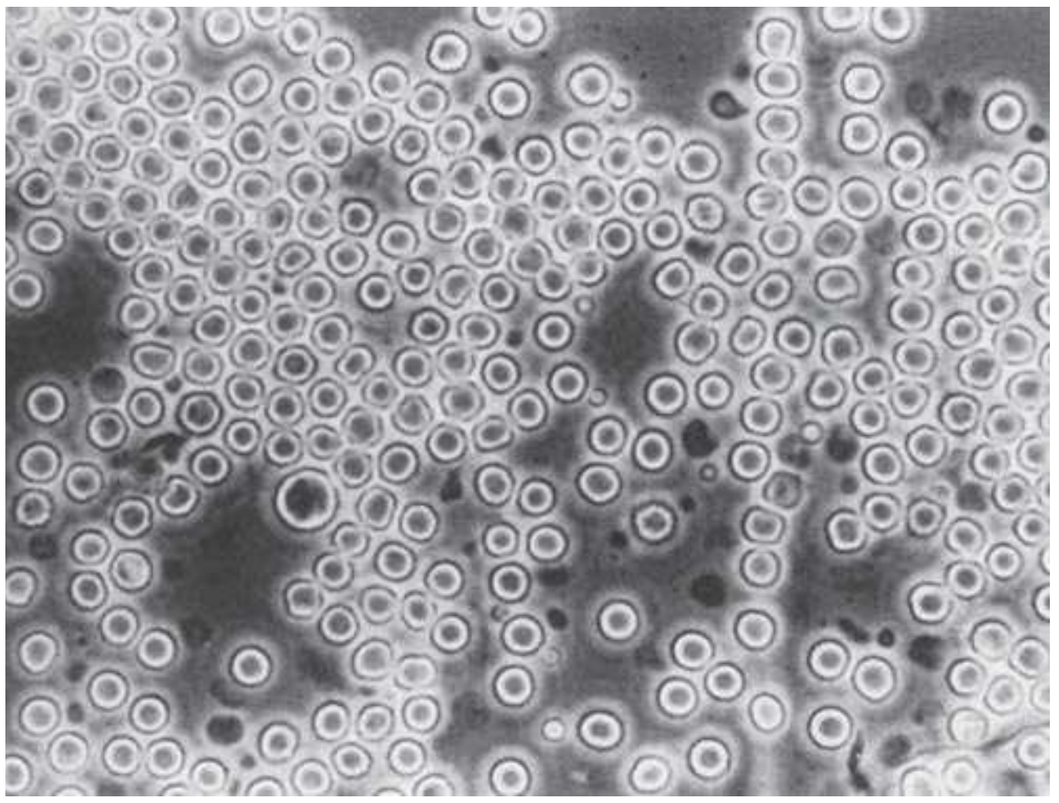


FIGURE 9.15 Monomorphic red blood cells seen in nonglomerular hematuria. Tumors, stones, menstrual contamination, lower urinary tract infection, or contamination of urine sample with blood may give this picture.

White Blood Cells. Small number of WBCs can be seen in urine in the absence of any disease.[461] The presence of five or more WBCs per HPF is usually considered abnormal and requires further evaluation.[462,463] Pyuria, or the presence of WBCs in the urine, implies inflammation in or around the kidneys or urinary tract. The most common cause of pyuria is urinary tract infection.[464] In addition, pyuria may also be seen in interstitial nephritis, glomerulonephritis, acute allograft rejection, and during conditions such as gastroenteritis or acute appendicitis that cause inflammation around the urinary tract. The presence of casts or heavy proteinuria suggests kidney involvement.

Neutrophils are the predominant WBCs found in the urine. They are easy to identify due to the presence of granular cytoplasm and multilobed nuclei (Fig. 9.16). Urine lymphocytes may be seen in acute allograft rejection but their detection requires special staining that is not widely used in clinical practice.[465,466] Urine eosinophils are known to be associated with allergic interstitial nephritis and can be identified using either Hansel's or Wright's stain.[467] Hansel's stain is preferred as it is much more sensitive than the standard Wright's stain.[468] Urine eosinophils are reported either qualitatively or as a percentage of total cells in the sediment. Greater than 1% is considered positive and the predictive value for acute interstitial nephritis increases with higher percentage.[467] For more than 1% urine eosinophils, the sensitivity and specificity for acute interstitial nephritis have been reported as 63% and 93%, respectively.[468] More recent data, however, suggests that sensitivity may be much lower and range between 25% and 40%.[469,470] These inconsistent data have raised doubts about the utility of testing for urine eosinophils.[470] It is advisable that clinicians consider urine eosinophil count as only one of the many diagnostic clues for allergic interstitial nephritis and interpret the results in the context of the patient's clinical presentation, drug exposure history, and other urinalysis and laboratory findings.

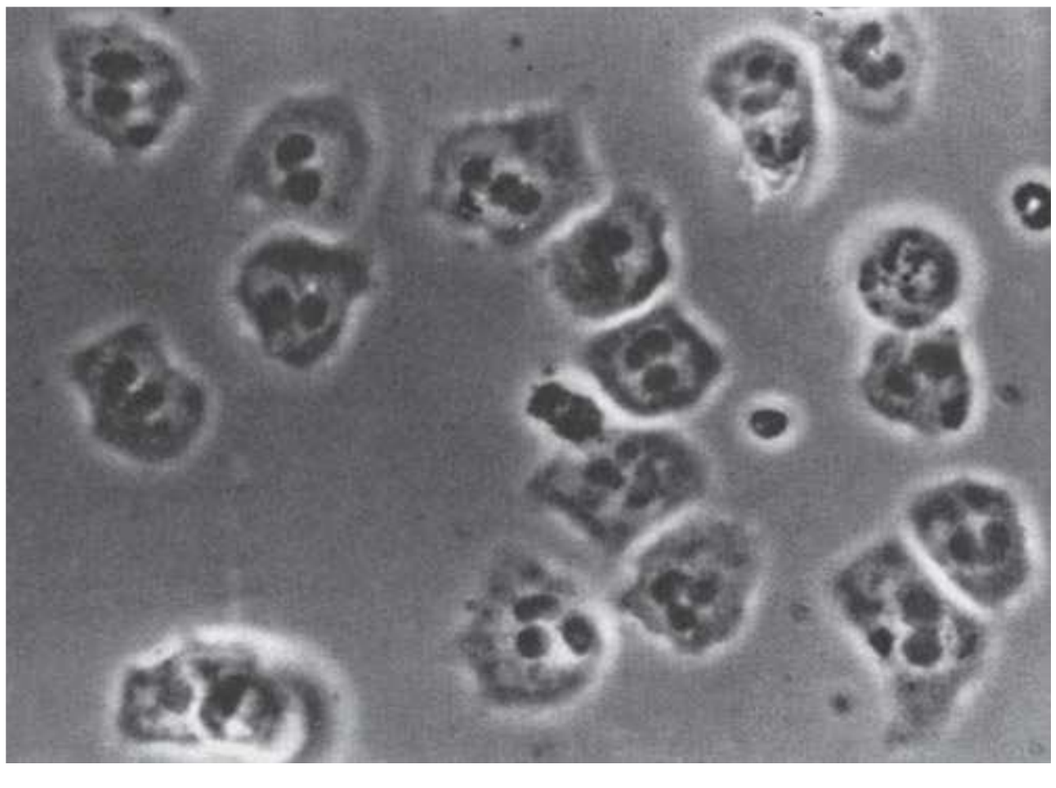


FIGURE 9.16 The multilobed nuclei of the leukocytes, clearly seen on phase-contrast microscopy.

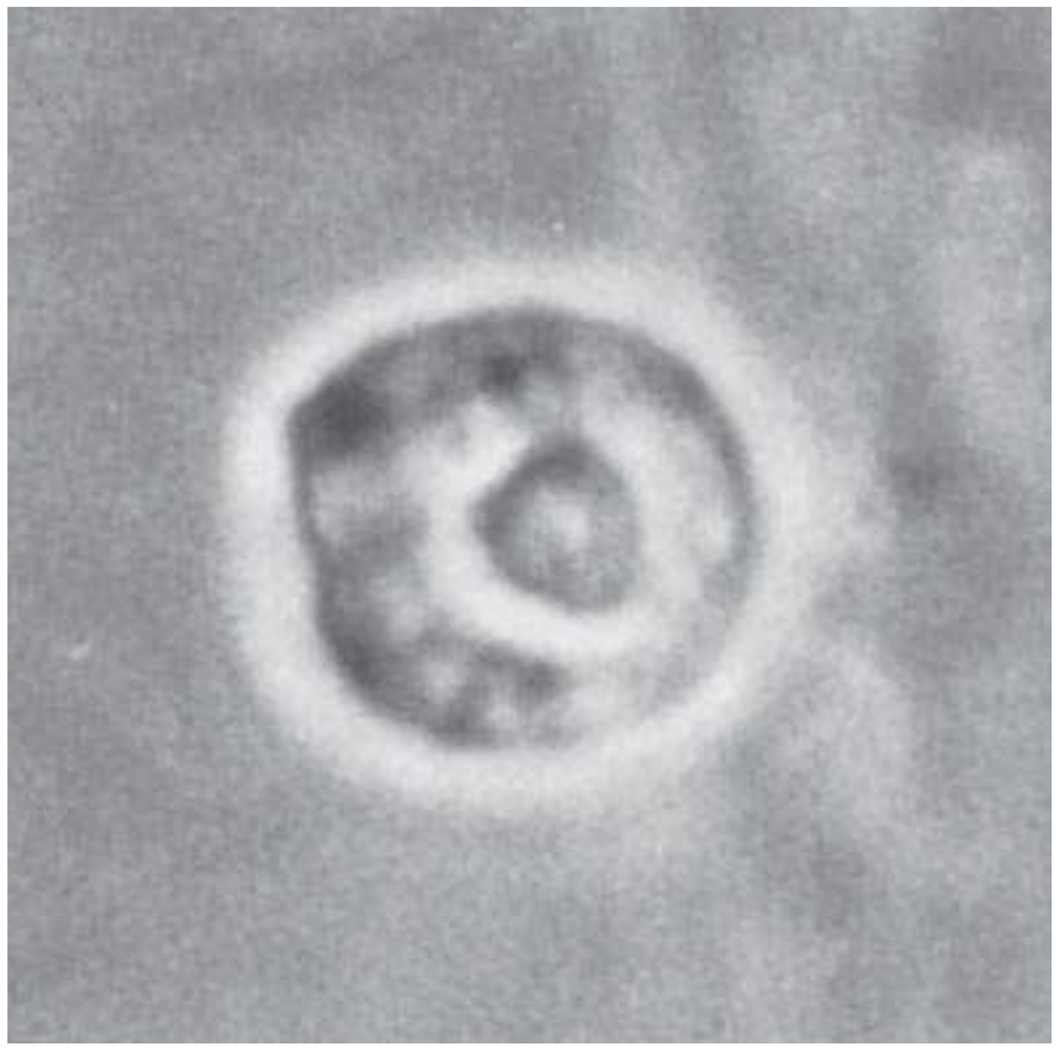


FIGURE 9.17 Renal tubular epithelial cell with a single nucleus, clearly seen on phase-contrast microscopy.

Epithelial Cells. Small numbers of epithelial cells may be present in normal urine. Renal tubular epithelial cells originate at the level of nephrons. They are mononuclear and are larger than neutrophils (Fig. 9.17). Higher numbers of tubular cells indicate tubular damage from conditions like acute tubular necrosis, interstitial nephritis, and allograft rejection. Transitional epithelial cells are derived from renal pelvis, ureters, or bladder. A higher number of transitional epithelial cells is associated with urinary tract infections, tumors, and stones. Squamous epithelial cells are of urethral or vaginal origin. They are large, flat cells with small nuclei. A higher number of squamous epithelial cells indicates urine contamination with the contents of skin, urethra, or vagina.

Casts

Casts are cylindrical structures formed when Tamm-Horsfall glycoprotein (uromodulin) secreted by the epithelial cells of the thick ascending limb of loop of Henle precipitates and takes the shape of renal tubules.[250] Casts are formed in distal tubules and collecting ducts as these are the areas where precipitation is most likely to occur. High tubular fluid concentration, low urinary flow rate, high sodium concentration, heavy proteinuria, and acidic milieu favor cast formation. Trapping of cells, other proteins, and fat within the cast matrix give rise to different types of casts with variable clinical significance (Table 9.13).

Hyaline Casts. Hyaline casts are colorless and are composed of THP alone. They may be seen in the absence of any kidney disease, especially during periods of volume depletion, diuretic use, fever, exercise, or stress.[250,471,472] Hyaline casts are usually seen with other types of cells or casts during disease states.

9.13 Clinical Significance of Cells and Casts in Urine Sediment

Clinical entity	Urinalysis finding									
	Hyaline casts	RBC	RBC Casts	WBC	WBC casts	Tubular cells	RTE casts	Granular casts	Fat[a]	Waxy casts
No kidney or urinary tract disease	+/−	−	−	−	−	−	−	−	−	−
Urinary tract disease not involving kidney	+/−	+/−	−	+	−	−	−	−	−	−
Cystic kidney diseases, urinary tract or kidney neoplasms	+/−	+	−	+/−	−	−	−	−	−	−
Tubulointerstitial nephritis, pyelonephritis	+/−	+/−	−	+	+	+/−	+/−	+/−	−	−
Acute tubular necrosis	+/−	+/−	−	+/−	−	+	+	+	−	−
Hereditary nephritis	+/−	+	+/−	−	−	+/−	−	+/−	−	−
Small vessel disease (microangiopathy)	+/−	+	−	−	−	+	+/−	+	−	−
Proliferative glomerulonephritis	+/−	+	+	+/−	+/−	+/−	+/−	+/−	+/−	+/−
Heavy proteinuria, nonproliferative glomerular diseases	+/−	+/−	−	−	−	+/−	+/−	+/−	+	−
Medium vessel diseases, noninflammatory tubulointerstitial disease	+/−	−	−	−	−	−	−	−	−	−
Severe chronic kidney disease	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+

[a]Free fat, oval fat bodies, fatty casts.
RBC, red blood cell; WBC, white blood cell; RTE, renal tubular epithelial cells.

Granular Casts. Granular casts are formed when proteins or cellular debris are trapped in the Tamm-Horsfall matrix.[473] In fine granular casts, granules are predominantly composed of filtered proteins and appear small and regular. In coarse granular casts, granules are predominantly composed of degenerated cells and appear large and irregular. A few fine granular casts may be seen in the absence of any kidney disease, but the presence of more than a few fine granular casts or of coarse granular casts indicates kidney disease.

Granular casts are usually nonspecific and can be seen in many different glomerular or tubular disorders. A special type of granular cast, called deeply pigmented or muddy-brown cast, is considered to be the characteristic urine sediment finding of acute tubular necrosis.

Red Blood Cell Casts. RBC casts are formed when RBCs leak into the tubules through damaged glomerular or tubular basement membrane gets trapped in the Tamm-Horsfall matrix.[443] RBC casts are considered pathognomonic for glomerular disease, and many RBC casts suggest glomerular inflammation associated with a proliferative glomerulonephritis. RBC casts can occasionally be seen in other parenchymal diseases like pyelonephritis and renal infarction. A careful examination of the sediment is essential when glomerular hematuria is suspected as RBC casts may be very sparse and contain only few cells. The cells within the casts may also show varying degrees of disruption and degeneration (Fig. 9.18) making it difficult to distinguish from more common coarsely granular casts.

White Blood Cell Casts. WBC casts are formed when WBCs leak into the tubules through damaged tubular or glomerular basement membrane and are trapped in the Tamm-Horsfall matrix.[443] The presence of WBC casts suggests tubulointerstitial inflammation, either from infection in the case of pyelonephritis or from toxins or drugs in the case of interstitial nephritis. Rarely, WBC casts may also be seen in acute glomerulonephritis.

Renal Tubular Epithelial Cell Casts. Renal tubular epithelial cell casts are formed when tubular cells slough from the tubular basement membrane and are trapped in the Tamm-Horsfall matrix. These casts are markers of tubular injury and most commonly are seen in acute tubular necrosis or interstitial nephritis.

Pigment Casts. Hemoglobin, myoglobin, bilirubin, and, rarely, melanin may form casts. Hemoglobin casts have a characteristic brownish hue and are coarsely granular in appearance. These casts are formed either from the degradation of RBC casts, or from free hemoglobinuria in patients with intravascular hemolysis. Myoglobin casts appear similar to hemoglobin casts and are associated with rhabdomyolysis. Bilirubin casts are yellow-brown in color and may be seen in patients with hyperbilirubinemia. Melanin casts are extremely rare and may be seen in patients with melanemia and melanotic tumors. They are coarsely granular and have dark brown or black color.

Broad and Waxy Casts. Broad casts are usually colorless and four to five times wider than typical hyaline casts. They are formed in tubules that are dilated as a result of atrophy and fibrosis of surrounding interstitium. Waxy casts (Fig. 9.19) are similar to broad casts in size, highly refractile, smooth, and waxy in appearance. Broad casts and waxy casts are seen in people with advanced kidney disease, and may reflect dilated and hypertrophic tubules in this condition.

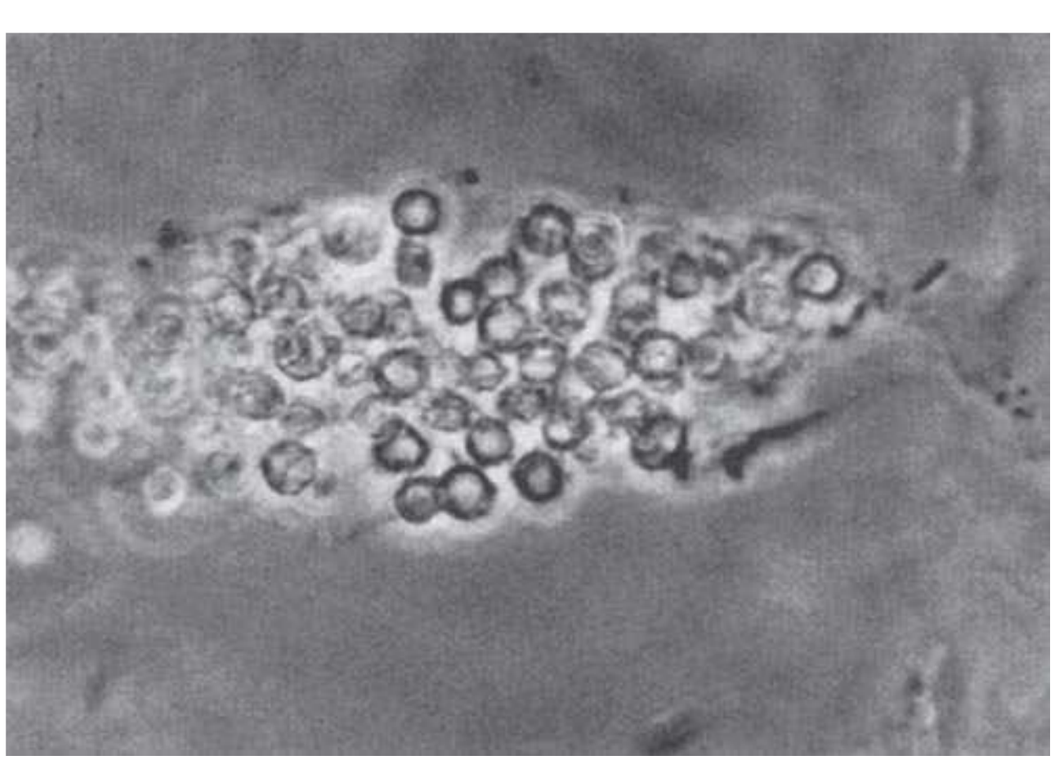


FIGURE 9.18 A red blood cell cast. Much of the hemoglobin from cells has already disappeared.

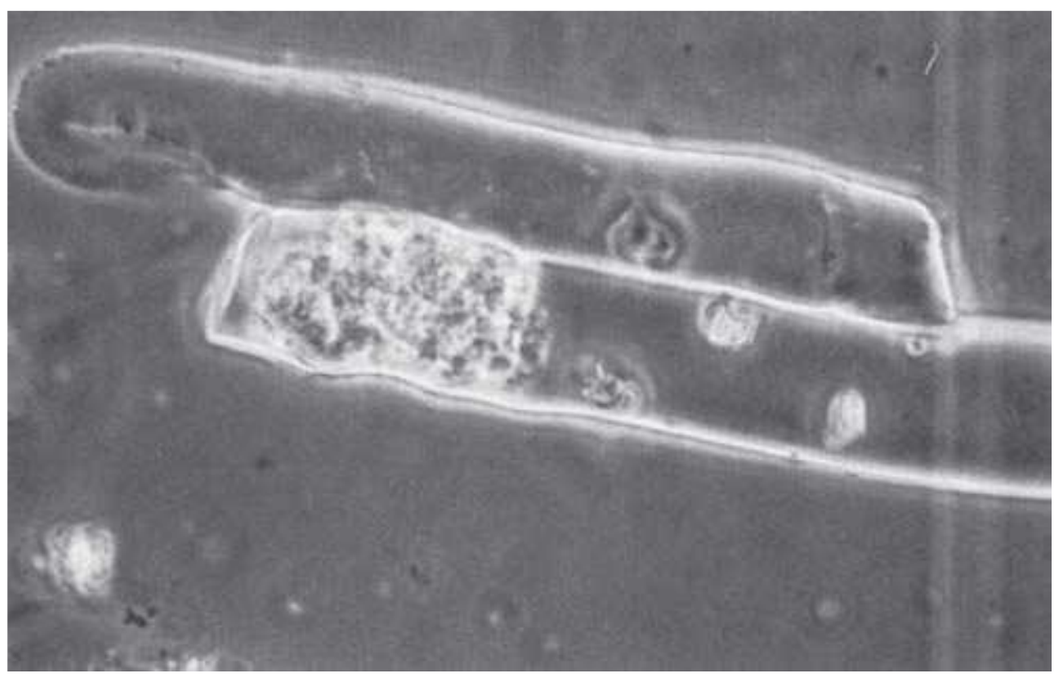


FIGURE 9.19 A waxy cast with sharply defined edges. A clump of disintegrating cells is seen at one end.

Urine Fat and Fatty Casts. Urine fat may be observed in the form of free fat globules, oval fat bodies, or fatty casts. Free fat globules are spherical in shape, yellowish in color, variable in size, and may exist in isolation or as fat clusters. When fat globules are taken up by tubular cells or macrophages, then they are described as oval fat bodies. When fat globules are trapped within the Tomm-Horsfall matrix then they are called fatty casts. Fat globules with cholesterol or cholesterol esters are anisotropic and have a characteristic "Maltese cross" appearance under polarized light (Fig. 9.20). Neutral fats like triglycerides are isotropic, do not polarize, and are identified using special stains like Sudan III or oil red O dye.

Urine fat is typically associated with heavy proteinuria and nephrotic syndrome.[474] It may also be

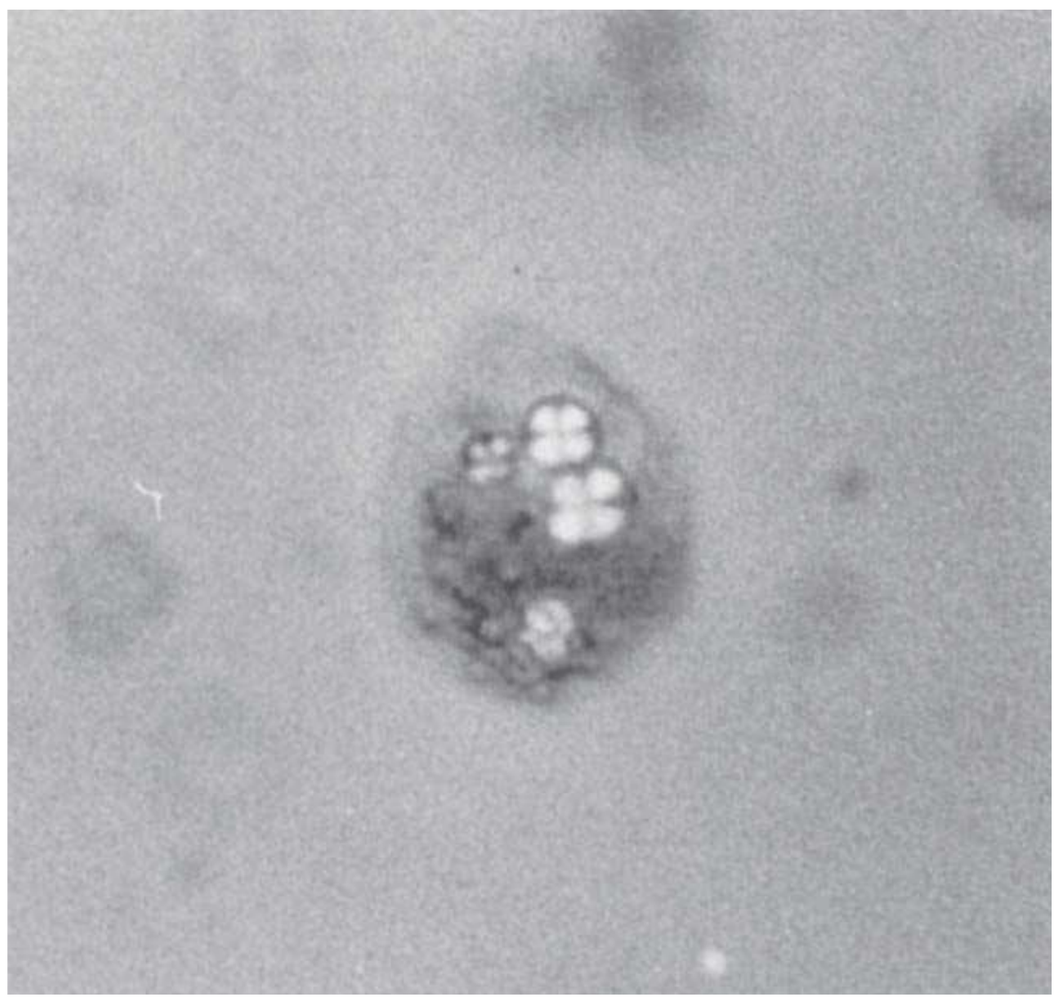


FIGURE 9.20 An oval fat body viewed under polarized light with a classic "Maltese cross" appearance.

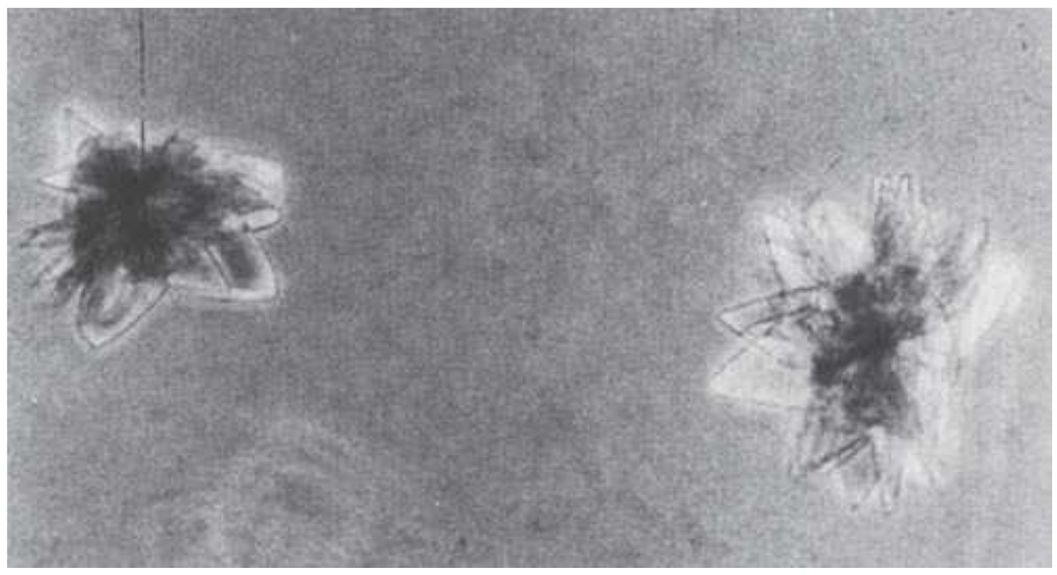


FIGURE 9.21 Uric acid crystals.

seen in polycystic kidney disease and fat embolization syndromes.[475,476]

Crystals

Crystals are common urine sediment findings that are of limited clinical importance in most settings. The identification of crystals, however, can provide important diagnostic clues while evaluating patients with nephrolithiasis, metabolic disorders, or toxin- or drug-induced AKI. Urine pH, crystal morphology, and examination under polarizing light helps to differentiate between different types of urinary crystals (Figs. 9.21 to 9.24). The characteristics of urinary crystals and their associated clinical conditions are described in Table 9.14.

Microorganisms

Bacteria, fungi, protozoa, and parasites may be seen in unstained urine either as a result of contamination or infection. The presence of WBCs suggests infection, and kidney involvement should be suspected if WBC casts or casts embedded with microorganisms are present. Estimation of

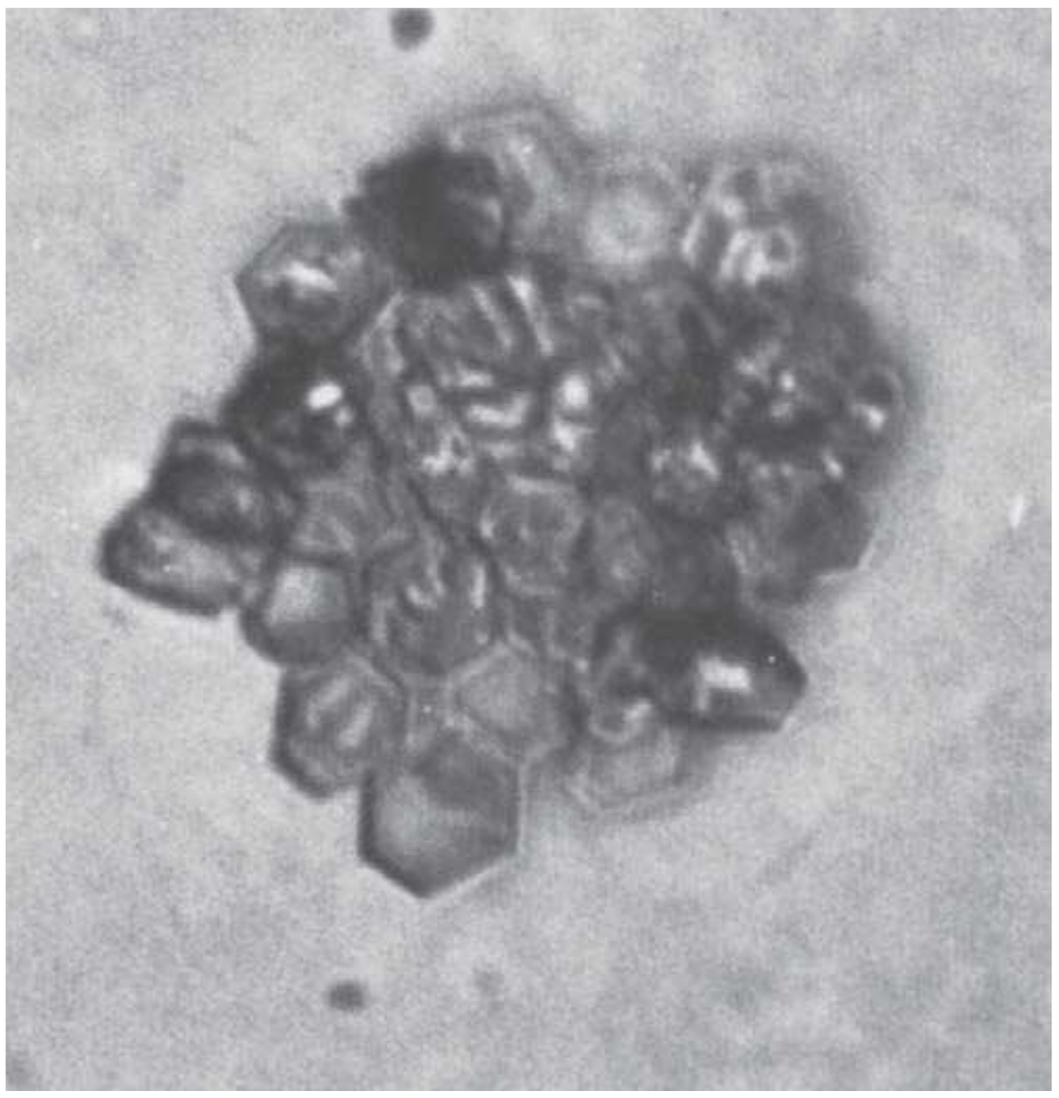


FIGURE 9.23 A conglomeration of cystine crystals from a patient with cystine stones. The typical benzene rings can be seen along the edges.

bacterial count is usually done after Gram staining of the uncentrifuged urine sample. Fungal elements like Candida may be seen both in its yeast and hyphal forms. Trichomonas vaginalis is the most common protozoan found in the urine and has a teardrop shape with motile flagellum. Schistosoma haematobium may be seen in urine in areas of Africa and the Middle East where schistosomiasis is endemic.

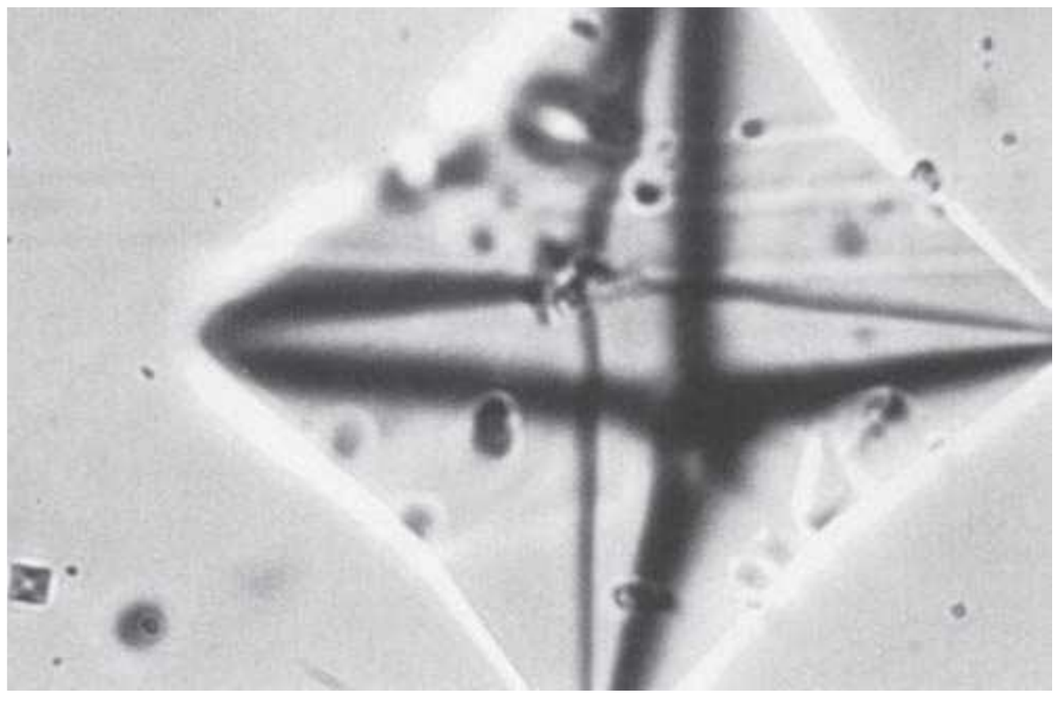


FIGURE 9.22 A very large calcium oxalate crystal. Its large size is obvious when this crystal is compared with the small calcium oxalate crystal (*bottom left corner*).

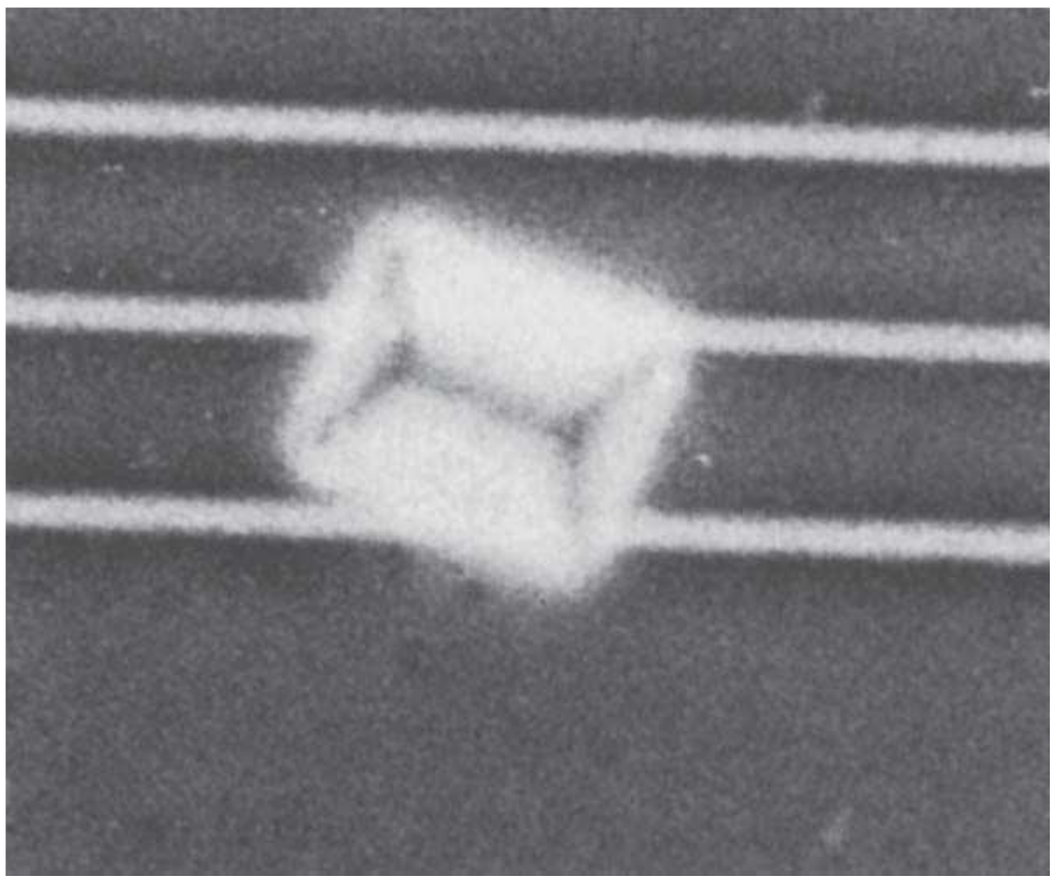


FIGURE 9.24 A triple phosphate crystal with a typical "coffin-lid" appearance, commonly seen in association with infection.

9.14 Urinary Crystals

Type	Description			Associated Clinical Condition
	Shape	Favorable pH	Polarizing Ability (Yes/No)	
Uric acid	Pleomorphic (amorphous, rhomboid prisms, rosettes, needles)	Acidic	Yes	Normal urine, nephrolithiasis (rarely)
Sodium urate	Amorphous	Acidic	Yes	Normal urine, nephrolithiasis (rarely)
Bihydrated calcium oxalate (Weddellite)	Bipyramidal or envelop	Acidic	No	Normal urine, nephrolithiasis (rarely)
Monohydrated calcium oxalate (Whewellite)	Dumb-bell, oval, or biconcave	Acidic	Yes	Nephrolithiasis (rarely), ethylene glycol intoxication
Amorphous phosphate	Amorphous	Alkaline	No	Normal urine, nephrolithiasis (rarely)
Calcium phosphate (hydroxyl-apatite or brushite)	Pleomorphic (prism, starlike, needle)	Alkaline	Yes	Nephrolithiasis (rarely), hyperparathyroidism (rarely), hypercalciuria (rarely)
Magnesium-ammonium-phosphate (triple phosphate or struvite)	Coffin lid or pyramid	Alkaline	Yes	Normal urine, urinary tract infection by urea-splitting bacteria
Calcium carbonate (Calcite)	Dumb-bell, small sphere with radial striations	Alkaline	Yes	Normal urine
Cystine	Hexagonal plate	Acidic	Yes	Cystinosis
Leucine and tyrosine	Yellow sphere (leucine), and brown needles (tyrosine)	Acidic	Yes	Severe liver disease, tyrosinosis, maple syrup disease
Cholesterol	Thin rectangular plate with a square notch corner	Non-pH dependent	Yes (slightly)	Heavy proteinuria
Bilirubin	Needle, red-brown sphere	Non-pH dependent		Conjugated hyperbilirubinemia
Ammonium biurate	Yellow-brown sphere with stria and spicules (thorny apple)	Alkaline	Yes	Normal urine, severe liver disease or portosystemic shunting (rarely)

(continued)

9.14 Urinary Crystals (continued)

	Description			
Type	**Shape**	**Favorable pH**	**Polarizing Ability (Yes/No)**	**Associated Clinical Condition**
Sulfadiazine	Pleomorphic, rosette	Acidic	Yes	Drug use or overdose
Sulfamethoxazole	Sphere, plate, rosette	Acidic	Yes	Drug overdose
Amoxicillin	Broom brush or sheave	Acid	Yes	Drug overdose
Ciprofloxacin	Pleomorphic (needle, sheave, star, fan, butterfly)	Alkaline	Yes	Drug use or overdose
Acyclovir	Needle	Acidic	Yes	Drug use or overdose
Indinavir	Platelike, fan-shaped, starburst	Acidic (may be seen in physiologic pH)	Yes	Drug use, may cause nephrolithiasis
Contrast agent	Platelike		Yes (slightly)	Contrast use

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CHAPTER

10 Ultrasonography and Nuclear Medicine

Frederic Rahbari-Oskoui • Andrew T. Taylor • William Charles O'Neill

Sonography and nuclear medicine are important tools in the evaluation of the kidneys and urinary tract. Due to its simplicity and ready availability, sonography is usually the initial imaging performed and, in many cases, the only examination required. The low cost and portability of modern equipment enable sonography to be an office-based or bedside procedure performed by the practitioner, adding to its convenience and attractiveness. Nuclear medicine can obtain important functional data and complements other imaging modalities that cannot provide this information. This functional assessment can be critical for clinical decisions and often avoids the necessity of invasive testing. This chapter reviews the advantages, disadvantages, and indications for each modality in the kidneys and urinary tract along with the basics of interpretation and common findings.

ULTRASONOGRAPHY

The introduction and extensive use of ultrasonography over the past four decades has dramatically simplified the diagnostic evaluation of the urinary tract. The acoustic properties, limited spectrum of pathology, and ease of visualization of the kidneys—coupled with the safety, simplicity, lack of radiation, and low cost—of sonography make it the initial modality of choice. The improved portability and affordability of equipment and availability of training provide an opportunity for nephrologists to become skilled at this technique, thereby enhancing the diagnosis and care of their patients.

Sonographic images are acquired by analyzing the amplitude and interval of reflected pulses of high-frequency sound. Time is converted to depth and amplitude is converted to brightness (echogenicity) to yield a pixel-based image. Highly reflective structures such as a stone appear bright and cast a dark, distal shadow. In contrast, fluid collections such as cysts do not reflect sound and appear dark but enhance the echogenicity of distal tissue (distal enhancement or through transmission).[1] Tissues such as renal parenchyma have an intermediate echogenicity related to backscatter of sound from the microscopic architecture. Indications for ultrasound imaging of the kidneys and the bladder are diverse and include evaluation of acute renal failure, chronic kidney disease (CKD), cystic diseases, pain, hematuria, severe hypertension, urinary tract infections, and guidance for kidney biopsies.

Imaging and Normal Appearance of the Kidneys

The adult kidney contains several lobules, each consisting of a rim of cortex surrounding a medullary pyramid that terminates in a papilla protruding into a minor calyx. The lobules fuse in utero or shortly after birth in most individuals. The cortex between two pyramids is called a column of Bertin. Minor calyces converge into major calyces that, in turn, converge to form the renal pelvis. The portion of the kidneys that is not parenchyma or urinary space is the sinus, which, in adults, is filled with adipose tissue (Fig. 10.1). Longitudinal views of the kidney are obtained in the supine position with the probe positioned so that the upper pole appears on the left side of the image. In the longitudinal plane, the normal kidney has a characteristic oval shape with a hypoechoic (dark) rim of cortex and medulla surrounding the echogenic (bright) sinus fat[2–4] that obscures the calyces and blood vessels (Fig. 10.2). The medullary pyramids are slightly less echogenic than the cortex[2] and can often be discerned. They are particularly prominent when cortical echogenicity is increased. Transverse images are obtained perpendicular to the longitudinal axis and the kidney appears circular at each pole and C-shaped through the center due to the break in the parenchyma where the ureter and vessels enter, thereby providing the best views of the renal pelvis and hilum.

The appearance of the kidneys is subject to normal variations related primarily to incomplete fusion of the ranunculi. These include complete duplication of the collecting system, which appears as a band of cortex separating the sinus fat into two compartments (Fig. 10.3) and is present in 5% of kidneys, and hypertrophied columns of Bertin, where the cortex extends into the sinus but does not completely bridge

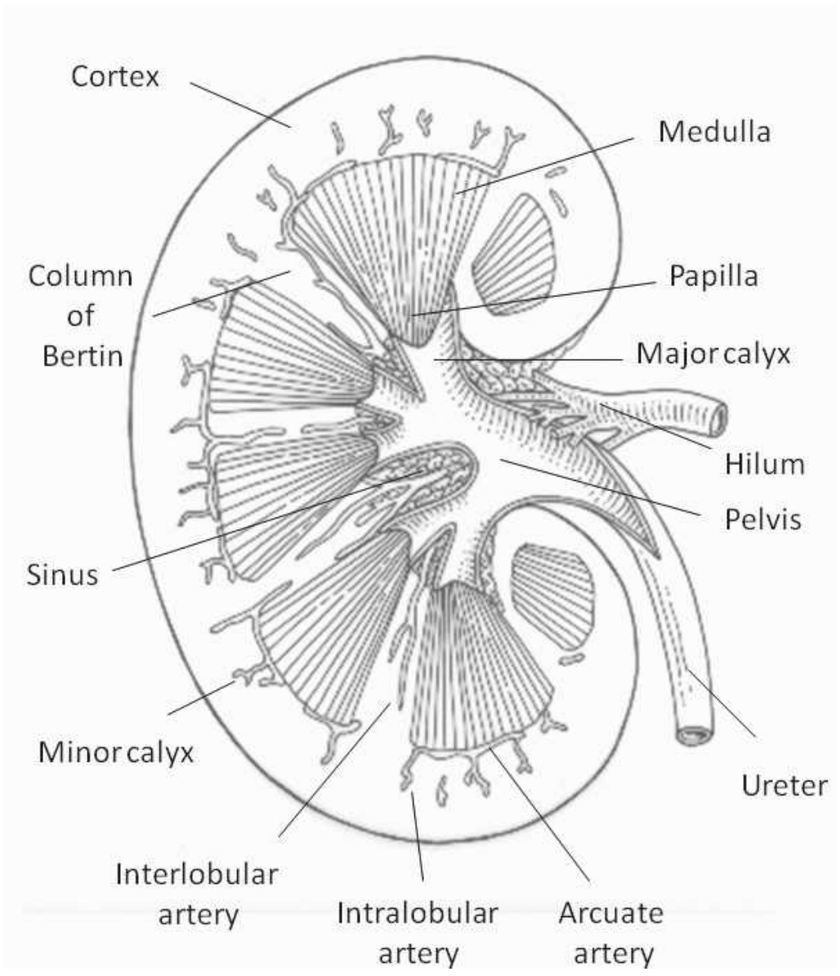


FIGURE 10.1 Intrarenal anatomy, midline coronal section. (Adapted from O'Neill WC. Sonographic evaluation of renal failure. *Am J Kidney Dis*. 2000;35:1021, with permission.)

it (Fig. 10.4) and is present in 15% of kidneys.[5,6] Junctional parenchymal defects are the most subtle manifestation of incomplete fusion, presenting as a wedge-shaped defect in the outer cortex filled with echogenic fat that is continuous with the renal sinus fat by a thin strand.[6] In some individuals, the lobules fail to completely fuse and persist into adulthood. These so-called fetal lobulations appear as regularly spaced convexities, each containing a pyramid.[7] Lobulation may reappear in chronic kidney disease due to atrophy of the columns of Bertin (Fig. 10.5).

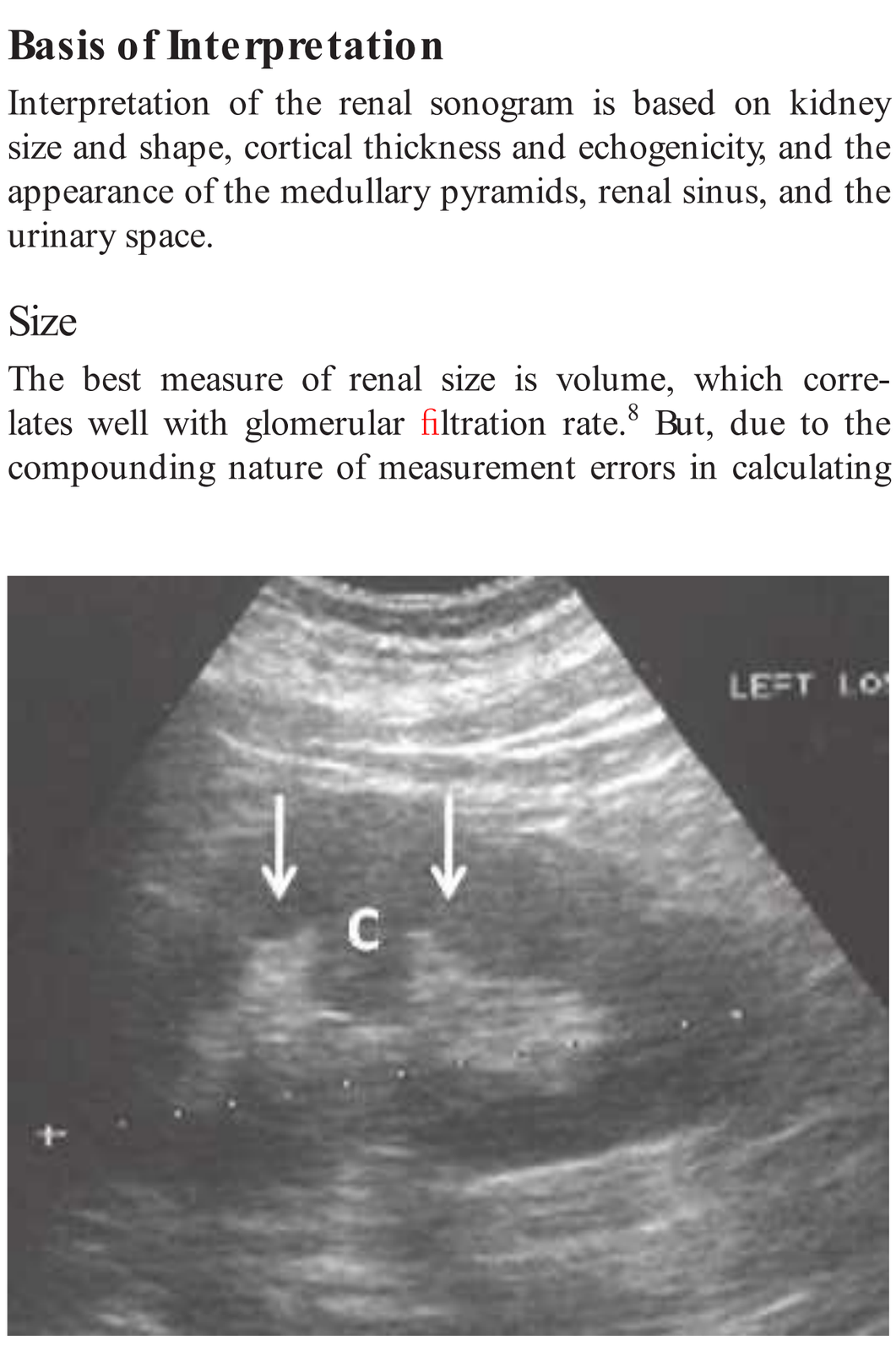


FIGURE 10.3 Duplication of the collecting system. Longitudinal view of the kidney showing a band of tissue (*arrow*) demarcating two separate renal sinuses (*S*).

Basis of Interpretation

Interpretation of the renal sonogram is based on kidney size and shape, cortical thickness and echogenicity, and the appearance of the medullary pyramids, renal sinus, and the urinary space.

Size

The best measure of renal size is volume, which correlates well with glomerular filtration rate.[8] But, due to the compounding nature of measurement errors in calculating

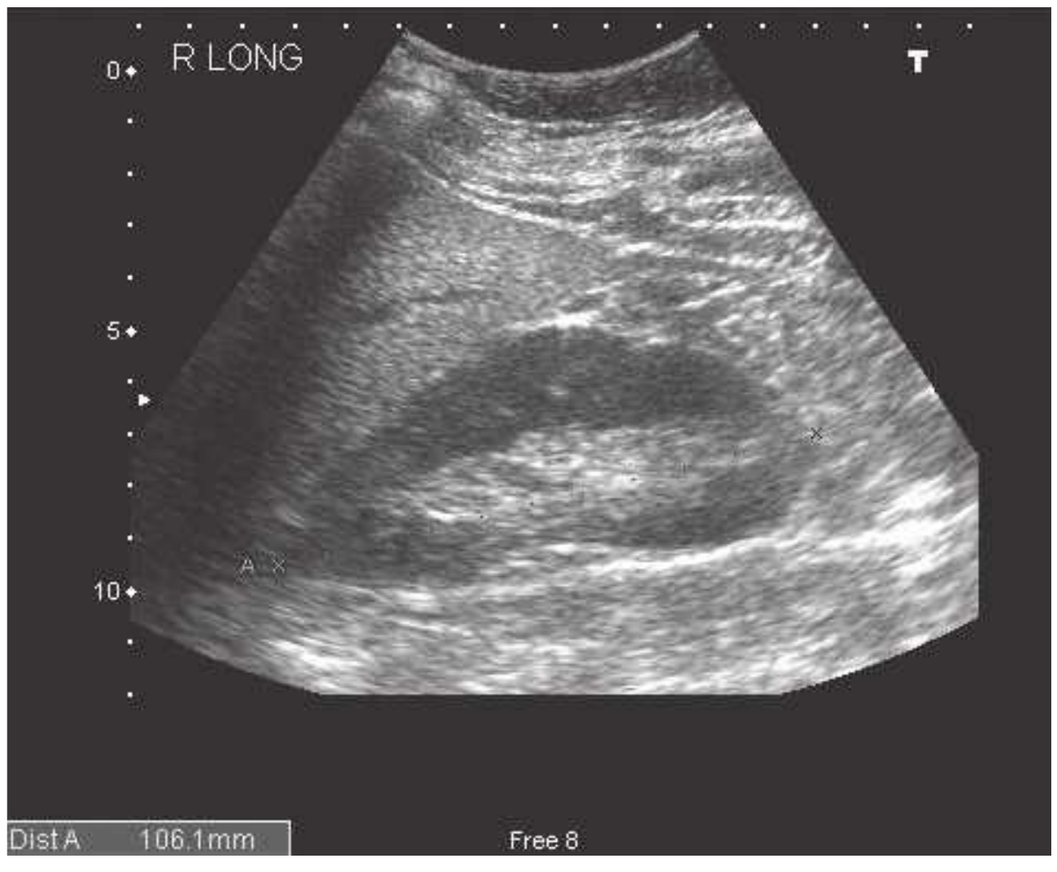


FIGURE 10.2 Longitudinal image of a normal right kidney. Compared to the liver, the renal parenchyma appears as a relatively hypoechoic, oval rim around the echogenic sinus fat.

FIGURE 10.4 Hypertrophied column of Bertin. Longitudinal image of the left kidney showing cortex (*C*) protruding into the renal sinus between two medullary pyramids (*arrows*).

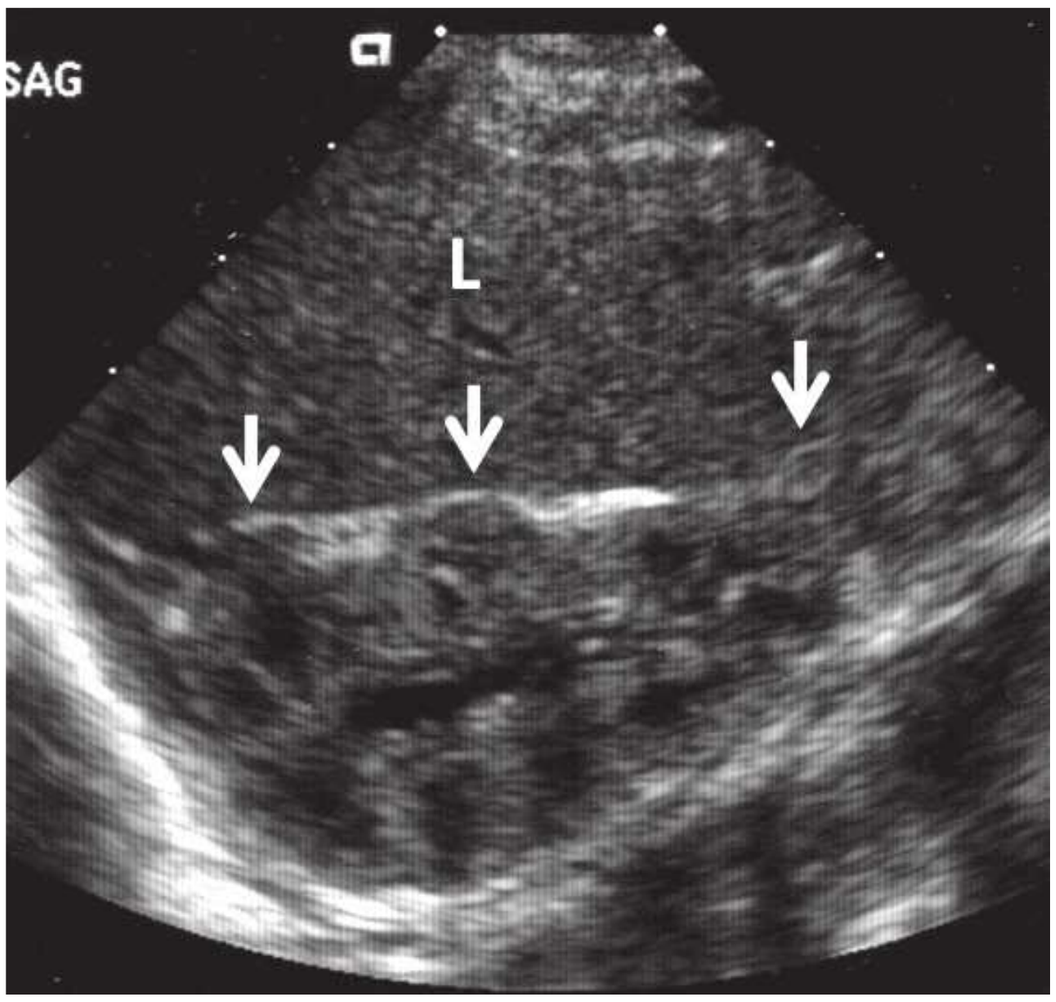


FIGURE 10.5 Normal neonatal kidney. Longitudinal view of the right kidney in a neonate showing accentuated lobules (*arrows*) and the medullary pyramid centered within each lobule. The cortex is more echogenic than the liver and there is no sinus fat.

volume, and the good correlation between maximum kidney length and renal volume,[9,10] maximum renal length is preferred for assessment of renal size on sonograms. Additional measurements in the transverse axes are very inaccurate and of no utility. Renal length averages 11 cm in adults,[10] and 10 cm to 12 cm is a useful range for normal renal length at average body height. Because the variability in measurements is 5%,[9] differences up to 1 cm may not be significant. The variability may be greater in children, comprising as much as 2 to 3 years in the comparison of kidney length to age.[11] Kidney length correlates best with body height in both adults and children (Fig. 10.6A)[10,12–14] and, after correction for body height, does not vary between sexes. Kidney length rapidly increases during the first year of life with a more gradual enlargement up to about 18 years[14,15] (Fig. 10.6B). Progressive enlargement occurs during pregnancy that resolves by 12 weeks postpartum, due primarily to parenchymal enlargement—although some pelvocaliceal enlargement occurs, particularly in the right kidney.[16] Proper interpretation of kidney size must take into account the effect of these nonpathologic factors. Compensatory hypertrophy is common in solitary kidneys in children (up to 80%–90% increase in volume),[17,18] and after nephrectomy in adults (5%–30% increase).[19,20] Enlargement of the kidneys occurs in nephritis and infiltrative diseases, often accompanied by a rounded shape and increased echogenicity.

Cortical Thickness

The thickness of the renal cortex is measured from the renal capsule to either the outer border of the medullary pyramids or to the arcuate arteries. A normal value of 9.3 ± 1.1 mm was obtained in 23 renal transplant donors.[21] If medullary pyramids are not discernible, the parenchymal thickness between the sinus fat and the renal capsule (mean value of 15 to 16 mm) may be used.[10] However, both parameters can vary within a kidney and are difficult to measure precisely. Cortical thinning is a sign of advanced chronic kidney disease. Increased cortical thickness is usually due to edema or

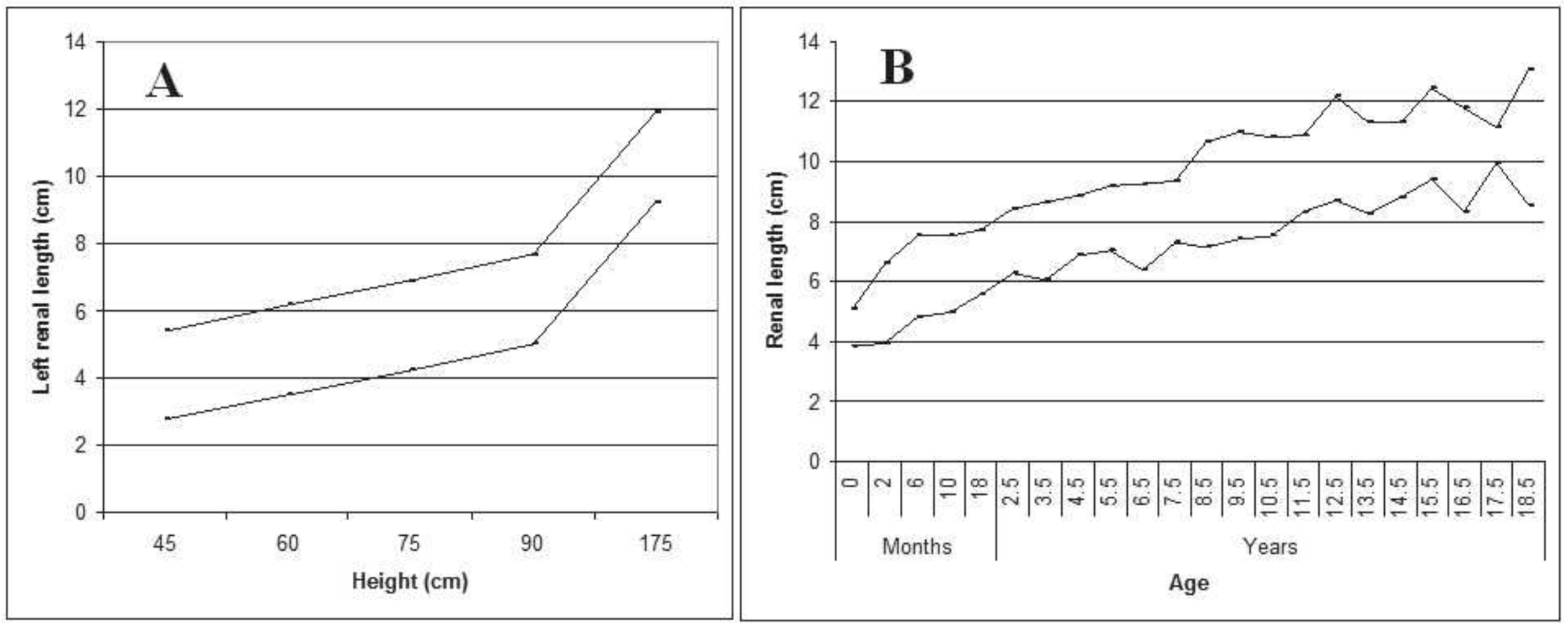


FIGURE 10.6 **A:** Nomogram of renal length based on body height showing 95% confidence limits for maximum length of the left kidney as a function of body height in children. (Adapted from Dinkel E, Ertel M, Dittrich M, et al. Kidney size in childhood: sonographic growth charts for kidney length and volume. *Pediatr Radiol.* 1985;15:38, with permission.) **B:** Nomogram of renal length and age: presenting 95% confidence limits for kidney length versus age in children. (Adapted from O'Neill WC. *Atlas of Renal Ultrasonography*. Philadelphia: WB Saunders; 2011, with permission.)

inflammation and is often accompanied by kidney enlargement, a globular shape, and obliteration of the sinus fat.

Cortical Echogenicity

Comparison of renal cortex to the liver or spleen, at the same depth, is the basis for determination of cortical echogenicity. Renal cortical echogenicity depends on age and is often greater than liver echogenicity in neonates but should be less than or equal to[22,23] that of the liver or spleen by 6 months of age.[24] After several years of age, the renal cortical echogenicity should always be less than the liver. Fibrosis, infiltrating cells, and tubular debris and dilation can increase echogenicity. Transmission artifacts related to overlying fluid and ribs, and increased hepatic echogenicity in steatosis or cirrhosis, can bias the interpretation of cortical echogenicity.

Medullary Pyramids

The medulla should be less echogenic than the cortex but visibility depends on overlying structures and the frequency of sound. Medullary disease usually causes increased echogenicity.

Renal Sinus

The neonatal kidney contains very little fat but the normal renal sinus in adults should exhibit only echogenic fat. Occasionally, the calyces are visible in otherwise normal kidneys, particularly during a brisk diuresis[25,26] or during pregnancy.[27,28] Blood vessels may also be visible, particularly in children and young adults or in states of increased central venous pressure.

Parenchymal Diseases

Glomerular or tubulointerstitial disorders usually present with diffuse changes in the kidneys. However, the changes can be similar in different disorders and the kidneys may appear normal. Thus, interpretation is very dependent on the clinical findings.

The most common parenchymal disorder that causes acute kidney injury is acute tubular necrosis (ATN). The renal sonogram can be normal in ATN[29–31] but increased cortical echogenicity and cortical expansion have been observed in both animal and human studies,[32–35] especially with nephrotoxic ATN,[32,35,36] whereas an enlarged hypoechoic cortex may be more typical of ischemic ATN.[6,32] The cortical enlargement presumably represents edema whereas increases in cortical echogenicity may be due to cellular and proteinaceous casts and debris within the tubules. The degree of renal enlargement has been shown to inversely correlate with recovery time from ATN.[34] In general, sonography is rarely useful in the workup of acute renal failure when the clinical picture suggests ATN and urinary obstruction is unlikely. However, it may be helpful in identifying underlying chronic kidney disease in this setting.

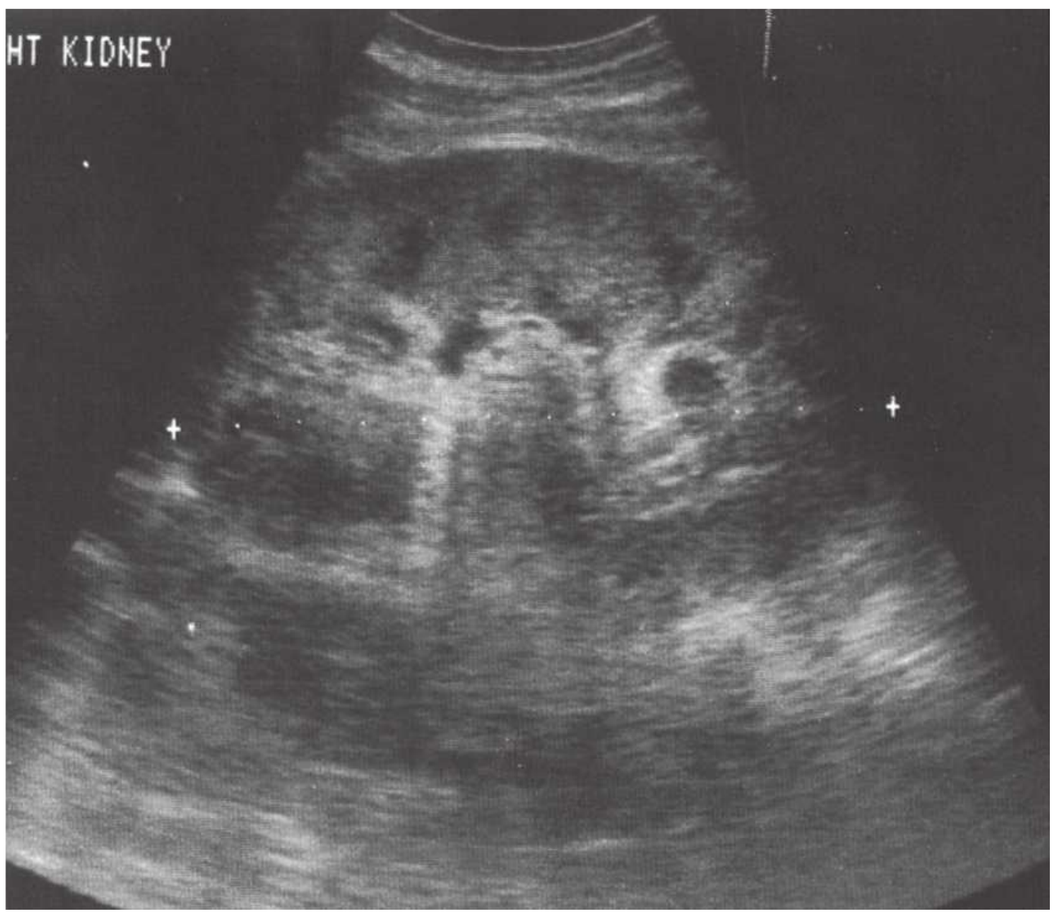


FIGURE 10.7 Acute glomerulonephritis. Longitudinal view of the right kidney showing a globular kidney with diminished sinus fat indicating cortical swelling. Note the prominence of the medullary pyramids due to increased cortical echogenicity.

Glomerulopathies

Although sonography is usually normal in glomerular diseases, acute glomerulonephritis and thrombotic microangiopathies can appear as cortical enlargement and increased cortical echogenicity.[30,31,37,38] When taken in the context of chronic renal failure, even a normal cortical thickness is suggestive of glomerular disease (diabetic nephropathy) because other disorders usually lead to cortical thinning.[39] The cortex is typically normal in membranous nephropathy or immunoglobulin A (IgA) nephropathy.[30,31,40] In severe glomerulonephritis, the kidney may take a rounded shape and echogenic appearance that is barely recognizable as a kidney (Fig. 10.7). Enlarged, echogenic kidneys can also be seen in HIV nephropathy,[41,42] amyloidosis, and preeclampsia.[43]

Tubulointerstitial Disease

Acute interstitial nephritis produces enlarged echogenic kidneys[30,44,45] that have the same appearance as glomerulonephritic kidneys. Chronic interstitial nephritis, particularly analgesic nephropathy (Fig. 10.8), presents with hyperechoic medullary pyramids, often with cortical atrophy.[31,46,47] At a more advanced stage, papillary necrosis and calcifications may be seen.[47–50] Medullary echogenicity can also be increased by uric acid deposition and nephrocalcinosis (Fig. 10.9), sickle hemoglobinopathies, Sjögren syndrome, and chronic hypokalemia.[51] Although acute pyelonephritis rarely produces renal failure, chronic pyelonephritis may cause chronic kidney disease and present with focal cortical scarring and thinning of the cortex often accompanied by caliectasis (Fig. 10.10). Xanthogranulomatous pyelonephritis may lead to enlarged, cystic appearing kidneys.[52,53]

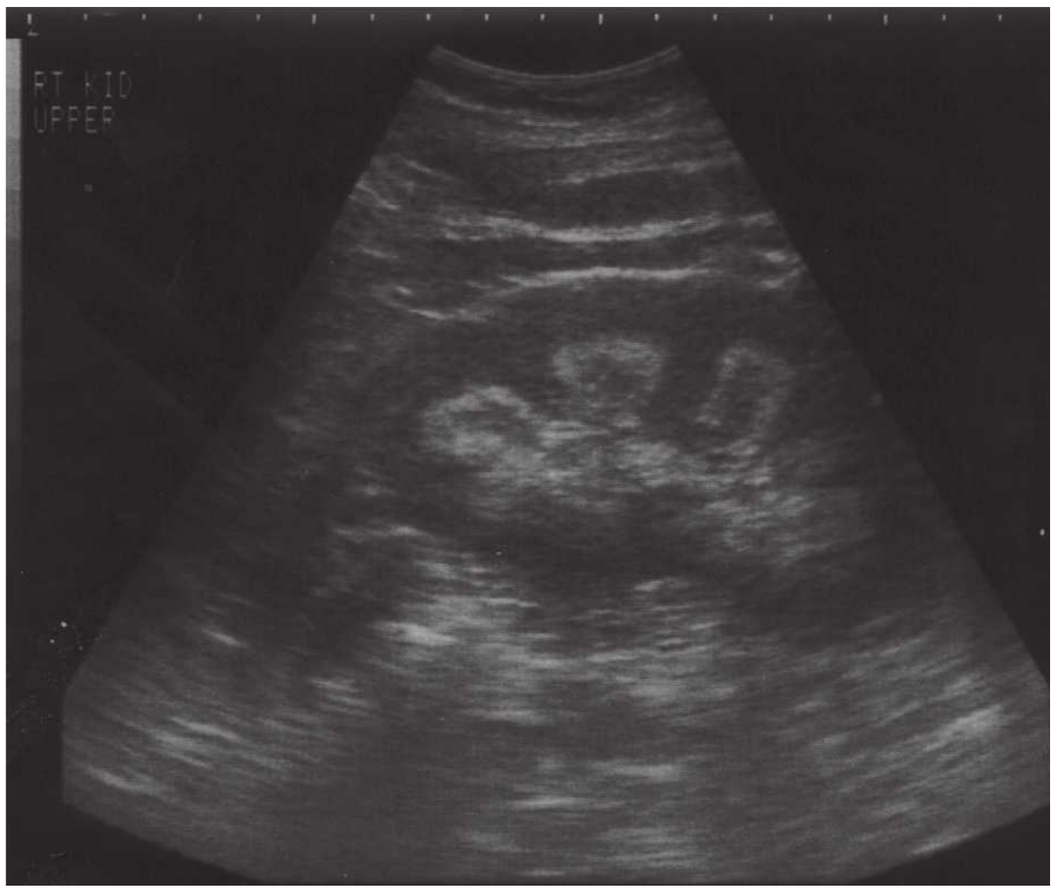


FIGURE 10.8 Analgesic nephropathy. Longitudinal view of the right kidney showing hyperechoic pyramids with relative sparing of the central portions.

Renal atrophy is usually the consequence of long standing renal disease or congenital defects, and presents as small kidneys with a thin cortex and accentuated lobulations (Fig. 10.11). The presence of renal atrophy does not provide any information on the underlying renal pathology but usually indicates that a renal biopsy will be uninformative.

Cysts

Cysts are fluid-filled structures with an epithelial lining usually originating from renal tubules. The typical sonographic features of cysts (anechoic structures with distal enhancement) make them very easily discernible by ultrasonography. The most common type of cyst is a sporadic acquired cyst, which can be present without any kidney disease. Cysts can be simple (Fig. 10.12) or complex (Fig. 10.13), with

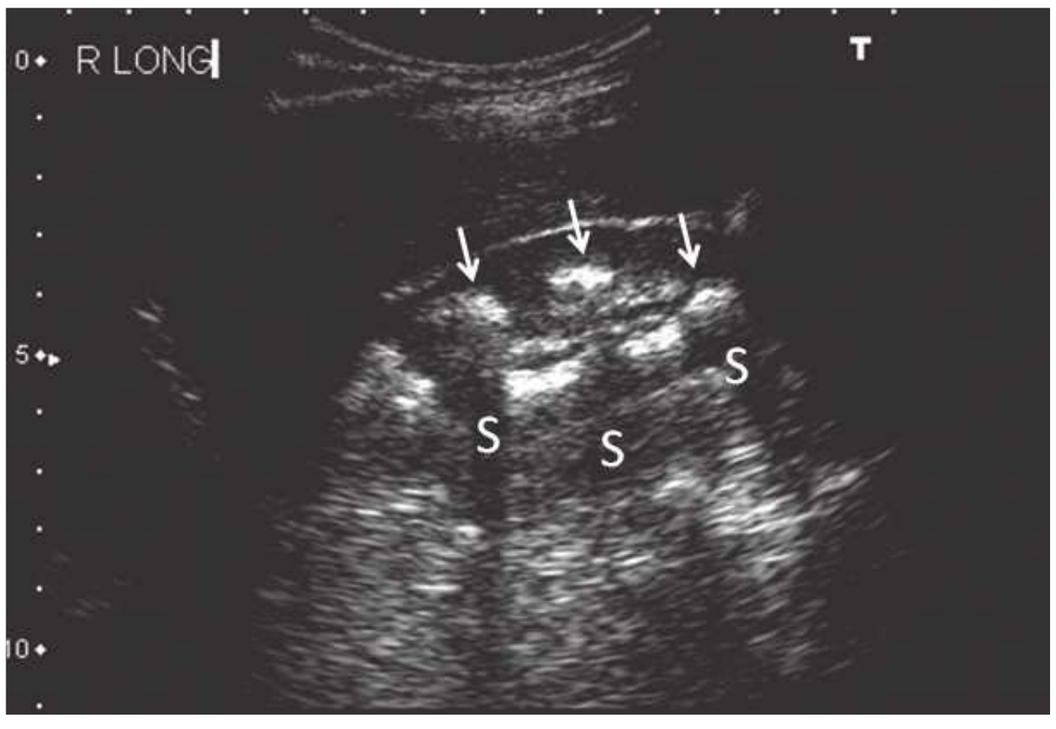


FIGURE 10.9 Nephrocalcinosis. Longitudinal image of the right kidney showing multiple calcifications in the inner medullae (*arrows*) producing acoustic shadows (*S*).

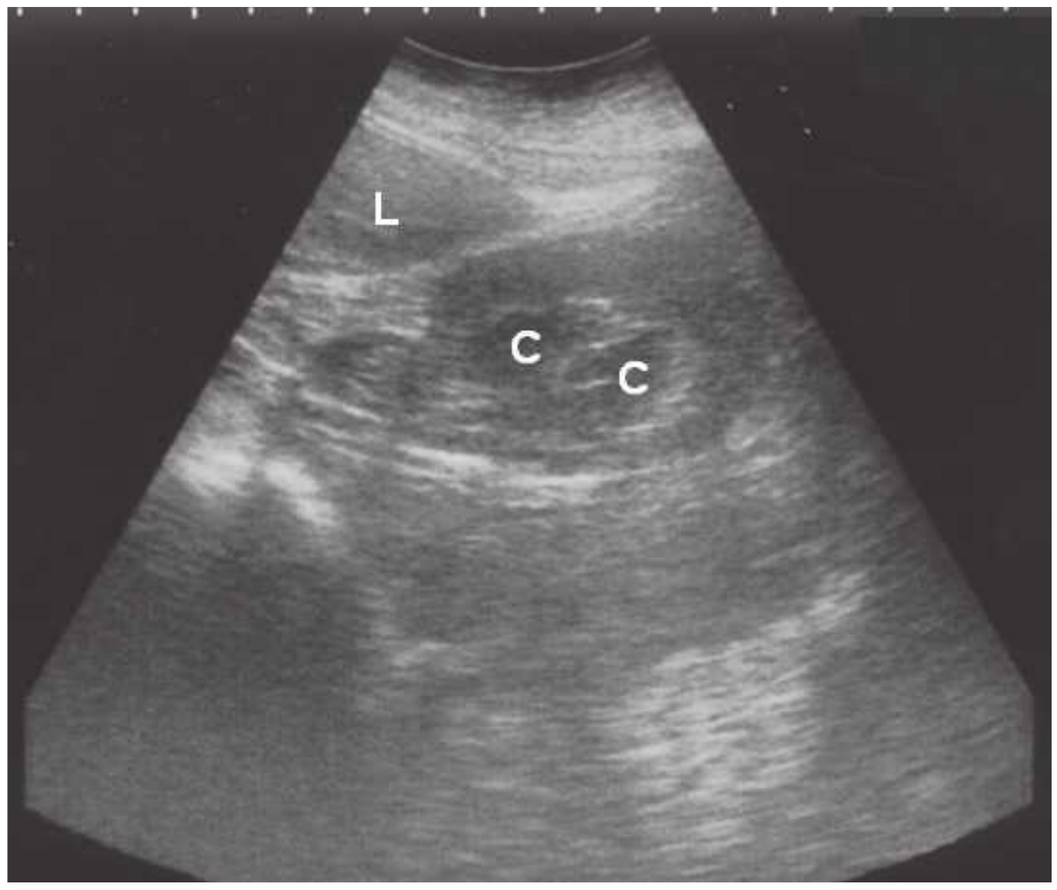


FIGURE 10.10 Chronic pyelonephritis. Longitudinal view of the right kidney shows caliectasis of the lower pole (*C*), and loss of parenchyma with scarring of the upper pole (*arrows*). *L*, liver.

the criteria for complexity including thickening of the wall, calcifications, more than two septations, and luminal echogenicity. Even though the great majority of complex cysts are benign, any complex cyst should be closely followed up with ultrasound or additional imaging studies (computed tomography [CT] and magnetic resonance imaging [MRI]), given the possibility of cystic renal cell carcinomas.[54]

Acquired cystic kidney disease (ACKD) and autosomal dominant polycystic kidney disease (ADPKD) are the most common types of multicystic renal disease. ACKD is often encountered in patients with advanced chronic kidney disease (CKD) or end-stage renal disease (ESRD). Cysts are

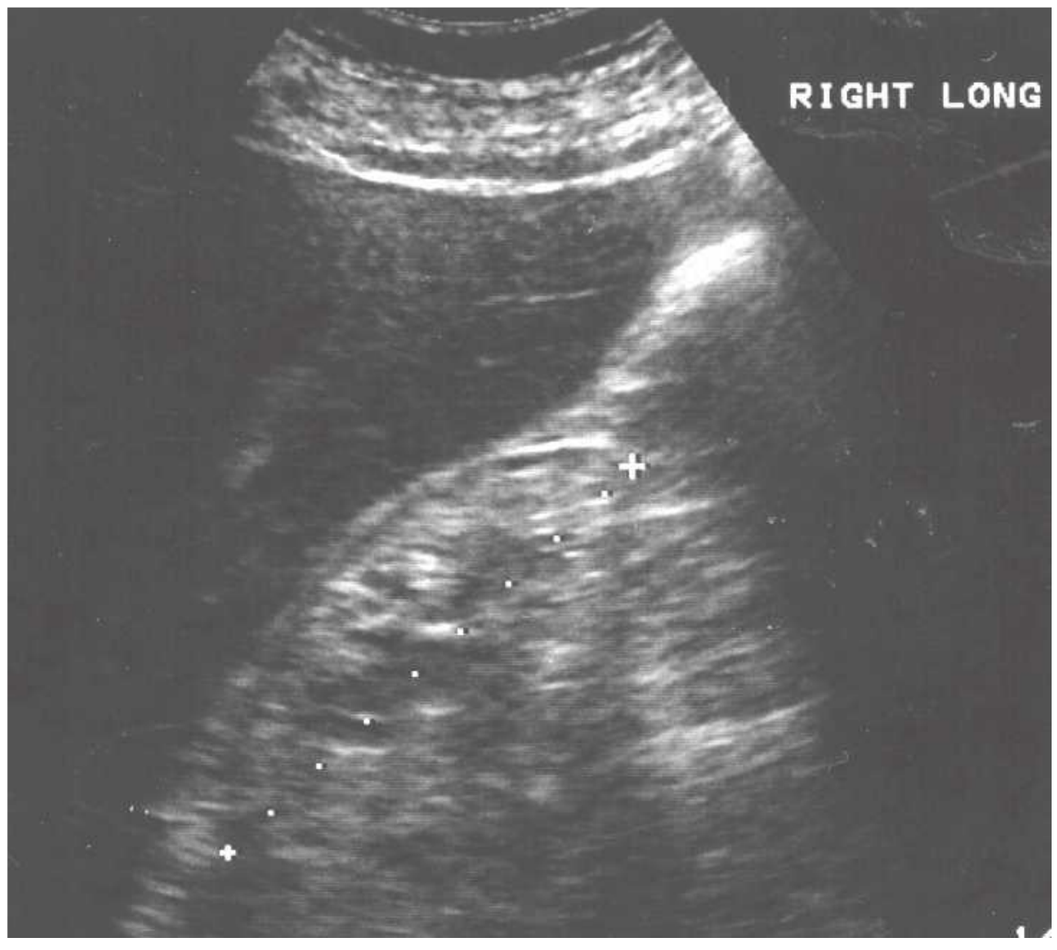


FIGURE 10.11 Renal atrophy. Longitudinal image of the right kidney showing a small kidney with a thin and hyperechoic cortex.

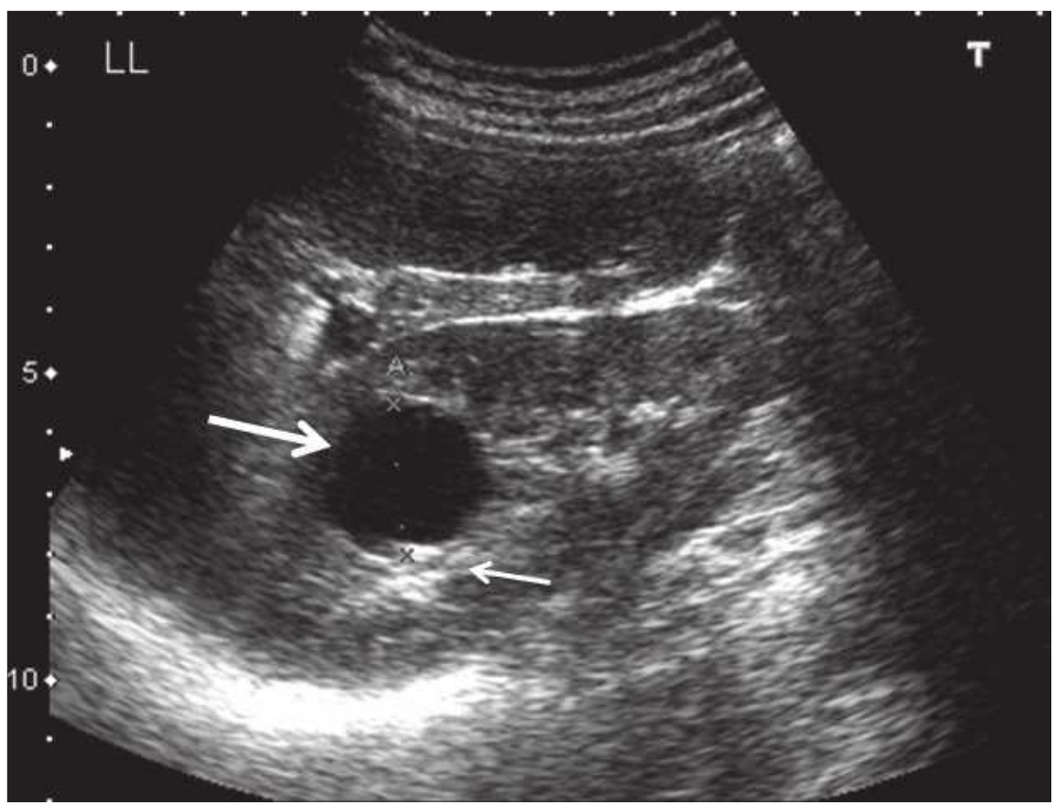


FIGURE 10.12 Simple cyst. Longitudinal image of the left kidney showing a round hypoechoic structure (*large arrow*) in the lower pole, which presents with distal acoustic enhancement (*small arrows*), consistent with a simple cyst.

typically of smaller size and the kidney is usually echogenic and small[55] (Fig. 10.14). ADPKD is the most common inherited cause of renal failure, and sonography is the cornerstone of diagnosis using specific criteria established by Ravine et al.[56] The performance of these criteria was found to be suboptimal in the PKD-2 genotype and revised unified criteria have recently been published by an international group of investigators (Table 10.1).[57]

The pathognomonic sonographic appearance of ADPKD includes presence of multiple bilateral renal cysts (Fig. 10.15) and liver cysts (in 83%–90% of patients)[58] with

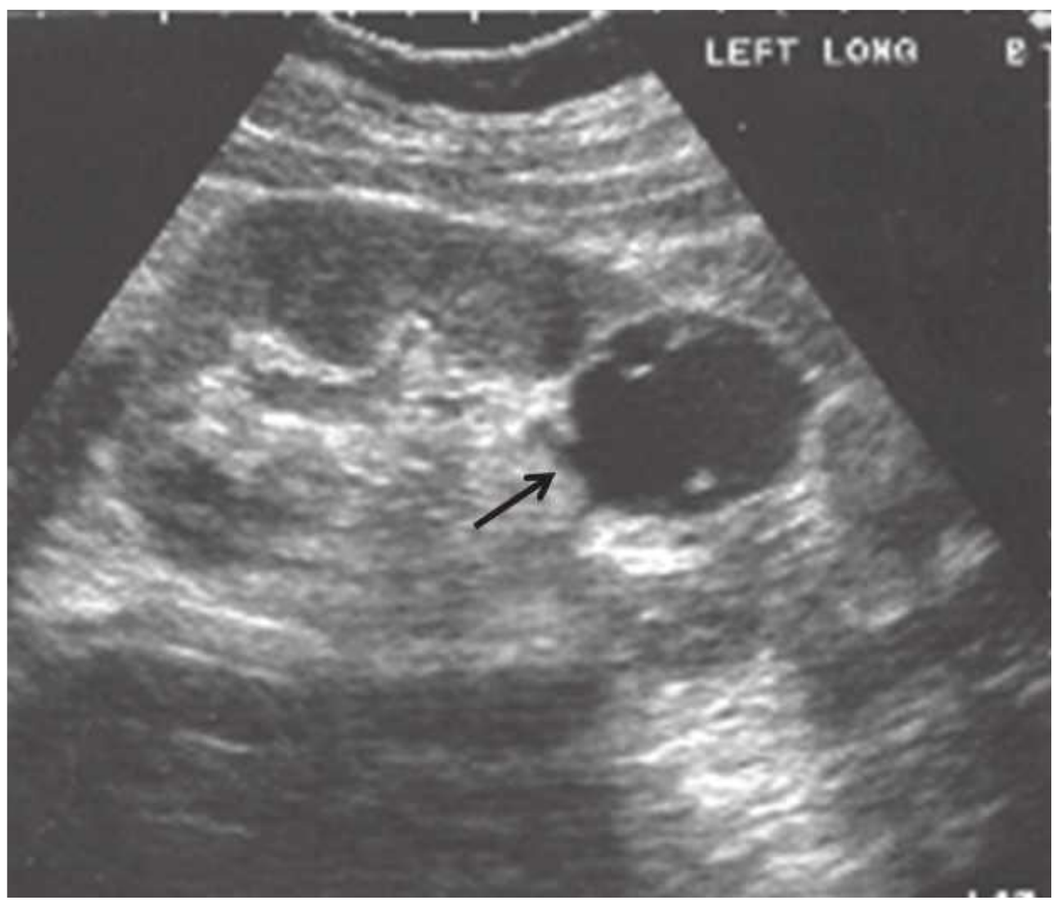


FIGURE 10.13 Complex cyst. Longitudinal view of the left kidney shows a cyst with an irregular wall (*black arrow*) and several internal echoes.

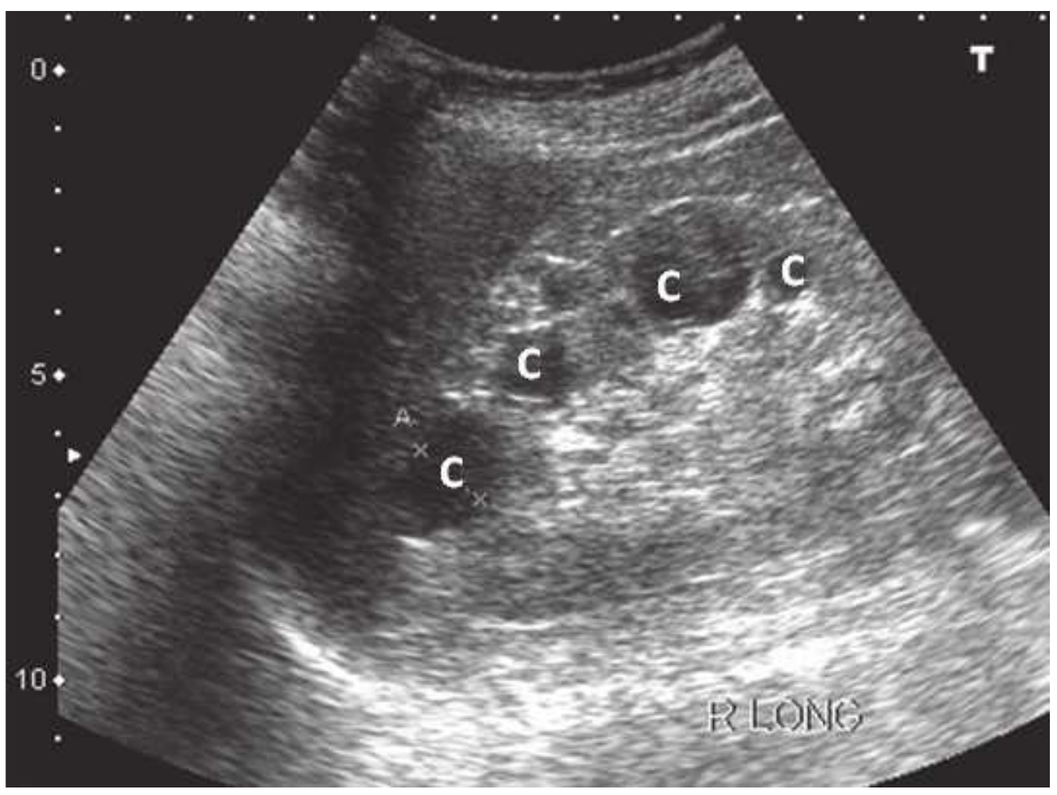


FIGURE 10.14 Acquired cystic kidney disease. Longitudinal view of the right kidney showing multiple cysts (*c*) with an intervening echogenic cortex.

significant renal enlargement. Other multicystic diseases such as medullary cystic kidney disease (MCKD), juvenile nephronophthisis, ACKD, or medullary sponge kidney[59,60] do not present with renomegaly. Multiple cysts with renal enlargement can be seen in von Hippel-Lindau disease, tuberous sclerosis, and multicystic dysplastic disease. Complex cysts are also commonly seen in ADPKD and the main etiologies are intracystic hemorrhage and infection. Even though renal cell carcinoma can be seen in this ADPKD, these patients are not at increased risk.[61]

Urinary Obstruction

Urinary obstruction typically results in hydronephrosis: a dilatation of the collecting system which may be predominantly seen in the minor calyces, the major calyces, or both. More atypical cases may present with minimal dilatation of the collecting system, particularly in the acute setting.[62] Hydronephrosis is only an anatomic diagnosis and may not indicate urinary obstruction. Brisk diuresis (such as in diabetes insipidus), papillary necrosis, and pregnancy can all result in nonobstructive calyceal dilatation.[28,62–64] Grading systems for severity of the hydronephrosis have proven to be of limited clinical utility because the degree of hydronephrosis may correlate poorly with the extent of obstruction. When obstruction is the primary cause of renal failure, it is always associated with hydronephrosis. In acute obstruction, the cortex is intact (Fig. 10.16) whereas chronic obstruction can lead to marked thinning of the cortex (Fig. 10.17). Failure to visualize the proximal ureter suggests obstruction at the ureteropelvic junction, whereas a dilated proximal ureter (hydroureter) (Fig. 10.16) indicates either obstruction at the level of the ureter or bladder. A large postvoid bladder indicates urinary retention, where sometimes the distal ureters can also be visualized (Fig. 10.18), whereas an empty bladder with dilatation of the distal ureters suggests obstruction

10.1 Performance Characteristics of Ultrasonographic Diagnostic Criteria for Autosomal Dominant Polycystic Kidney Disease in At-Risk Individuals Without Information on Genotype

Age Group (years)	Diagnostic Criterion	NPV	PPV	Accuracy
15–29	≥1 renal cyst	0.908	0.966	0.934
	≥2 renal cysts	0.877	0.992	0.924
	≥3 renal cysts	0.855	1.0	0.912
30–39	≥2 renal cysts in each kidney	0.875	1.0	0.922
	≥1 renal cyst	0.983	0.94	0.962
	≥2 renal cysts	0.97	0.979	0.974
	≥3 renal cysts	0.964	1.0	0.984
40–59	≥2 renal cysts in each kidney	0.948	1.0	0.965
	≥1 renal cyst	1.0	0.897	0.960
	≥2 renal cysts	1.0	0.967	0.988
	≥3 renal cysts	0.984	0.965	0.978

NPV, negative predictive value; PPV, positive predictive value.

Adapted from Pei Y, Obaji J, Dupuis A, et al. Unified criteria for the ultrasonographic diagnosis of ADPKD. J Am Soc Nephrol. 2009;19:205–212, with permission.

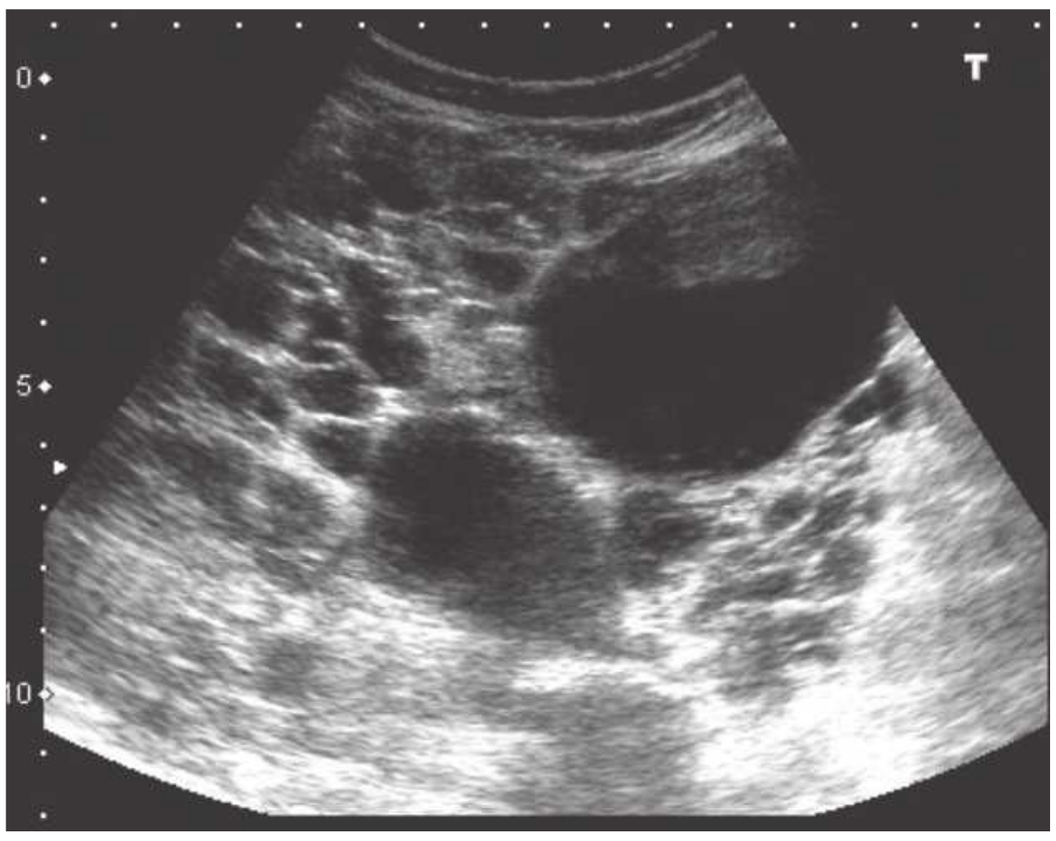


FIGURE 10.15 Autosomal dominant polycystic kidney disease. Longitudinal view of the right kidney showing an enlarged kidney with numerous cysts (hypoechoic structures) of different sizes.

at the bladder inlet. Recognition of hydronephrosis can be difficult in polycystic kidney disease (PKD), where massive cyst formation can prevent or obscure calyceal dilatation and more subtle signs should be sought (Fig. 10.19). This is an important cause of acute renal failure and can be due to stones or blood clots from cyst rupture. Radioisotope scanning may confirm obstruction when sonographic findings are not characteristic.

Occasionally, peripelvic cysts may also mimic hydronephrosis (Fig. 10.20). These are actually dilated lymphatics and, because they track with the blood vessels, can sometimes have a branching pattern. A rim of sinus fat separating the "cysts" from the parenchyma and the absence of a dilated ureter are useful hints toward peripelvic cysts. Another differential diagnosis for hydronephrosis is venous engorgement often seen in cases of volume overload or renal vein thrombosis. In general, the renal vein branches before entering the renal sinus (bush appearance) whereas the collecting system branches in the sinus (pruned tree appearance). The presence of venous pulsations and the tracking of the vein medially to the vena cava, as well as the

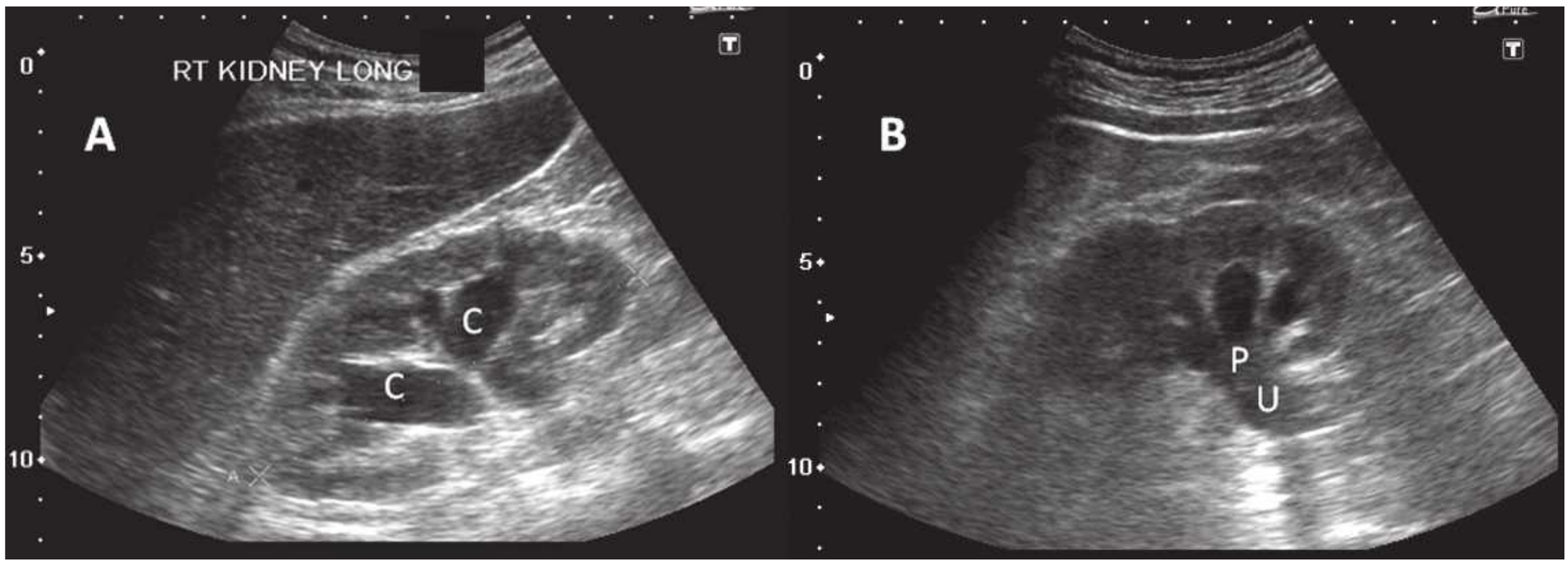


FIGURE 10.16 Acute hydronephrosis with hydroureter. Longitudinal image of the right kidney showing dilatation primarily of the major calyces (*C*) with some enlargement of the minor calyces and with conserved parenchymal thickness. The dilatation of the pelvis (*P*) and ureter (*U*) is easily visualized.

use of Doppler sonography, can differentiate blood vessels from the urinary tract. Occasionally, the renal pelvis is situated outside the sinus (extrarenal pelvis) appearing as a dilated proximal ureter but without any calyceal enlargement (Fig. 10.21).

The urinary bladder should be carefully examined by sonography in all patients with hydronephrosis. Additional indications are anuria, hematuria, pain, and urinary tract infections. The bladder is located in the midline, posterior to the symphysis pubis, and contains no luminal structures. When full, it appears as an anechoic fluid collection that is oval in transverse plane and becomes more elongated in the sagittal plane. The bladder volume is calculated by using the following formula for ellipsoid structures[65]:

$$\text{Volume} = 0.523 \times \text{length} \times \text{width} \times \text{depth}$$

A normal bladder is usually empty after a complete void and should not contain more than 10 mL of urine. A postvoid residual volume of more than 50 mL is associated with a threefold risk of urinary retention in males and is often used as a threshold for the diagnosis of urinary retention.[66] Hyperechoic structures such as stents, Foley catheters, bladder stones (Fig. 10.22), tumors, and blood

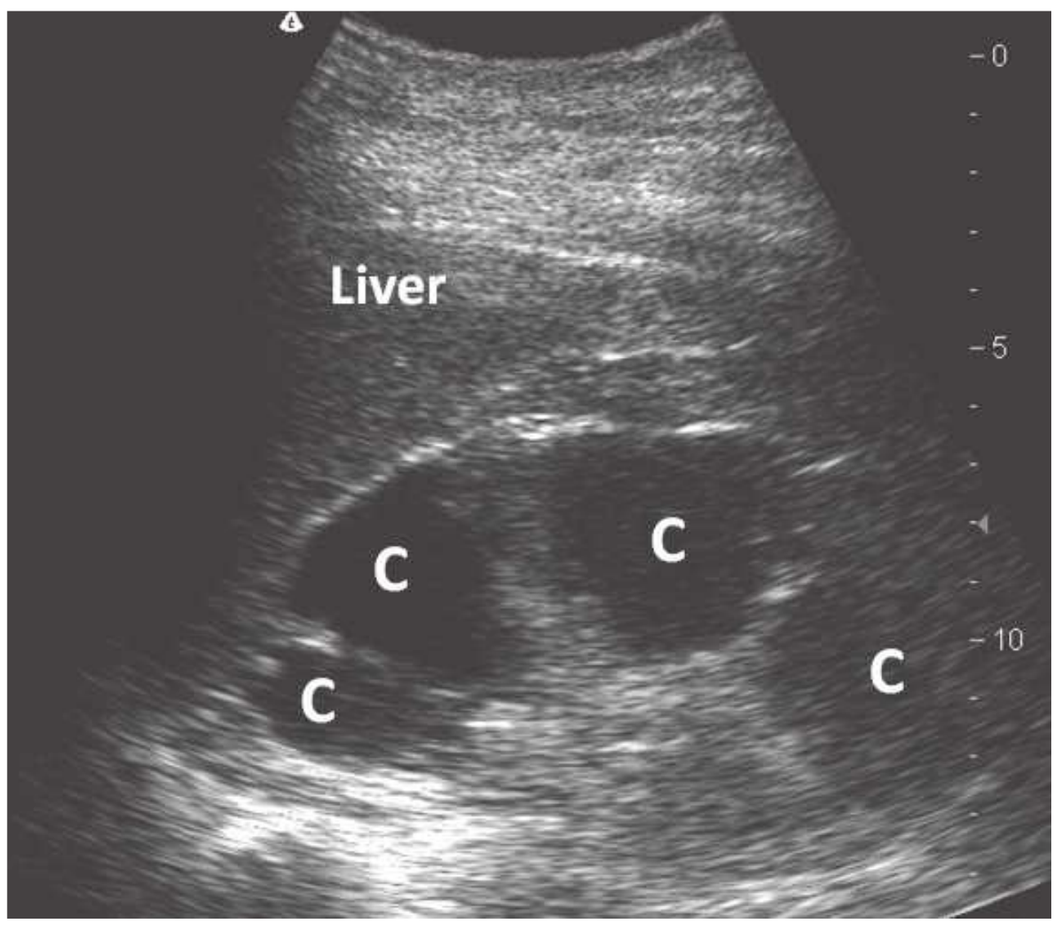


FIGURE 10.17 Chronic hydronephrosis. Longitudinal view of the right kidney showing dilated calyces that extend to the renal capsule, indicative of an extremely thin cortex.

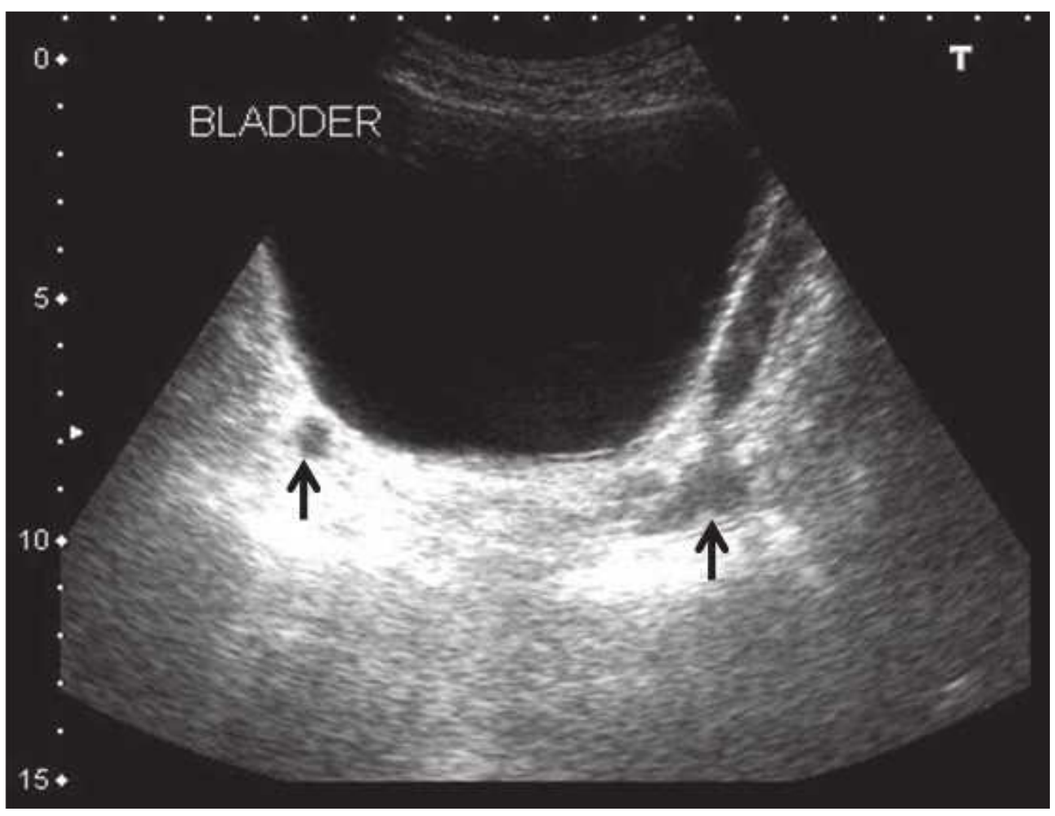


FIGURE 10.18 Urinary bladder retention. Transverse view showing an extremely dilated bladder. The distal ureters (*arrows*) are visible posterior to the bladder.

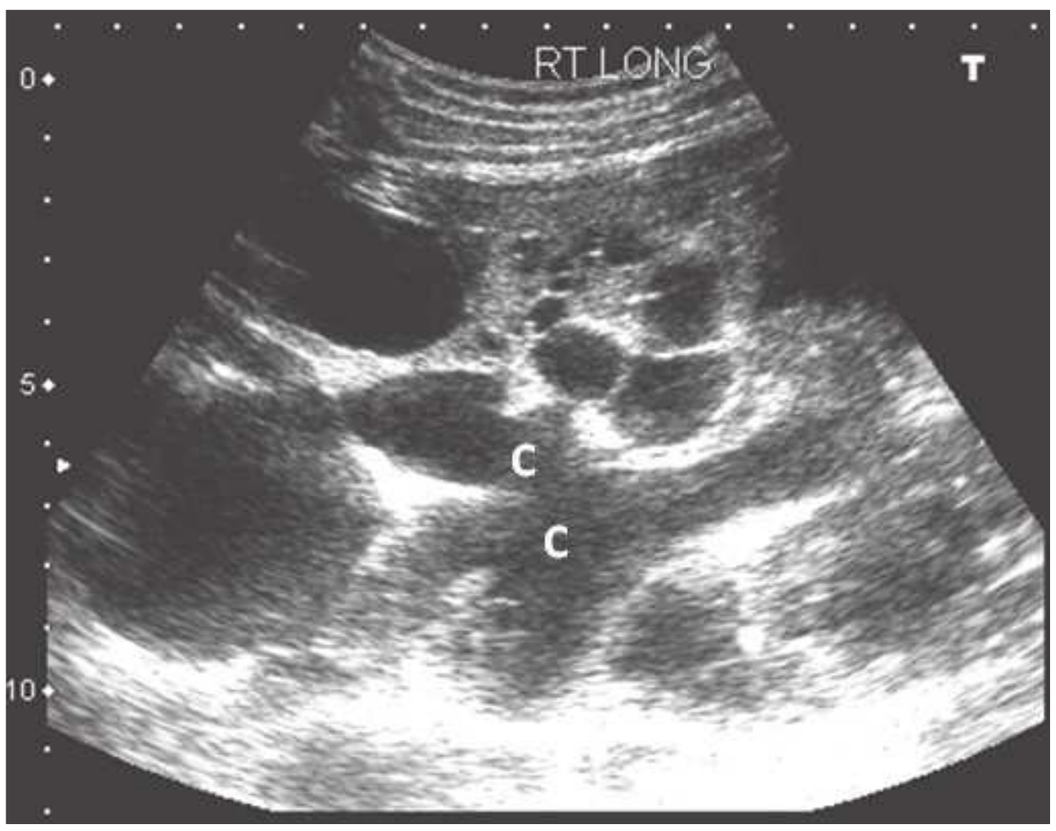


FIGURE 10.19 Hydronephrosis in autosomal dominant polycystic kidney disease. Converging dilated calyces (*C*) are apparent in this young patient who presented with major hemorrhagic cyst rupture and acute renal failure due to ureteral clots. Hydronephrosis resolved after ureteral stent placement. The multitude of cysts and disruption of the normal anatomy often make detection of hydronephrosis difficult.

clots (Fig. 10.23) may be visible in the bladder. The presence of urinary jets from a ureter proves its patency. Ureteral stents may transmit bladder pressure back to the kidney, resulting in hydronephrosis. Thus, the diagnosis of stent obstruction requires an empty bladder.

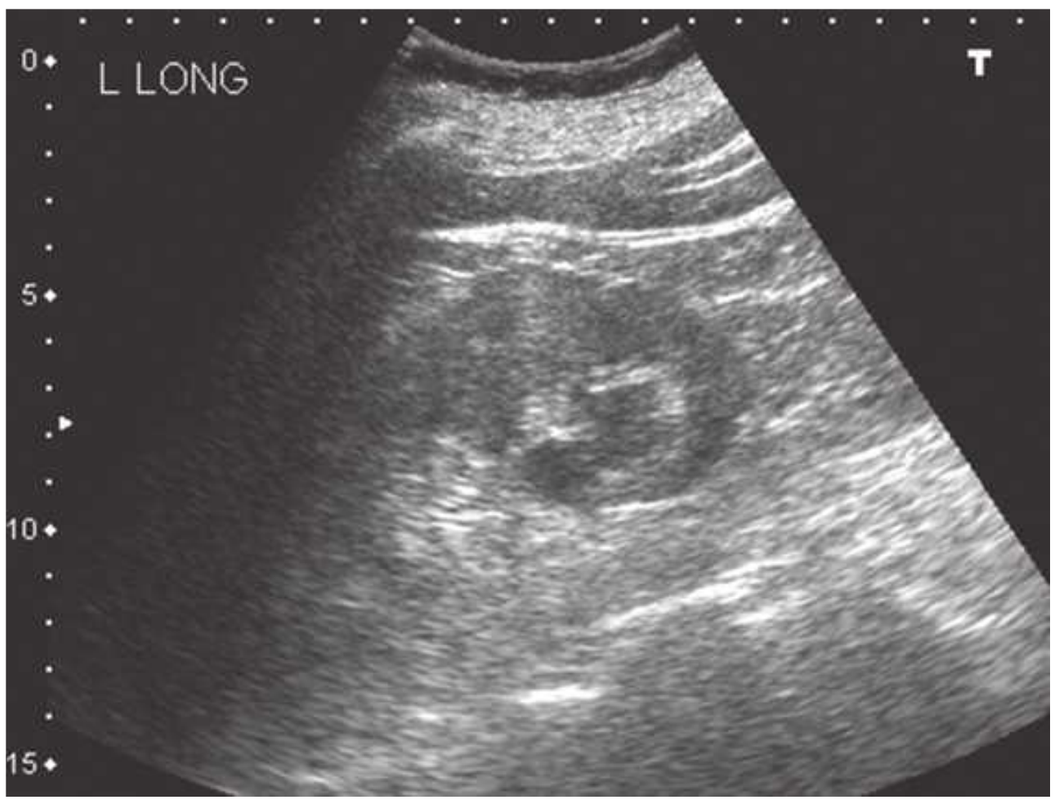


FIGURE 10.20 Peripelvic cyst. Longitudinal view of the left kidney showing a hypoechoic structure where the renal pelvis is expected to be seen. The absence of calyceal dilatation and presence of the echogenic sinus fat interposed between the cyst and the parenchyma are indicative of peripelvic cysts rather than hydronephrosis.

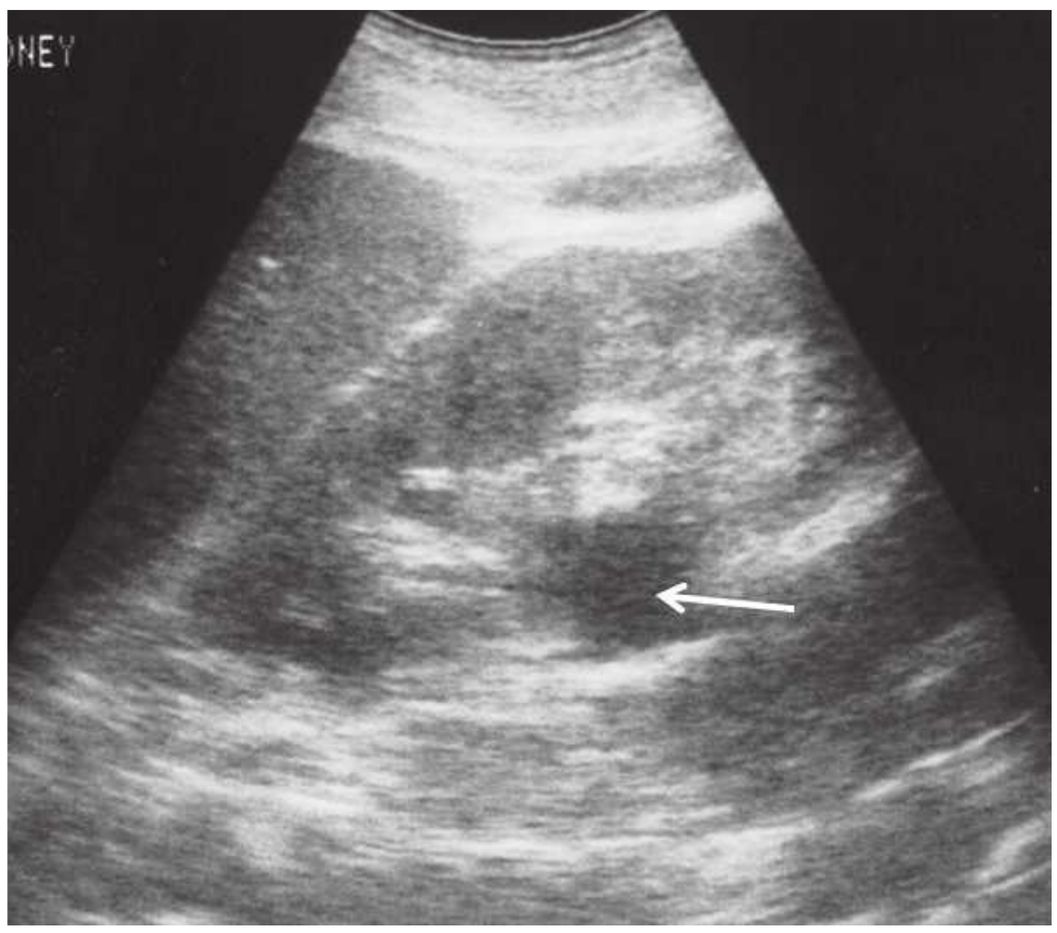


FIGURE 10.21 Extrarenal pelvis. Longitudinal view of the left kidney. There is apparent dilatation of the initial proximal ureter with no further hydroureter. Although the major calyces can be seen converging into the extrarenal renal pelvis, there is no enlargement of the minor calyces.

Kidney Stones and Calcifications

Stones typically reflect most of the sound, rendering them echogenic with a distal shadow and very easily discernible by ultrasonography no matter what the composition. However, both findings may not be present and small kidney stones

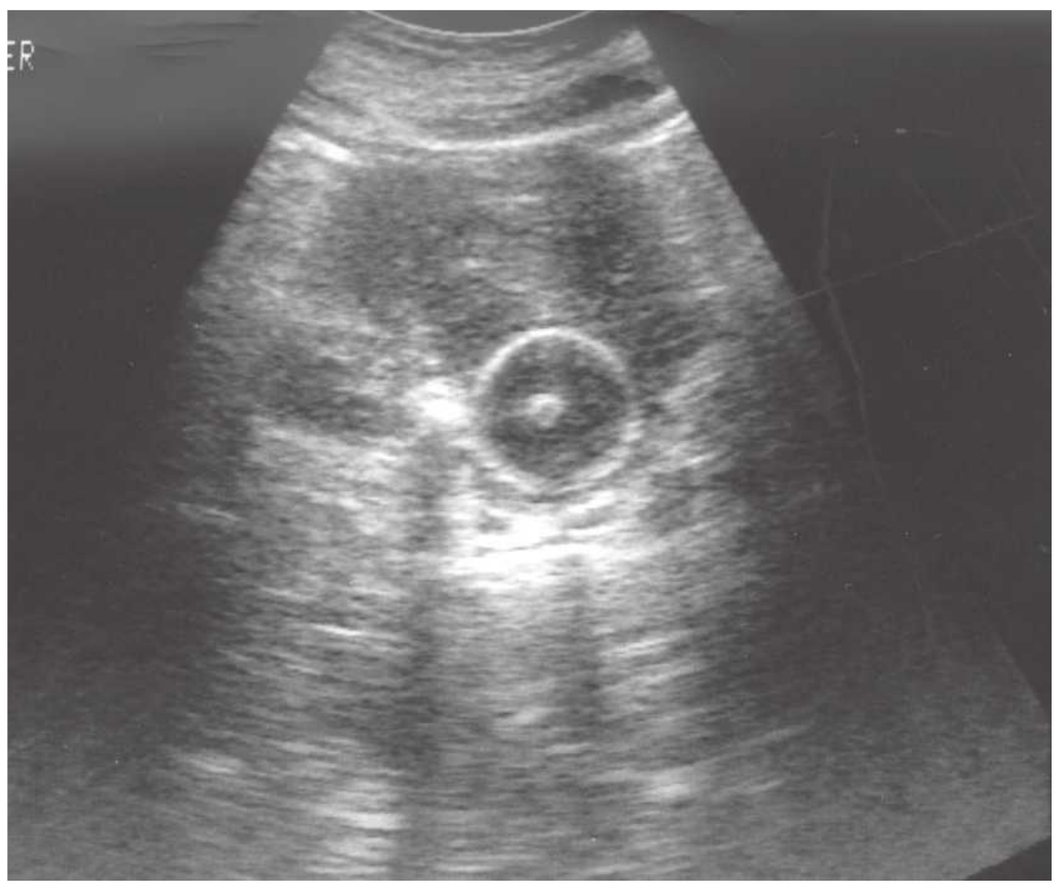


FIGURE 10.22 Foley catheter and bladder stone. Transverse view of the bladder shows a perfectly round shaped circle with echogenic walls (inflated balloon) surrounding the central echogenic catheter. Adjacent to the left side of the Foley catheter, a small opacity with a posterior shadow represents a bladder stone.

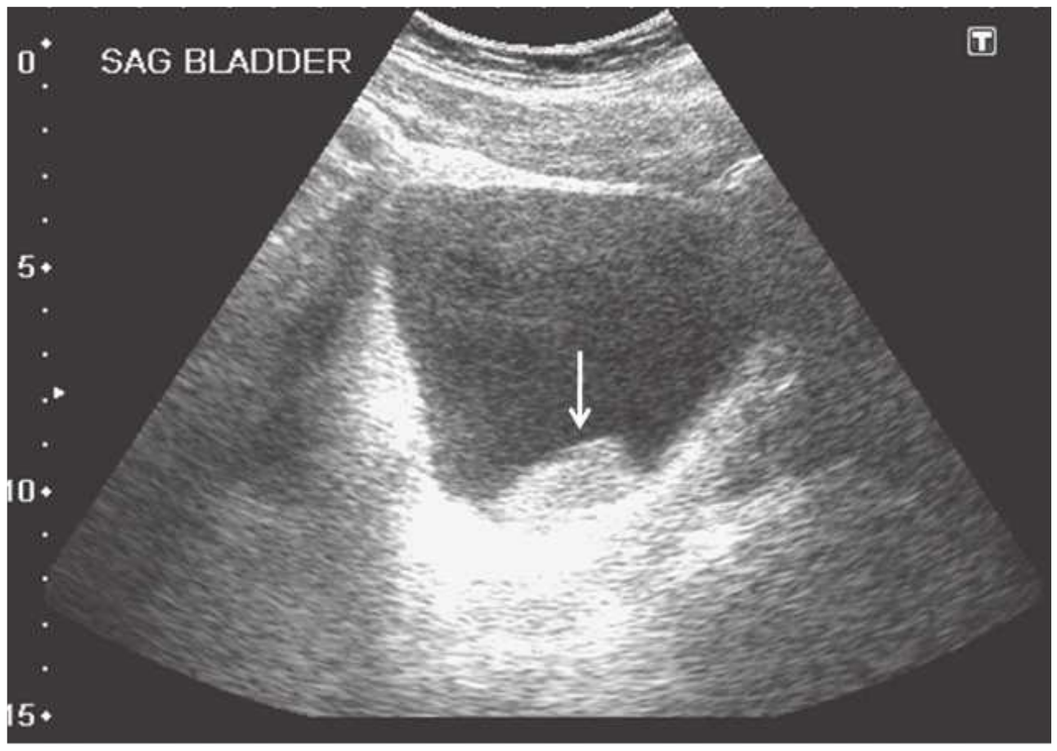


FIGURE 10.23 Clot in the bladder. Sagittal view of a distended bladder containing a hyperechoic mass, representing a blood clot (*arrow*).

and ureteral stones may not be visible. Stones are seen in the urinary space (Fig. 10.24) and may be associated with urinary obstruction, hydronephrosis, and urinary tract infection. Staghorn calculi typically fill the entire calyceal system but can appear as multiple stones on single images (Fig. 10.25).

In nephrocalcinosis (Fig. 10.9) and papillary necrosis, the calcification is in the renal medulla and not the urinary space but this distinction can sometimes be difficult. The differential diagnosis also includes ureteral stents, which typically are not as echogenic and yield less distinct shadows.

Neoplasms

Renal neoplasms are usually discovered incidentally or during the workup for pain or hematuria. Special attention

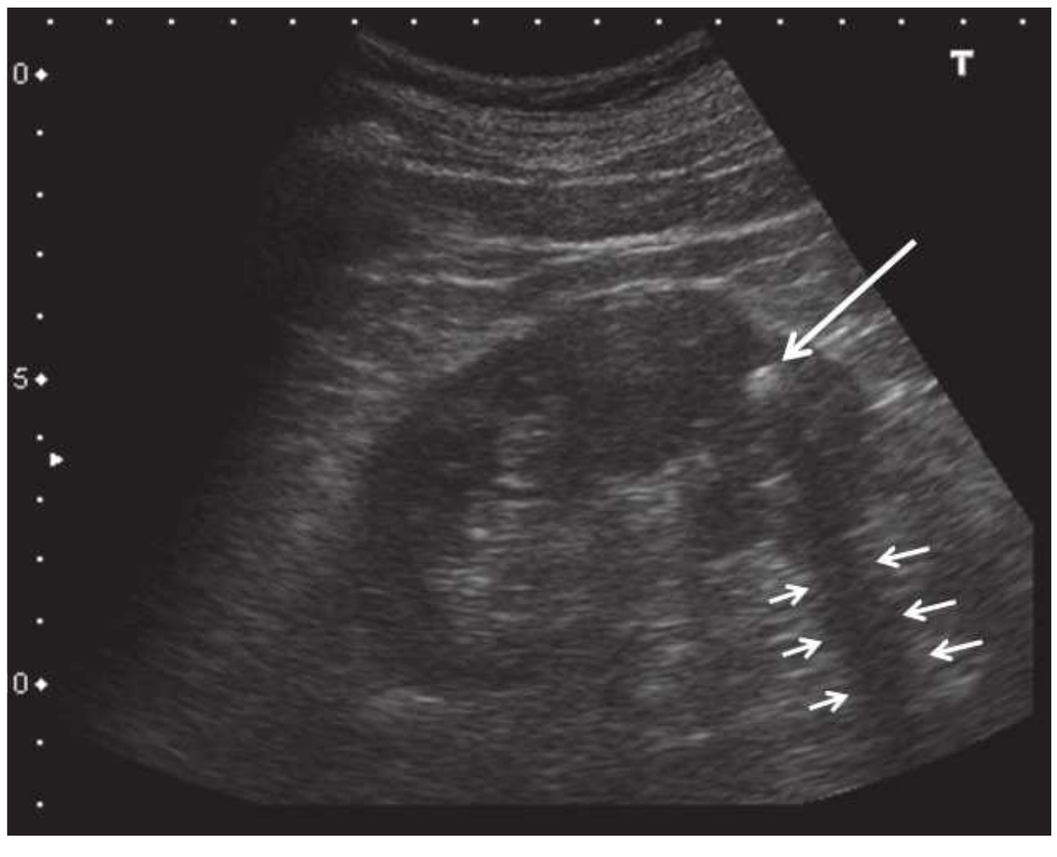


FIGURE 10.24 Kidney stone. Longitudinal view showing a hyperechoic stone (*large arrow*) in the lower pole that casts an acoustic shadow (*small arrows*).

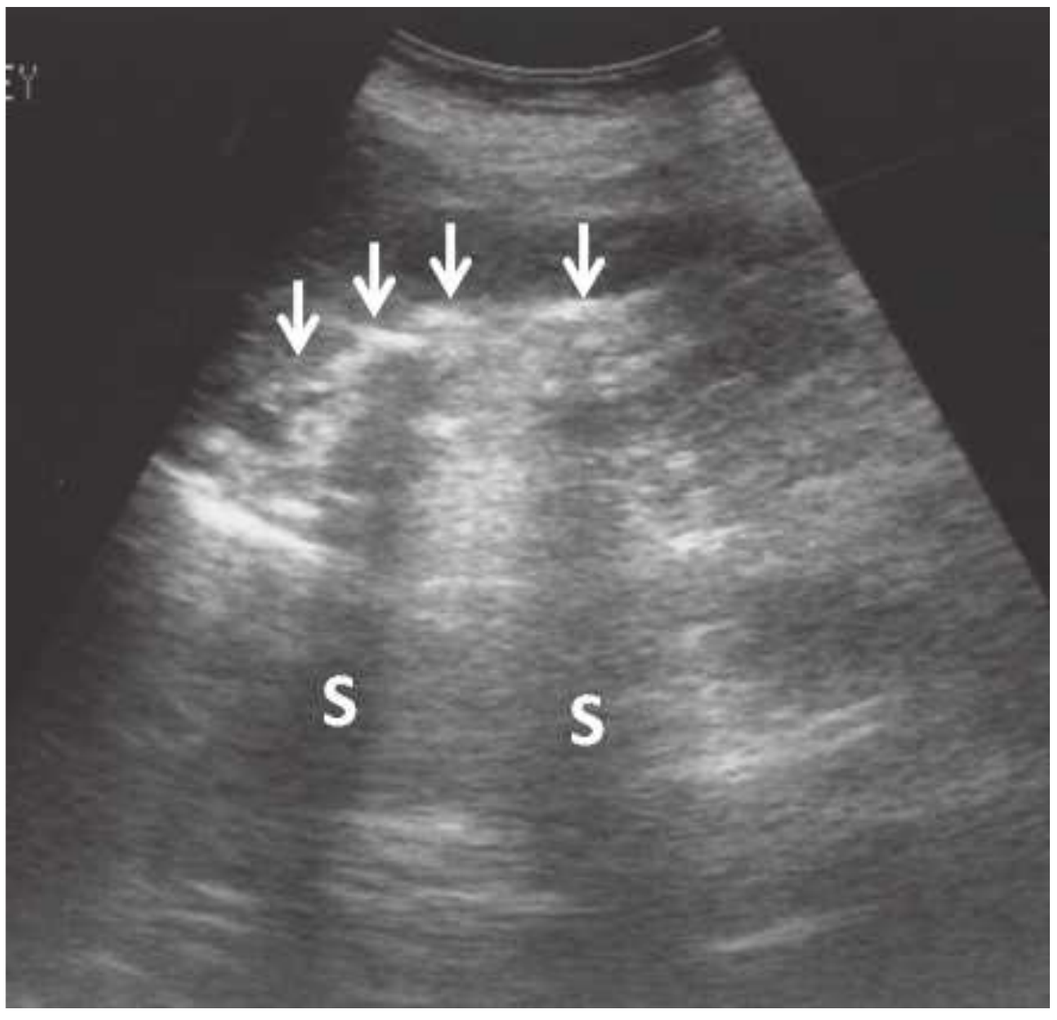


FIGURE 10.25 Staghorn calculus. Longitudinal image of the right kidney showing a long curvilinear echogenicity in the renal sinus with dense acoustic shadows (*S*).

should be given to rule out cysts, transmission artifacts, and normal variants such as lobulation and hypertrophied columns of Bertin.[67] Although the absence of blood flow by Doppler ultrasound may point to a cyst, additional imaging is almost always indicated because sonography can rarely identify the cause of a solid lesion.

Renal Cell Carcinoma

The typical sonographic appearance of renal cell carcinoma is of a well-demarcated hypoechoic mass that distorts the renal contour (Fig. 10.26), but tumors can be isoechoic (and more difficult to visualize) and can be hyperechoic when small (<3 cm). About 10% of renal cell carcinomas have a cystic appearance.[68]

Transitional cell carcinoma is a tumor of the renal pelvis that typically presents as a relatively hypoechoic mass within the renal sinus, separated from parenchyma by fat tissue.[69–71] They can also contain echogenic areas and occasionally can be diffusely infiltrative.

Angiomyolipoma

Sporadic angiomyolipomas (AMLs) are the most commonly encountered tumors in kidneys (autopsy incidence of 11%, more commonly seen in middle-aged women)[72] but multiple AMLs can be observed in tuberous sclerosis. They are not malignant but may cause hemorrhage. The typical presentation is of a very echogenic parenchymal mass (due to the high fat content) with an acoustic shadow in 33% of cases[73] (Fig. 10.27).

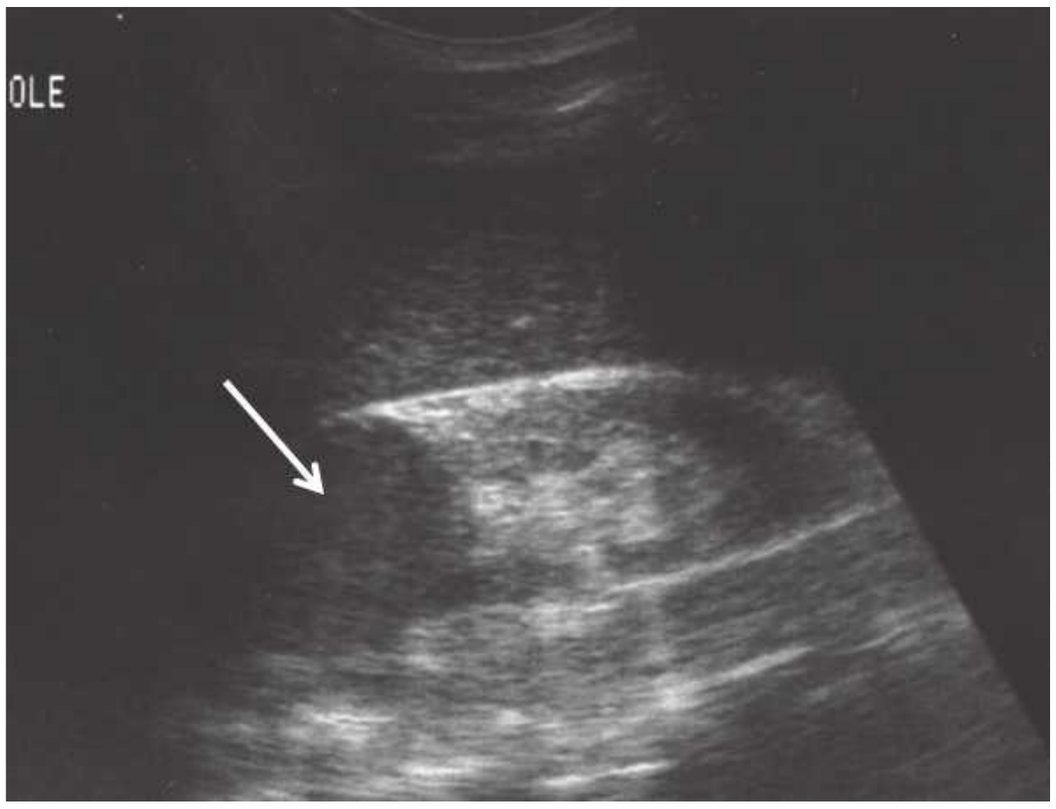


FIGURE 10.26. Renal cell carcinoma. Longitudinal view of the left kidney shows a hypoechoic mass (*arrow*) in the upper pole which was confirmed to be a renal cell carcinoma.

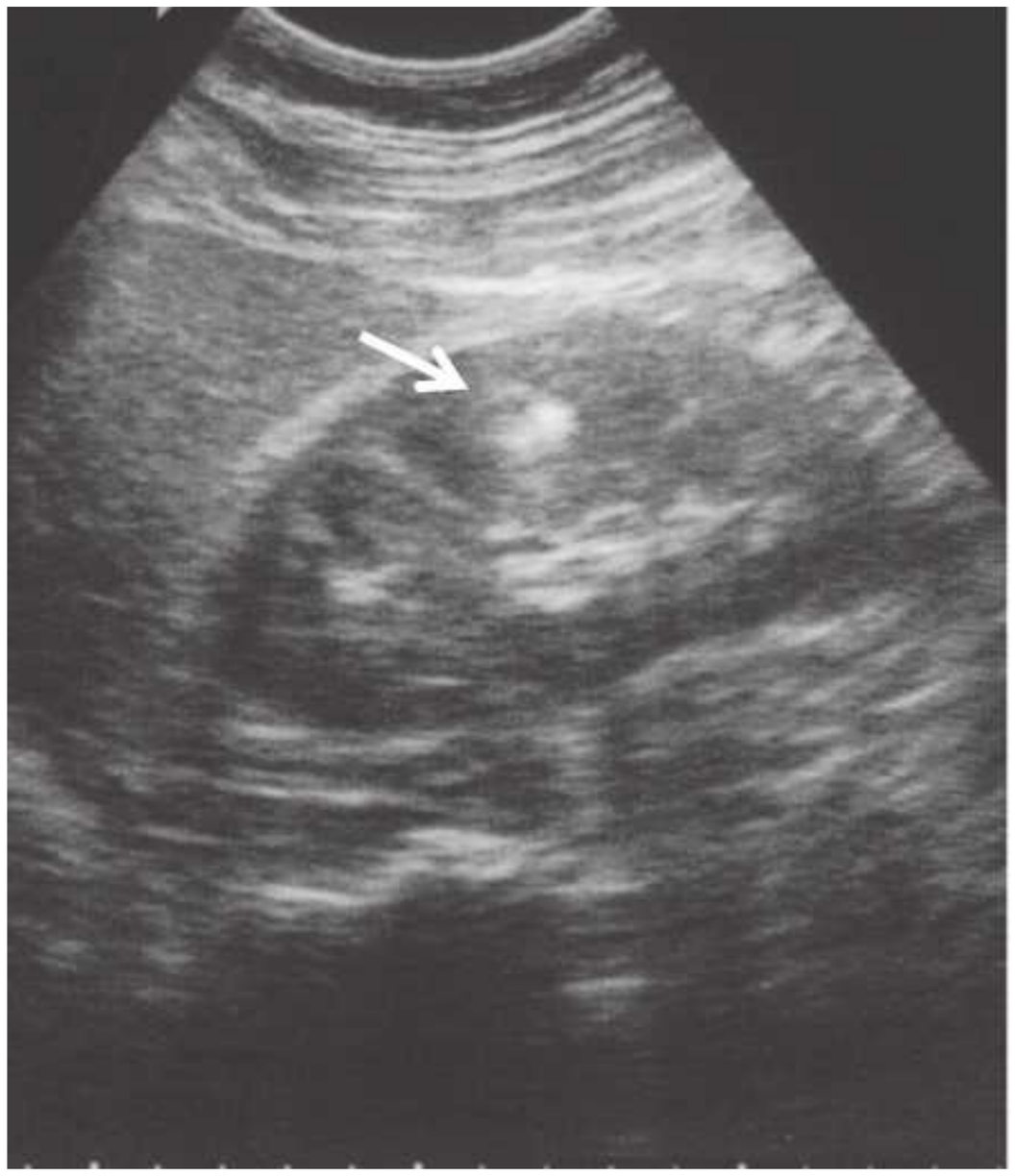


FIGURE 10.27 Angiomyolipoma. Longitudinal view of the left kidney shows a small, brightly echogenic mass in the cortex (*arrowhead*) without any distal shadowing.

Metastasis and Infiltrative Neoplastic Diseases

Infiltration of the kidneys is common in lymphomas (20% on CT scan,[74] 50% on autopsies[75]). They can cause acute renal failure by obstruction or diffuse infiltration.[76] The typical appearance is of multiple hypoechoic masses but a "perirenal halo" is also a classic but more unusual occurrence.[77] Leukemias can also infiltrate the kidney. Other solid tumors (e.g., lung cancer) may metastasize to the kidney, in which case they tend to be focal and nodular and are not distinguishable from other tumors.

Infections

Sonography is not indicated in most routine cases of pyelonephritis. Indications include male gender, children, failure to resolve, complications, and frequent recurrence. Pyelonephritis is usually lobular, appearing as a poorly defined hypoechoic area corresponding to a lobule, but it occasionally is diffuse. This can progress to abscesses which are usually single or multiple, heterogenous masses. The differential diagnosis includes complex cysts and neoplasms, which may require CT or MR for diagnosis.

Hemorrhage

Renal hemorrhage usually appears as perirenal or subcapsular hematomas and usually results from percutaneous kidney biopsy, surgery, or trauma. Initially they may appear as anechoic fluid collections but typically they are heterogeneous with fluid and solid components.

Transplanted Kidneys

Although many aspects of sonography are similar in native and transplanted kidneys, important differences exist due to anatomic considerations. The allograft is usually placed in the right pelvic fossa, aligned along the incision with the hilum oriented inferiorly and posteriorly, but a variety of other orientations can be encountered especially in obese patients, repeat transplantations, and combined kidney-pancreas transplantation. The donor artery and vein are anastomosed to the external iliac (or the common iliac) vessels and the ureter is anastomosed to the superolateral wall of the bladder (Fig. 10.28). When two kidneys are transplanted en bloc, portions of the donor aorta and vena cava are retained and anastomosed to the recipient vessels.[78,79] The kidney is often placed within the peritoneum when combined with a pancreas transplant. In very young children, the kidney may be placed posterior to the cecum with anastomosis of the donor vessels to the great vessels.[79] The anatomic relationships are readily apparent on sonograms. The psoas muscle and iliac vessels lie posteriorly and are usually imaged transversely on longitudinal scans of the allograft. The former can be identified by its contraction when the leg is flexed at the hip, and the latter are frequently pulsatile. The ureter and renal vessels are often visible even in normal allografts. The ureter courses medially, usually lying directly under the lower (medial) pole, whereas the renal vein travels posteriorly. The bladder lies medially and can easily be mistaken for a perirenal fluid collection. The peritoneum is superior and occasionally anterior to the allograft, typically appearing as a

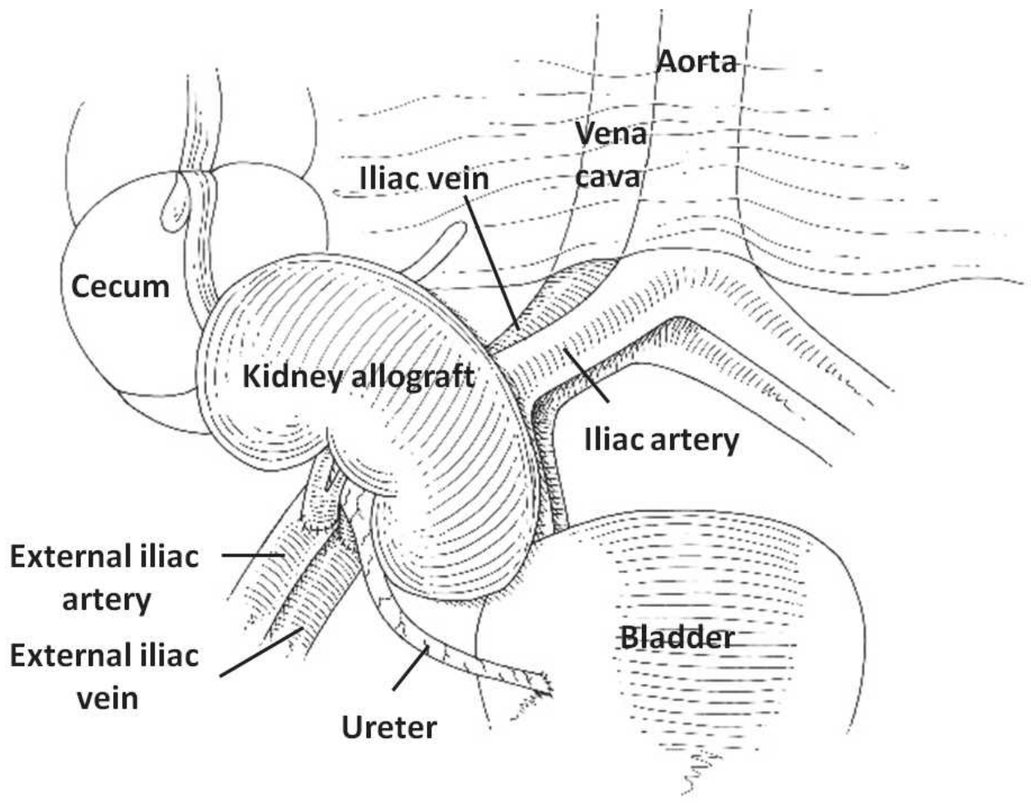


FIGURE 10.28 Anatomic relationships of the kidney. (Adapted from O'Neill WC. *Atlas of Renal Ultrasonography*. Philadelphia: WB Saunders; 2011, with permission.)

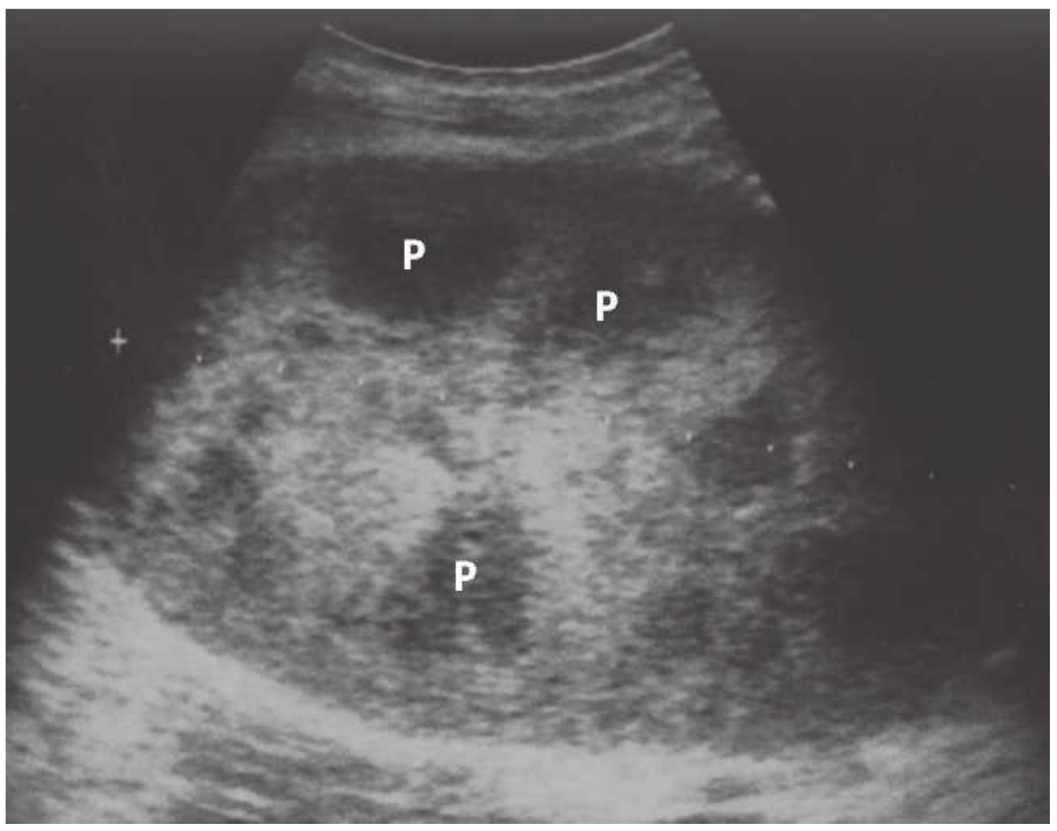


FIGURE 10.29 Acute rejection, longitudinal scan. The kidney is swollen and globular and the cortex is brightly echogenic, rendering medullary pyramids (*P*) very prominent.

beaklike projection over the allograft, and is easily identified by the peristalsis of the bowel loops.

Sonography of the transplanted kidney is indicated in most cases of acute renal failure[80] and can easily diagnose thrombosis of the renal artery or vein, urinary obstruction, and urine leaks in the immediate postoperative period. Other, nonspecific findings can suggest acute rejection. Sonography can be helpful in guiding percutaneous biopsy, diagnosing fluid collections, measuring residual bladder volume, and identifying ureteral stents.

Allograft Parenchymal Disease

The visualization of renal allografts is easier than native kidneys but suffers from the inability to evaluate two important parameters: renal size and echogenicity. Measurement of renal length is problematic due to the frequent inability to capture the entire length of the kidney in one view and the uncertainty of its significance due to both donor-dependent and recipient-dependent variables. Renal size can increase up to 40% during the 6 first months after transplantation.[81–83] Evaluation of echogenicity is difficult due to the lack of an adjacent reference organ. The only useful clues are prominence of the medullary pyramids and, when echogenicity is markedly increased, blending of the allograft with the surrounding tissue. Because of these limitations and also the simplicity of allograft kidney biopsy, size and echogenicity are usually not considered in clinical decisions.

The most common causes of allograft failure are acute tubular necrosis (related to harvesting or storage), acute rejection, drug toxicity, chronic allograft nephropathy, and recurrent disease, but sonographic findings lack sensitivity and specificity in diagnosing any of these. In particular, kidneys often appear normal in mild and even moderate cases of rejection. When present, findings consist of cortical swelling and increased echogenicity (due to cellular infiltration). Allograft enlargement is fairly specific for acute rejection[83–90] but can occur with ATN and recurrent nephritis (Fig. 10.29). Infections (pyelonephritis and BK virus) can also cause allograft failure but sonographic findings are nonspecific.

Urinary Obstruction of the Allograft

The frequent occurrence of urinary tract obstruction is the principal reason for performing sonography in most transplant patients presenting with acute renal failure. In the immediate postoperative period, the ureter may be obstructed by intraluminal blood clots, kinking, or external compression by edema or hematomas. Urinary retention, lymphoceles, and ureteral strictures are responsible for most other cases,[91,92] but are rarely seen when ureteral stents are routinely placed during transplantation. Acute rejection within the ureter may also obstruct urine flow.[93,94] Ultrasonography is an excellent test to diagnose obstruction in allografts with a sensitivity of almost 100%.[95] Specificity is lower because hydronephrosis is not always an indication of obstruction because small degrees of calyceal dilatation are easily and commonly seen in otherwise normal allografts. Mild, and even moderate, dilatation may not be a manifestation of urinary obstruction,[92,96] but dilatation of the minor calyces and ureter are usually indicative of obstruction (Fig. 10.30). Sonography is also very useful in pinpointing the site of obstruction. Hydronephrosis without dilatation of the proximal ureter indicates obstruction at the ureteropelvic junction, which may be caused by extrinsic compression by a lymphocele or ureteral strictures.[97] In both cases, there is usually abrupt tapering of the renal pelvis. A common site for strictures is in the distal segment or near the anastomosis

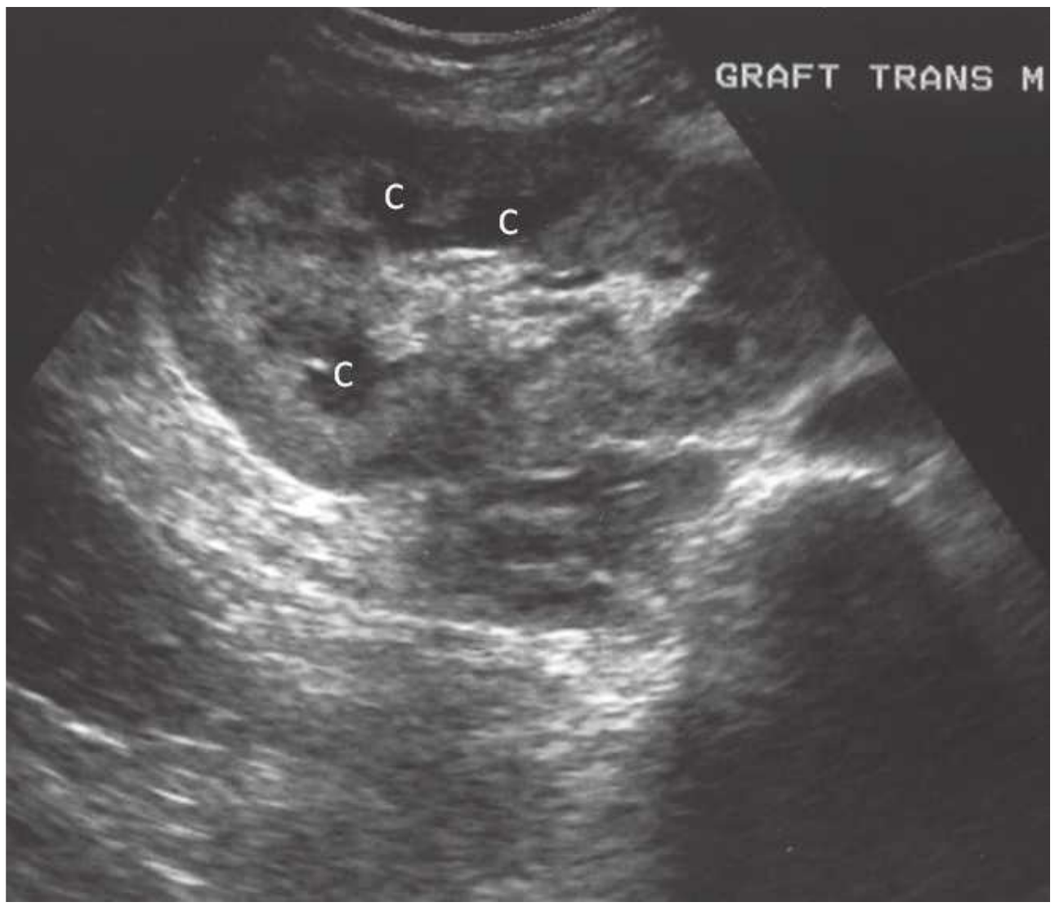


FIGURE 10.30 Mild hydronephrosis of the allograft kidney. Transverse view of the allograft shows mild dilatation of the calyces (*C*), consistent with mild hydronephrosis.

with the bladder.[91,97] In this setting the dilated ureter can be followed to the bladder and remains dilated when the bladder is emptied.

Thickening of the calyceal or ureteral walls is not uncommon and can be mistaken for luminal dilatation. This is usually caused by edema (Fig. 10.31) and has a fine echo pattern as opposed to anechoic urine. A fine central line presumably representing apposition of the luminal surfaces is occasionally seen.[98] Edema is frequently associated with stents and may also be caused by ureteral rejection.[93] Calyceal thickening was initially thought to be a sign of acute rejection but is now known to be nonspecific.[99,100] Additional causes of echogenicity within the collecting system are stents, hemorrhage, stones, and infection.

Fluid Collections and the Kidney Allograft

Fluid collections consisting of blood, urine, or lymph can be complications of transplant surgery.[95] Sonography cannot distinguish different types of fluid but the location, shape, internal structure, and the clinical presentation can all help in making the correct diagnosis. Additional studies such as examination of aspirated fluid and pyelography or radionuclide scanning to detect extravasation of urine may be needed. Care must be taken to distinguish fluid collections from the bladder or ascites.

Lymphoceles are the most common fluid collections encountered near renal allografts, with a frequency as high as 20%.[91,101] Half occur in the first 10 months after surgery but they can occur as late as 4 years.[101] They are usually not diagnosed unless they compress the ureter or the iliac vein and spontaneous resolution is a common feature.[101] In general, two types of lymphoceles are observed, consistent with different origins.[91,92,101] Most commonly they arise from donor lymphatics and are seen immediately adjacent to the allograft and have a propensity to obstruct the proximal ureter. They often appear as triangular-shaped fluid collections adjacent to the normal pelvis (Fig. 10.32) but can attain any shape and even surround the allograft when large. Less commonly, lymphoceles arise from native lymphatics disrupted during anastomosis of the donor vessels. They are in proximity to the iliac vein and have a tendency to obstruct the venous drainage of the leg, producing ipsilateral edema.[92,102]

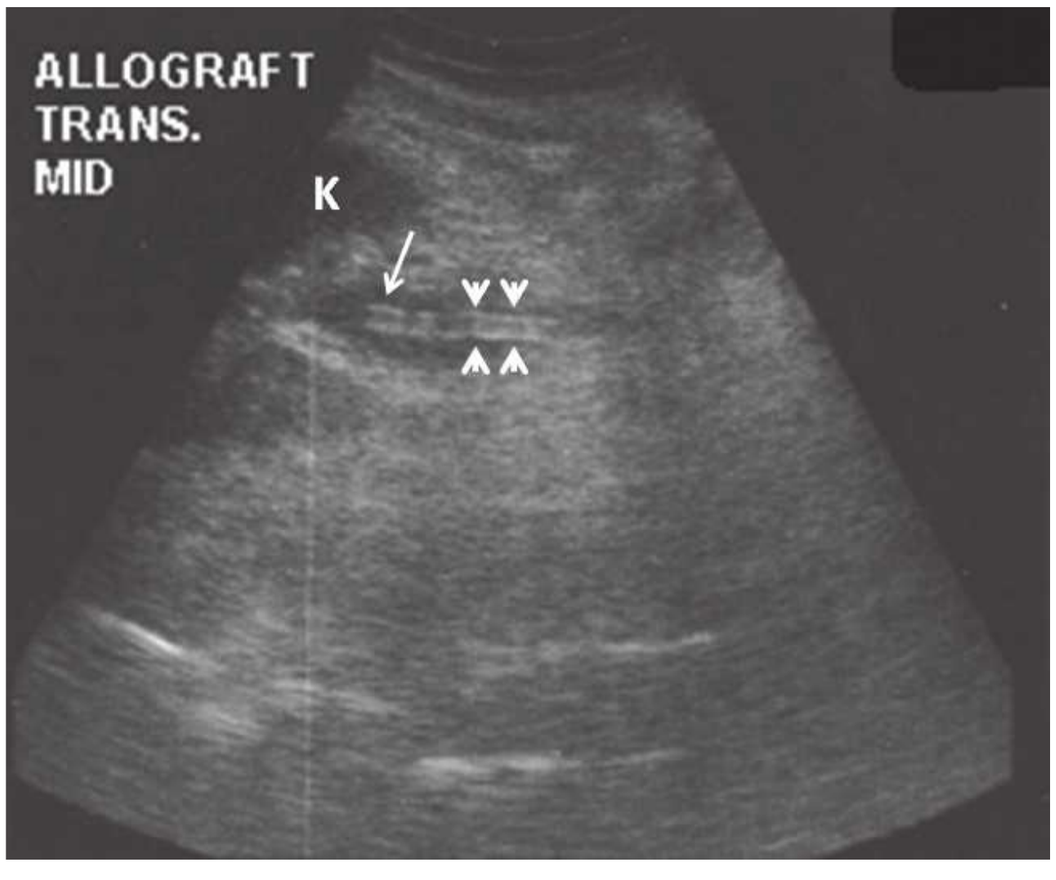


FIGURE 10.31 Urinary leak and ureteral stent. Transverse view of the allograft kidney (*K*) shows a small urine leak (*arrow*) surrounding a ureteral stent (*arrowheads*), medial to the allograft.

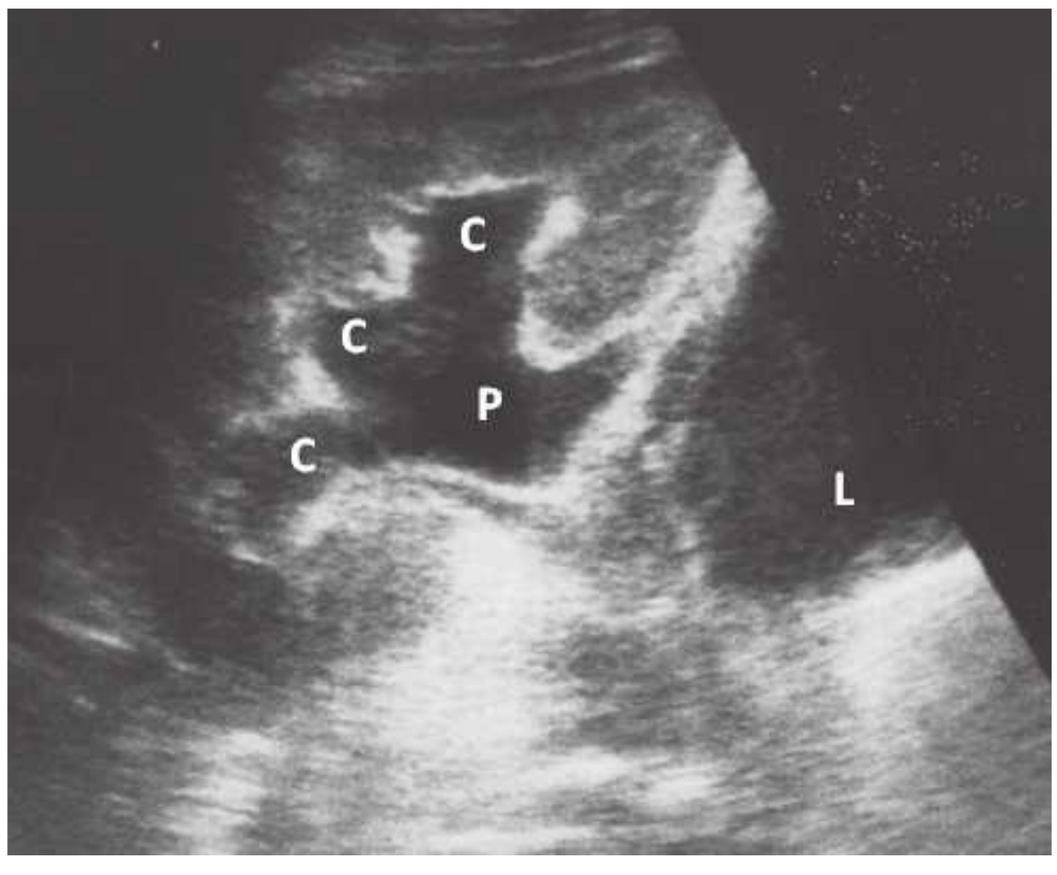


FIGURE 10.32 Large lymphocele with hydronephrosis. The lymphocele (*L*) presents as a hypoechoic collection medially situated vis-à-vis the allograft and causing obstruction of the ureter resulting in dilatation of calyces (*C*) and renal pelvis (*P*).

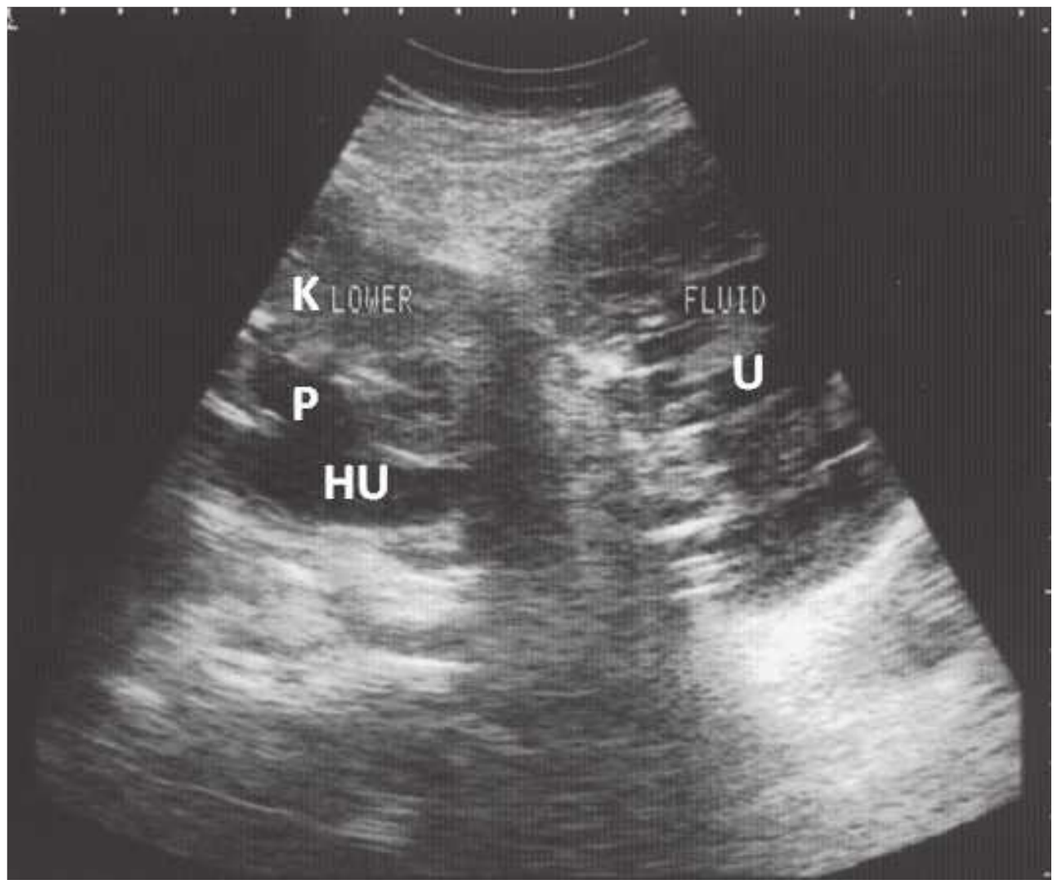


FIGURE 10.33 Large urinoma, longitudinal scan. The urinoma (*U*) appears as a large, complex fluid collection medial to the allograft (*K*). Hydroureter (*HU*) and renal pelvis dilatation (*P*) are common with urine leaks.

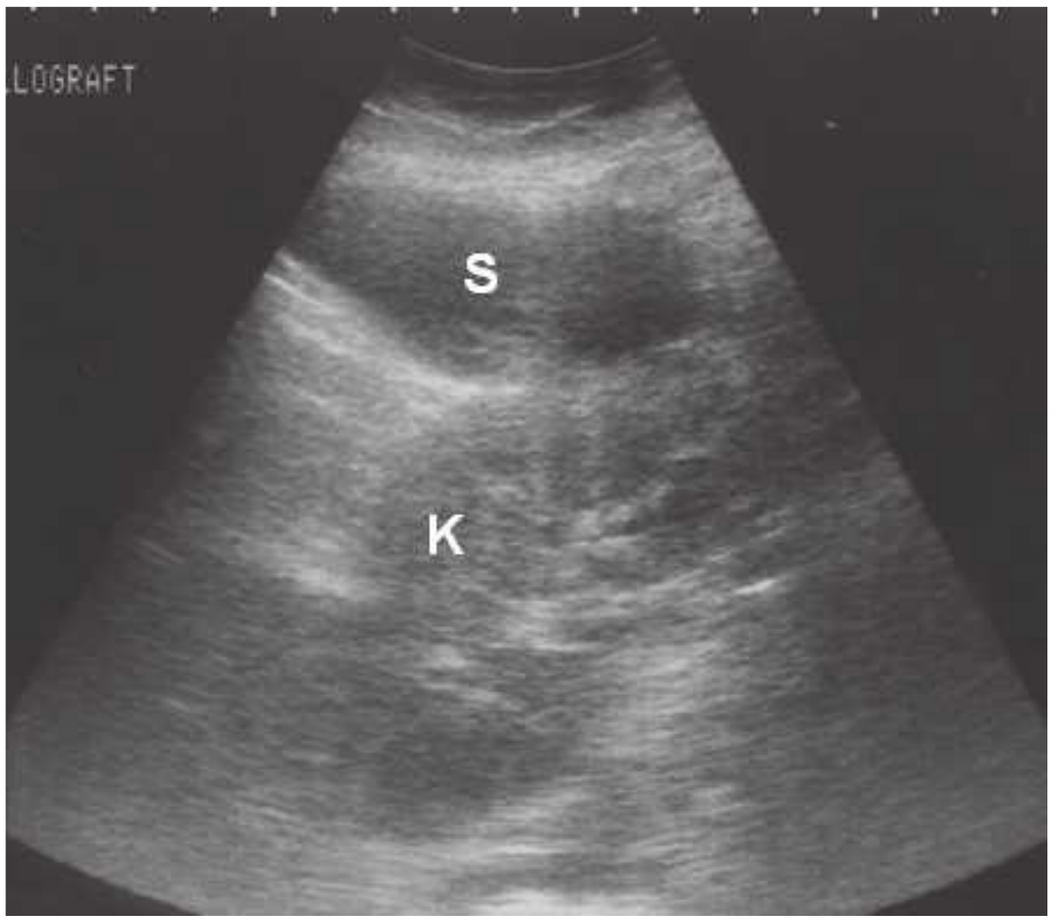


FIGURE 10.34 Seroma (*S*) appears as a complex fluid collection containing echogenic material in the subcutaneous tissue anterior to the allograft (*K*).

Urinomas, arising from urine leaks, need to be considered when fluid collections are seen within several weeks after surgery.[91] Tenderness and fever occur in one half and one quarter of patients, respectively,[103] but acute renal failure or delayed graft function can also be seen. Urinomas are usually adjacent to the ureter, but they can dissect along tissue planes and form seemingly separate collections, usually medial or anterior to the allograft and even surrounding it (Fig. 10.33). The appearance can be identical to that of a lymphocele, but often the margins are irregular and indistinct. Dilatation of the collecting system proximal to the leak is common.[102,103]

Hematomas are commonly seen in sonograms performed in the first few weeks after transplantation[91,92] but can also be seen after percutaneous biopsy. The typical appearance is of a heterogeneous mass containing both liquid (anechoic) and solid (echogenic) components. Echogenicity may increase with the level of organization of the clot.

Seromas are related fluid collections that are extremely common in the immediate postoperative period. These serosanguineous collections usually follow tissue planes anterior to the allograft and thus appear linear, often with septations (Fig. 10.34). They generally have little clinical significance and resolve spontaneously, and are important only in the differential diagnosis of other types of fluid collections.

Doppler Ultrasonography of the Kidneys

Doppler ultrasound measures the shift in frequency when sound strikes a moving object. This shift is dependent on the angle of the sound beam relative to the direction of flow, so that it is maximal when the sound is parallel to the flow and absent when the sound is perpendicular. In practice, the scanning angle should ideally be no greater than 60 degrees because large errors in velocity occur above this. This becomes critically important in evaluating the renal artery. The Doppler signal can be displayed in several formats. A small region can be selected (e.g., the lumen of a blood vessel) and velocity versus time displayed as a graph (pulse wave Doppler). Alternatively, a larger region can be selected and the velocities displayed on a color scale superimposed on the B-mode image (color flow Doppler). These modes can be combined (duplex Doppler). Lastly, the signal can be displayed in a vectorless form (power Doppler) where the color scale indicates the speed but not the direction. This last mode is useful for demonstrating the presence or absence of flow, and has the advantage of being less dependent on the angle of insonation.

In the kidney, Doppler sonography is used primarily to determine whether masses are vascular and to diagnose vascular disorders, particularly renal artery stenosis. The main renal artery and vein branch into segmental (also called interlobular) vessels at the renal hilum, which is located just outside of the medial aspect of the mid kidney. The segmental vessels travel through the renal sinus directly to the parenchyma, usually without further branching. They course through the columns of Bertin between the medullary pyramids, branching into the arcuate vessels at the corticomedullary junction. Evaluation of the renal vasculature usually consists of two separate examinations: (1) direct visualization and measurement of velocity in the renal artery or vein; and (2) analysis of wave forms in intrarenal arteries.

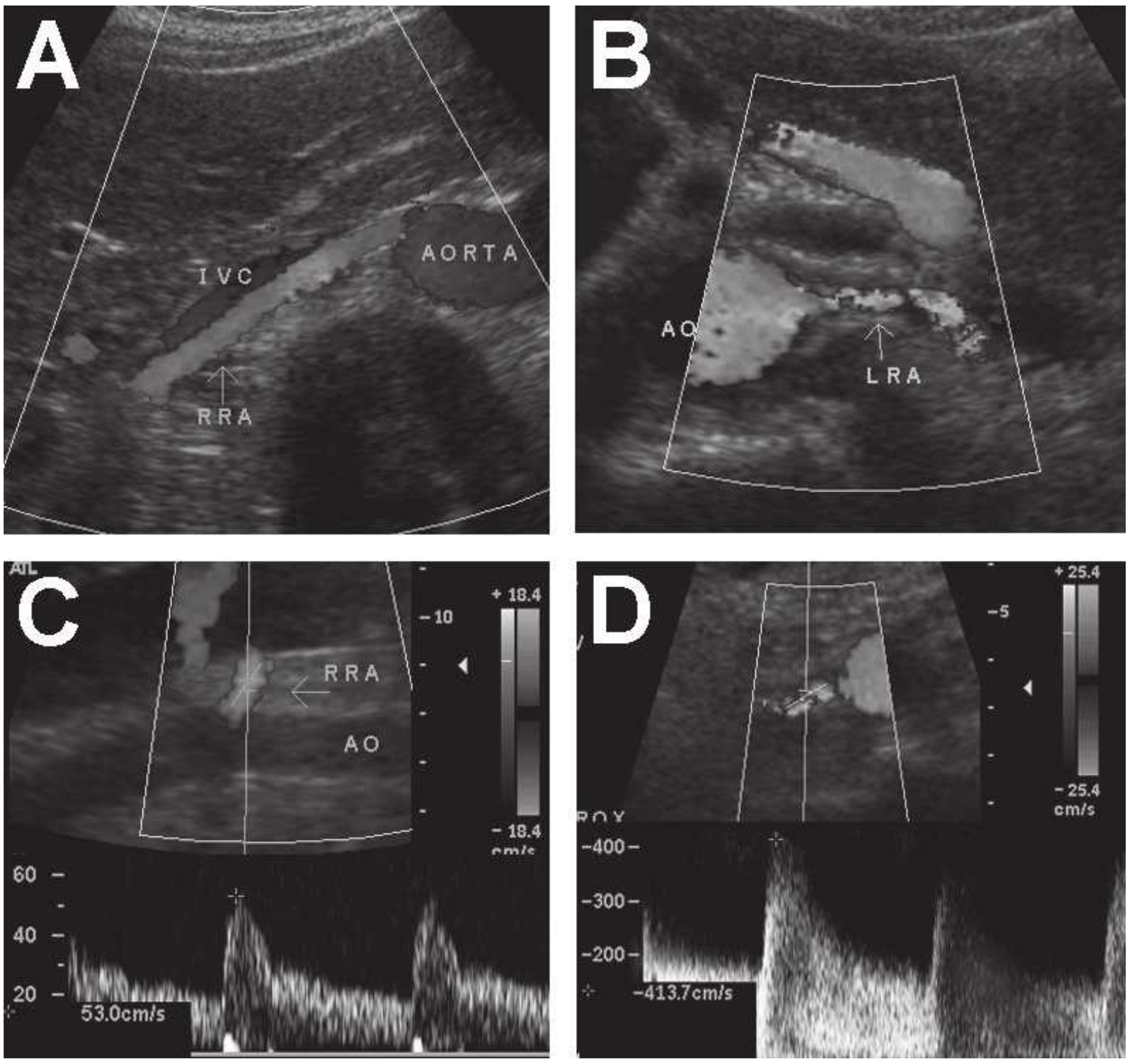


FIGURE 10.35 Renal artery Doppler. **A:** Normal right renal artery, midline transverse image. **B:** Stenotic left renal artery, midline transverse image. Note the turbulent flow (speckled color pattern) and the poststenotic dilatation. **C:** Normal right renal artery, coronal view, with a peak systolic velocity of 53 m per second. **D:** Stenotic right renal artery, midline transverse view. The peak systolic velocity averages well over 300 m per second. (Images courtesy of Sue Zellman, RDMS, RVT.)

Renal Artery Doppler

Examination of the renal artery is usually performed to rule out stenosis but should be reserved for patients with a high index of suspicion due to the poor performance of Doppler ultrasound. This can be a challenging examination in many patients because of overlying bowel gas, difficulty in achieving an angle less than 60 degrees, and the presence of multiple renal arteries in 30% of individuals. As many as a third of examinations may be inadequate.[104] The artery is examined by color flow Doppler from the aorta to the renal hilum, looking for narrowing, poststenotic dilatation, and turbulent, high velocity flow (Fig. 10.35). The diagnosis of significant renal artery stenosis is suggested by peak blood velocities greater than 2.0 meters per second (Fig. 10.36), and/or a ratio of peak systolic velocities in the renal artery and aorta above 3.5.

Intrarenal Arteries

Examination of the intrarenal arteries (segmental or interlobular) is limited to analysis of the waveforms, which can provide information on blood flow in the larger vessels. In renal artery stenosis, systolic flow can be dampened, leading to a delay in the peak flow (Fig. 10.36). In renal vein thrombosis, increased distal resistance can reduce diastolic flow. There may even be reversal of diastolic flow, which is virtually pathognomonic for renal vein thrombosis. Because most waveform analysis is based on indices (one portion of the wave compared to another) or on time, rather than on absolute velocity, it is independent of the angle of insonation. It is therefore a far easier study to perform but the evidence obtained is indirect.

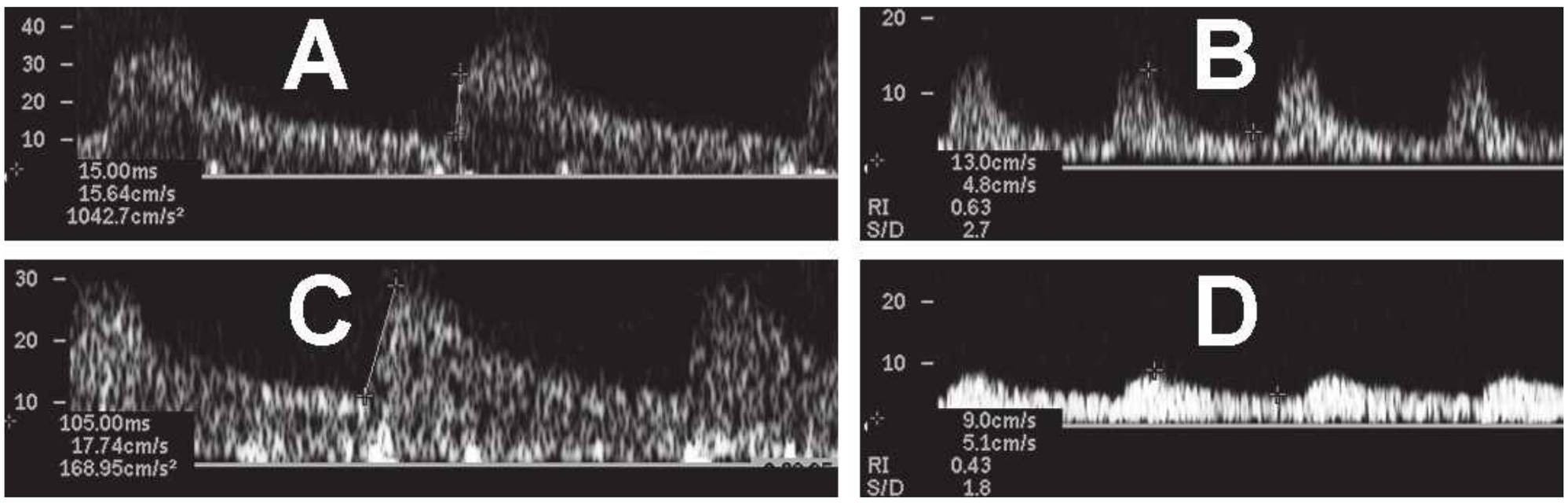


FIGURE 10.36 Intrarenal Doppler waveforms. **A:** Normal segmental artery with maximum systolic acceleration of approximately 1000 m per sec^2. **B:** Normal intraparenchymal artery. **C:** Segmental artery with maximum systolic acceleration less than 200 m per sec^2, indicative of renal artery stenosis. **D:** Intraparenchymal artery showing marked delay and blunting (tardus-parvus) of the systolic peak consistent with severe renal artery stenosis. (Images courtesy of Sue Zellman, RDMS, RVT.)

Renal Artery Stenosis

A number of Doppler parameters have been applied to the diagnosis of renal artery stenosis including direct measurements and indices of velocity and acceleration, indices of resistance and pulsatility, or combinations of these.[105] Although good results have been published using renal artery Doppler as a screening test for renal artery stenosis, a large meta-analysis showed that CT angiography and MR angiography performed significantly better.[106]

Parenchymal Disease

Much attention has been focused on duplex Doppler as a diagnostic tool for renal disease but the findings are nonspecific. Resistive index has been proposed as a parameter to predict outcomes after revascularization of stenotic kidneys,[107] but this approach is flawed because the resistive index is influenced by systemic parameters such as heart rate and pulse wave velocity.[108,109] Doppler indices are of no utility in the diagnosis of acute rejection in transplanted kidneys.[110,111]

Renal Vein Doppler

The main renal veins are often visible in adults and can be prominent in children or young adults, in patients with heart failure, and in inflamed kidneys. The right renal vein tracks directly from the inferior vena cava to the right kidney whereas the left renal vein has a longer course, passing just underneath the origin of the superior mesenteric artery. Renal vein thrombosis presents as enlarged, echogenic kidneys with clot sometimes visible within the lumen of the renal vein, best detected by power Doppler. When limited to the intrarenal vessels, the thrombus may not be visible. Duplex Doppler may reveal absent flow in the renal vein although this can be difficult to determine due to the lack of pulsatility in venous flow.

The Role of the Nephrologist

Because sonography is the most common imaging modality in patients with kidney disease and is essential to their management, nephrologists should consider incorporating this tool into their practice. The simplicity, the low cost and portability of the equipment, and the availability of training all make this a realistic goal. Certification in the performance and interpretation of renal sonograms can be obtained from the American Society of Diagnostic and Interventional Nephrology (www.ASDIN.org). Even if they are not performing the procedure themselves, nephrologists must have a comprehensive understanding of sonography of the kidneys and the urinary tract because interpretation frequently requires clinical input and correlation.

NUCLEAR MEDICINE IMAGING OF THE KIDNEYS

Radionuclide renal scintigraphy provides important functional data to assist in the diagnosis and management of patients with a variety of suspected genitourinary problems. Requesting a renal scan, however, is not always straightforward because there are five different renal radiopharmaceuticals and several imaging protocols. Renal scintigraphy is also complicated by the fact that there can be marked differences in scan quality at different institutions despite using the same radiopharmaceutical, the same equipment, and identical billing. To ensure that the patient receives the most appropriate study, the clinical question must be clearly specified. In addition, referring physicians should be familiar with the elements that make up a quality study, the different procedures and their limitations, the principal radiopharmaceuticals and the rationale for their use, as well as the terminology and quantitative parameters often included in the report. To achieve these goals, this section describes the available radiopharmaceuticals, the basic renal scan, and the quantitative indices used to interpret the study; it also reviews clearance measurements, the primary scan indications (Table 10.2), essential information needed by patients, radiation exposure, and includes a short discussion of renal scan applications in selected clinical settings.

10.2 Scans and Primary Clinical Indications

I. Basic Renogram
- To assess renal function and urodynamics
- To determine the percent of total renal function contributed by each kidney

II. Diuresis Renogram
- To diagnose or exclude urinary tract obstruction

III. ACE Inhibition (RVH or Captopril) Renogram
- To diagnose or exclude renovascular hypertension

IV. Renal Transplant Scintigraphy
- To evaluate arterial flow and function
- To help diagnose rejection and acute tubular necrosis
- To detect urinary leak, infarct, or outflow obstruction

V. Renal Cortical Scintigraphy
- To detect or exclude pyelonephritis
- To determine the percent of the total renal function contributed by each kidney

VI. Radionuclide Cystography
- To detect, quantitate, and monitor reflux
- To evaluate asymptomatic siblings of children with reflux

ACE, angiotensin-converting enzyme; RVH, renovascular hypertension.

Radiopharmaceuticals

The radiopharmaceuticals available for assessment of renal function and anatomy can be grouped into three broad categories: radiopharmaceuticals filtered by the glomerulus, tracers primarily secreted by the renal tubules, and those retained in the renal tubules for long periods of time.

^{99m}Tc-DTPA (Glomerular Filtration)

^{99m}Tc-DTPA (technetium-99m diethylenetriamine pentaacetic acid) is the only renal radiopharmaceutical available for routine imaging that is purely filtered by the glomerulus; consequently, it is the only imaging radiopharmaceutical that can be used to measure glomerular filtration rate (GFR).[112] In normal subjects, the extraction fraction of DTPA (the percentage of the tracer extracted with each pass through the kidney) is approximately 20%; this extraction fraction is relatively low compared to the extraction fraction of tubular tracers (40%–50%).

^{51}Cr-EDTA (Glomerular Filtration)

^{51}Cr-EDTA (chromium-51 ethylenediamine-tetraacetic acid) is used to measure GFR.[112] Chromium-51 does not emit enough photons to be used for imaging and the tracer is not available in the United States.

^{125}I-iothalamate (Glomerular Filtration)

^{125}I-iothalamate is used to measure GFR.[112] Iodine-125 does not emit a photon of sufficient energy to be useful for renal imaging.

^{99m}Tc-MDP (Glomerular Filtration)

^{99m}Tc-MDP (methylene diphosphonate) is a bone imaging tracer but it is cleared by glomerular filtration. Measurements of relative renal uptake (relative GFR) can be obtained at the time of a bone scan using standard renal software.

^{123}I- and ^{131}I-OIH (Tubular Secretion)

Iodine-123 and iodine-131 orthoiodohippurate (OIH) are cleared primarily by the proximal tubules although a small component is filtered by the glomeruli. The clearance of OIH is approximately 500–600 mL per minute in subjects with normal kidneys; this clearance is often described as the effective renal plasma flow (ERPF).[113] There is a common misconception that the ERPF (OIH clearance) is equivalent to renal plasma flow or, at least, proportional to renal plasma flow. The clearance of OIH and the clearance of ^{99m}Tc tubular tracers have two components: (1) delivery to the kidney (renal plasma flow) and (2) extraction from the plasma and transport to the tubular lumen (tubular function). These parameters do not always change in a proportional fashion. Because of poor imaging characteristics, the potential for delivering a high radiation dose, and/or unfavorable logistics, ^{123}I- and ^{131}I-OIH are no longer commercially available in the United States.[114,115]

^{99m}Tc-MAG3 (Tubular Secretion)

^{99m}Tc-mercaptoacetyltriglycine (MAG3) is highly protein-bound and is removed from the plasma almost exclusively by the organic anion transporter 1 (OAT1) located on the basolateral membrane of the proximal renal tubules.[113,116,117] The extraction fraction is 40% to 50%,[116] more than twice that of DTPA. Because of its more efficient extraction, MAG3 is preferred over DTPA in patients with suspected obstruction and impaired renal function and is used in approximately 70% of the renal scans performed in the United States.[118–122]

The MAG3 clearance averages about 320 mL/min/1.73 m^2 in adults under age 40 and decreases by approximately 1% per year after age 40.[123,124] The clearance of MAG3 is highly correlated with the clearance of OIH, and MAG3 clearance can be used as an independent measure of renal function.[112,113]

^{99m}Tc-L,L and D,D-EC (Tubular Secretion)

^{99m}Tc-L,L and D,D ethylene dicysteine (EC) are enantiomers—both are excellent renal radiopharmaceuticals with clearances

slightly higher than MAG3.[125,126] Although D,D-EC is cleared more rapidly than LL-EC,[127] LL-EC was first described and is available in several countries as a kit formulation.

^{99m}Tc-DMSA (Cortical Retention)

^{99m}Tc-DMSA (dimercaptosuccinic acid) is an excellent cortical imaging agent that is used primarily in pediatrics to evaluate relative function, pyelonephritis, and renal scarring.[128,129] Approximately 40% of the injected dose is retained by the renal tubules within 1 hour after injection; the remainder is slowly excreted in the urine over the subsequent 24 hours.

^{99m}Tc-GH (Cortical Retention and GFR)

^{99m}Tc-GH (glucoheptonate) is cleared both by glomerular filtration and tubular secretion. Most of the dose is rapidly excreted but 10% to 15% of the injected dose remains in the renal tubules allowing delayed, high-resolution static images to be obtained. GH tends to be used if DMSA is unavailable.

Basic Renal Scan and Renogram Curve

The basic renal scan is performed by injecting 1 to 10 mCi (37–370 MBq) of ^{99m}Tc-MAG3 or ^{99m}Tc-DTPA into a peripheral vein with the patient supine. Images are acquired dynamically for 20 to 30 min and postvoid views of the kidneys and bladder are often obtained at the conclusion of the study. Images are usually displayed at 1-, 2-, or 3-minute intervals as the radioactive tracer is removed from the blood, transits the kidney, and enters the bladder (Fig. 10.37A). Data are recorded on the computer for subsequent analysis, and renogram curves are obtained by placing a region of interest (ROI) over each kidney and generating time-activity curves for each ROI from the time of injection to end of the study.

Quantitative Indices

The more common quantitative indices and their clinical relevance are discussed here.

1. Relative function: The relative uptake of the radiopharmaceutical provides a measure of differential renal function (the specific function depends on the radiopharmaceutical) and should be reported. For MAG3 and DTPA, the measurement is usually made by placing an ROI over each kidney and measuring the radioactivity during the 1 to 2, 1 to 2.5, or 2 to 3 minute period postinjection. Because of radiotracer present in blood and the interstitial space of the kidney, as well as in the tissues anterior and posterior to the kidney, a background correction needs to be performed to correct for nonrenal counts present in the renal region of interest. Automated perirenal backgrounds (see Fig. 10.37A,B) and C-shaped backgrounds are preferred over background regions placed inferior to the kidney.[119,130] The 95% confidence interval for the relative uptake of MAG3 in the presence of normal renal function ranges from 42% to 58%.[123]
2. GFR, ERPF, and MAG3 clearance measurements: Measurement of GFR, effective renal plasma flow (ERPF), or MAG3 clearance may be obtained, depending on available software, protocols, and expertise (see discussion of clearance measurements).
3. Whole kidney versus cortical ROIs: The whole kidney ROI consists of an ROI placed around the entire kidney including the renal pelvis. Quantitative values generated using this ROI will be affected by retention of tracer in both the kidney parenchyma and renal pelvis. Thus retention may be due to pathologic states such as diabetic nephropathy or obstruction but may also occur in nonpathologic states such as a nonobstructed dilated collecting system or mild dehydration. To obtain a better assessment of parenchymal function, ROIs can be limited to the renal cortex (parenchyma), thereby excluding any activity retained in the pelvis or calyces (Fig. 10.37).
4. Time-to- peak or T_{max}: The time-to-peak or T_{max} simply refers to the time from radiopharmaceutical injection to the peak height of the renogram curve. MAG3 and DTPA renograms normally peak by 5 minutes and decline to half-peak height by 15 minutes postinjection; however, physiologic retention of the tracer in the renal calyces or pelvis can alter the shape of the whole kidney renogram curve in normal kidneys and lead to prolonged values for the time-to-peak, 20 min/max count ratio, and T1/2.
5. The T1/2: The T1/2 refers to the time it takes for the activity in the kidney to fall to 50% of its maximum value (see suspected obstruction).
6. Postvoid kidney counts/maximum or 1 to 2 minute kidney counts: In evaluating suspected obstruction, simple ratios that incorporate gravity-facilitated drainage from the kidneys such as counts in the postvoid kidney divided by the maximum counts in the kidney or counts at 1 to 2 minutes appear to provide more robust measurements of drainage than the T1/2.[131–134]
7. 20 min/max count ratio: The 20 min/max count ratio is the ratio of the kidney counts at 20 minutes to the maximum (peak) counts; this measurement provides an index of the transit time and parenchymal function and is often obtained for both whole kidney and cortical (parenchymal) ROIs. For MAG3, the normal 20 min/max count ratio for cortical ROIs averages 0.19 with standard deviations of 0.07 and 0.04 for the right and left kidneys, respectively.[123] If the patient is not dehydrated and the 20 min/max count ratio for the cortical ROI exceeds 0.35 (greater than two to three standard deviations above the mean), the kidney is likely to be abnormal. With the tubular agents, this index can be useful in monitoring patients with suspected urinary tract obstruction and renovascular hypertension.[135–137]

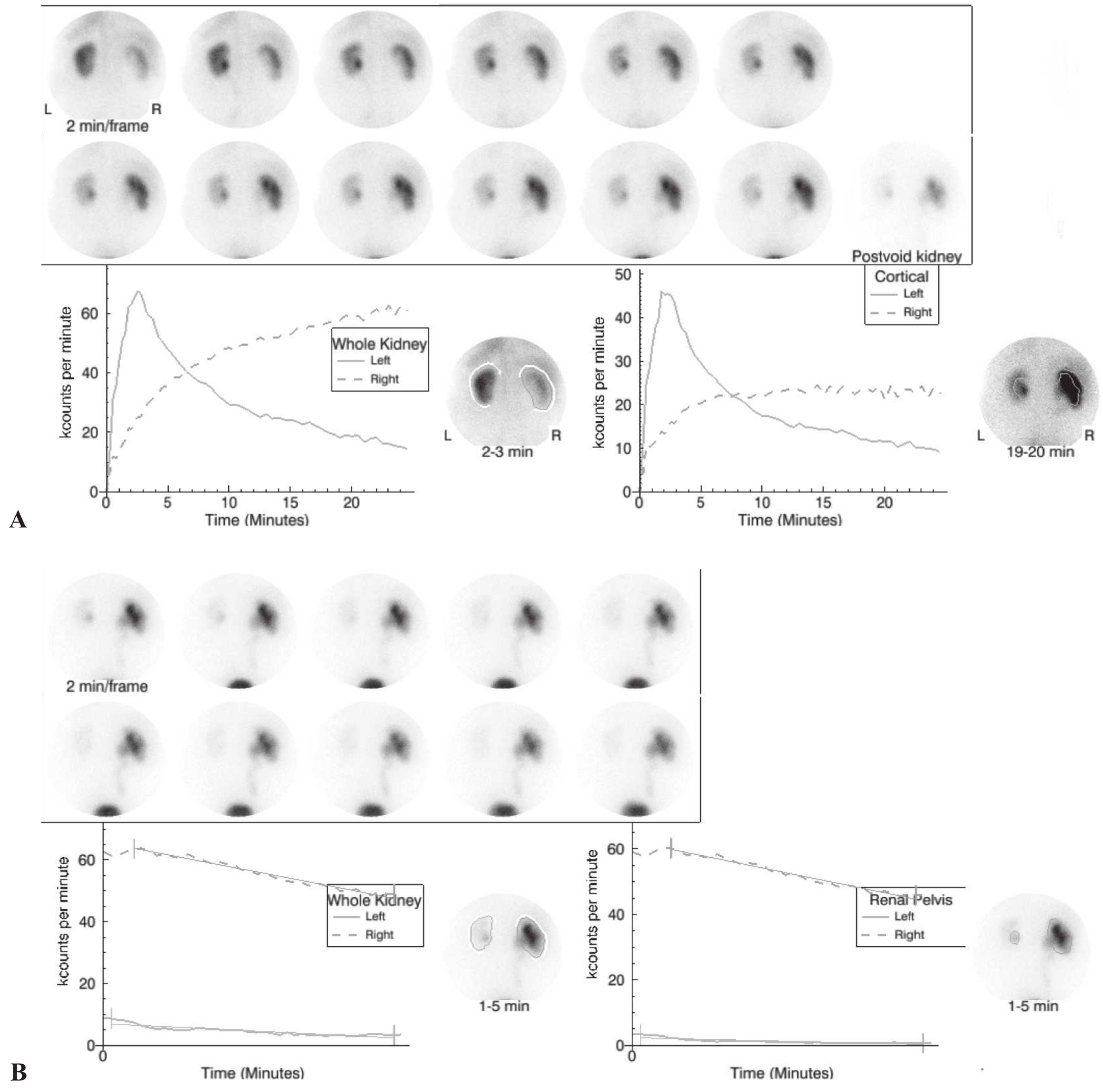


FIGURE 10.37 A 70-year-old man was referred for a renal scan because of suspected obstruction of the right kidney. The patient received an intravenous injection of 9.2 mCi (340 MBq) of ^{99m}Tc MAG3. **A:** The upper panel shows sequential 2-minute MAG3 images in a posterior projection; the final image in the series is a postvoid image. The lower left panel shows the whole kidney renogram curves (*blue*, left kidney; *green*, right kidney); the lower right panel shows cortical renogram curves. The relative uptake was 74% in the left kidney, 24% in the right kidney; the MAG3 clearance was 231 mL/min/1.73 m^2. The images and renogram curve for the left kidney are normal. There is retention of the MAG3 in the right renal collecting system with a rising renogram curve. **B:** To further investigate the possibility of obstruction in the right kidney, the patient received an intravenous injection of 40 mg furosemide followed by an additional 20 minutes of imaging. The upper panel shows sequential 2-minute images following furosemide administration. The lower left panel shows the whole kidney renogram curve and the lower right panel shows the renogram curve with the regions of interest placed around the retained tracer in the collecting system. There is very little MAG3 remaining in the left kidney (*blue*); the post-furosemide images show continuing retention in the right renal collecting system with very slow washout (T1/2 = 36 minutes for the region of interest over the collecting system) consistent with obstruction. (See Color Plate.)

8. Residual urine volume: Residual urine volume can be measured based on the counts in pre- and postvoid ROIs over the bladder and a measurement of the voided volume.[138] This is a relatively easy measurement to perform, is routine at some institutions, and may detect unsuspected urinary retention. The main source of error is tracer remaining in the renal pelvis which subsequently drains into the bladder after voiding; this problem can be detected by looking at the last kidney image in the study.
9. Urine flow rate: The rate of urine flow can be useful in assessing the adequacy of hydration and the adequacy of diuresis following furosemide administration. Assuming a constant postvoid volume, urine flow

rate can be calculated by dividing the voided volume at the conclusion of the study by the time interval between voiding prior to and at the conclusion of the study.

Renal Function and Clearance Measurements

Because of inaccuracies associated with estimation of GFR based on serum creatinine or creatinine clearance, a more accurate measurement of renal function may be needed in patients at the extremes of age and body mass and in patients with severe malnutrition, grossly abnormal muscle mass (amputation, paralysis), high or low intake of creatinine or creatine (vegetarian diet, dietary supplements), rapidly changing renal function, prior to kidney donation, and prior to dosing with toxic drugs excreted by the kidney. Measurement of renal function at the time of the scan can aid in the interpretation of a radionuclide study, provide a measurement of renal function independent of serum creatinine, and serve as a baseline for monitoring changes. The two most widely used techniques to measure clearances are plasma sampling and camera-based techniques.

Multisample Plasma Clearances. The gold standard for measuring renal clearances is a constant infusion technique where the plasma concentration of the tracer remains constant.[112] A variant of this technique uses a subcutaneous injection; however, both of these methods are cumbersome, time consuming, expensive, and limited to research applications. An alternative approach that does not require urine collections is the plasma clearance obtained using the single injection, two-compartment model.[139] For tubular tracers, the plasma disappearance curve can be determined from multiple blood samples obtained over 90 minutes. Because GFR tracers have a much lower extraction fraction and a lower clearance, plasma samples typically need to be obtained for 4 hours to adequately characterize the curve.

Single and Dual Plasma Sample Clearances. Simplified techniques based on one or two plasma samples have been developed to estimate the multisample clearance.[112] The MAG3 clearance can be estimated from the dose injected and the amount of radioactivity in a single blood sample obtained approximately 45 minutes after injection[112,124,140] and the GFR can be estimated from the dose injected and the activity in one or two plasma samples obtained 1 to 4 hours after injection.[112,141–143] These techniques provide reliable results and can be performed at the time of a standard renal scan using MAG3 or DTPA.

Plasma Sample Techniques: Sources of Error and Availability. Plasma sampling techniques assume a normal volume of distribution. If a patient has ascites, marked edema, or a large effusion, the tracer can diffuse into these extra fluid spaces and the plasma clearance will not provide an accurate measure of renal function. Plasma sample clearances require meticulous technique and attention to detail. If the measurement is performed by a poorly trained or inexperienced individual, technical errors are often made and the results are spurious. Because of the need for specialized training, the necessity of handling plasma samples, extra effort, and lack of reimbursement, plasma sample clearances are not widely available in the United States.

Camera-Based Clearances. Camera clearance methods have been developed for DTPA, OIH, and MAG3 that do not require blood or urine samples.[141–145] The principle is based on the fact that the initial tracer accumulation by the kidneys is proportional to the clearance. Camera-based techniques determine the tracer accumulation (counts) in the kidneys at a defined period shortly after injection and divided by the counts injected to obtain a percent injected dose in the kidneys. The percent injected dose is converted to a clearance measurement using a validated nomogram.[145]

Camera-based clearances appear to be reproducible and have been reported to be superior to creatinine clearance for monitoring changes in renal function.[146] Although camera-based clearances are considered to be less accurate than plasma sample clearances,[112] they avoid sources of error (timing of plasma samples, correction for radioactive decay, dilution of standards, pipetting small volumes) inherent in plasma sample techniques.

Camera-based Clearances: Sources of Error. To obtain the percent injected dose in each kidney, kidney counts have to be corrected for background, infiltration, attenuation, and renal depth. Two common sources of error are background subtraction and the estimation of renal depth. Because MAG3 is extracted more than twice as efficiently as DTPA, the kidney-to-background ratio will be much higher for MAG3 than DTPA and any potential error introduced by background subtraction will be minimized.

Renal depth is usually estimated from a nomogram based on height and weight.[147,148] To the degree that a population-derived nomogram fails to fit a particular individual, the clearance measurement will vary from the true clearance. The sources of error due to background, self-attenuation, and renal depth tend to be reflected in a wider confidence interval associated with the accuracy of camera-based clearance compared to plasma sample clearances; however, these parameters tend to be constant on sequential studies and have less effect on reproducibility. Commercial camera-based techniques are currently available for measuring GFR (DTPA), effective renal plasma flow (OIH), and the MAG3 clearance. In evaluating the reliability of commercial software, it is important to confirm that the vendor has (1) incorporated the appropriate quality control features and (2) provided citable validation studies to confirm that the software is performing as claimed.

General Patient Preparation and Information

Patients appreciate knowing what to expect when they are referred for an unfamiliar test. The following information should be communicated to the patient.

Procedure and Time Required for the Study

The patient will receive an intravenous injection of the radiopharmaceutical and will lie quietly on an imaging table for 20 to 30 minutes. Depending on the protocol, there may be two imaging sessions. Unlike radiographic contrast, there is essentially no risk of an allergic or anaphylactic reaction.

Hydration

The patient should be told to arrive well hydrated because good hydration minimizes the radiation dose to the bladder and facilitates interpretation of the exam.

Diet and Medication

For a basic renogram, there are no medication or dietary restrictions. Medication and dietary restriction for angiotensin-converting enzyme (ACE) inhibition renography are discussed later.

Radiation Exposure

Depending on the radiopharmaceutical and the amount administered, the radiation exposure from a typical MAG3 or DTPA scan ranges from 5% to 70% of the background radiation a patient receives each year from cosmic rays and naturally occurring radioactive sources in the environment; it is less than 5% of the yearly radiation dose considered safe for doctors and technologists who work with radiation. In patients with normal kidney function, over 95% of the radiation leaves the body by 3 hours. Patients can go to public places and use a bathroom without risk to others (see later text for a more detailed discussion of radiation).

Pregnancy

If the patient is pregnant or thinks she may be pregnant, she should discuss this possibility with the nuclear medicine physician prior to arrival for the test.

Radiation Exposure to the Nuclear Medicine Patient

Radiopharmaceuticals are designed to target specific organs, tumors, or pathways and to avoid others; consequently, they do not distribute uniformly throughout the body. Different tissues receive different exposures depending on the isotope, its half-life, its tissue distribution, and its retention time. For example, ^{99m}Tc has a half-life of about 6 hours; even if none of a ^{99m}Tc tracer was eliminated from the body after intravenous administration, the amount remaining in the body 24 hours later (four half-lives) would be only about 6% of the administered dose.

The concept of effective radiation dose has been developed to define a single quantity that could express the overall potential deleterious effect of radiation exposures. This concept allows the risk of the different radiation doses to different tissues throughout the body to be expressed in a single measurement. The effective radiation dose equivalent is the whole-body radiation dose that would have to be given to produce a risk equivalent to the sum of the risks from the individual radiation doses to the various organs. The effective dose from a MAG3 or DTPA scan ranges from 15 to 20 mrem (0.15–0.2 mSv) per millicurie injected. The typical administered dose is 1 to 10 millicuries (37–370 MBq); consequently, the effective dose for a renal scan ranges from 15 to 200 mrem (0.15–2.0 mSv).[115] For comparison, background radiation is estimated to be approximately 300 mrem (3.0 mSv) per year.

Suspected Obstruction (Diuresis Renography)

Obstruction to urinary outflow may lead to obstructive uropathy (dilatation of the calices, pelvis, or ureters) and obstructive nephropathy (damage to the parenchyma). Urine outflow obstruction may be suspected based on clinical findings, the incidental detection of a dilated renal collecting system, or diagnosis of previous obstruction in a patient referred for follow-up. Diuresis renography is noninvasive, widely available, and can evaluate renal function and urodynamics in a single test. This noninvasive test is based on a high endogenous rate of urine flow stimulated by the administration of furosemide. Interpretation of the test is based on the washout of the radiopharmaceutical from the collecting system in the upper urinary tract.

Acquisition Protocols, Radiopharmaceutical Choice, and the Timing of Furosemide

The patient should arrive well hydrated and void immediately prior to the examination because a full bladder may affect upper tract emptying and give false-positive results.[120] Adult and pediatric consensus groups recommend tubular agents for diuresis renography because tubular tracers are much more efficiently extracted by the kidney than DTPA.[120,122,134,149] Several protocols are available for diuretic renography that differ mainly in the timing of furosemide administration and in the use of a single acquisition or a baseline scan followed by post-furosemide acquisitions if the baseline scan does not exclude obstruction.[120,122] Typical times for furosemide administration are the F – 15, F = 0, and F + 20 protocols where the furosemide is administered 15 minutes before, simultaneously with, or 20 minutes after the tracer administration. Each protocol has its advocates but all are acceptable and appear to give comparable results in the majority of patients.[134,150–153]

Dose of Furosemide

Furosemide is secreted by the proximal tubule and reaches its site of action in the tubular lumen of the thick ascending loop of Henle via the tubular fluid.[154] Secretion of

furosemide is reduced in patients with impaired renal function; consequently, the standard 40-mg adult dose may not be sufficient to induce an adequate diuretic response in a kidney with moderate or severely impaired renal function and a larger dose of furosemide may be required.[154,155]

Diagnostic Criteria, the T1/2, Gravity-Assisted Drainage, and Postvoid Images

Drainage is often assessed quantitatively by measurement of the T1/2 following furosemide administration. Although the T1/2 calculated from an ROI placed around retained activity in the collecting system rather than around the whole kidney provides a better assessment of drainage, T1/2 measurements are not standardized and they vary depending on timing, ROI assignment, and method of calculation.[120,122,156,157] There is general agreement that prompt clearance of the radiopharmaceutical from the renal collecting system with a T1/2 under 10 minutes excludes obstruction. On the other hand, a prolonged T1/2 should never be the sole criterion for determining the presence of obstruction and must be interpreted in the context of the whole set of images, curves, and quantitative indices as well as any clinical information or diagnostic studies that may be available. Techniques that consider renal function, utilize gravity-assisted drainage, and incorporate postvoid images appear to provide more robust alternatives to the T1/2.[131,133,134,136,158,159]

False-Positive and Indeterminate Studies

A poor diuretic response due to dehydration or impaired renal function may result in false-positive or indeterminate findings. Measuring the urine flow rate alerts the nuclear medicine physician to an inadequate diuresis.

Relative Renal Function

Unless obstruction is acute, it usually causes a loss of function in the affected kidney. If the relative renal function is approximately the same in both kidneys in a patient with suspected unilateral obstruction, the likelihood of obstruction is reduced even if the quantitative data such as the T1/2 are abnormal. In these cases, it may be appropriate to observe the patient and repeat the study at a later date or to combine the study with sonography to determine if the size of the renal pelvis is increasing.

Does a Patient Presenting with Flank Pain have Acute Renal Colic? If so, Can He be Managed Conservatively?

Knowledge of the size of the obstructing calculus is important because calculi less than 5 mm generally pass spontaneously; as the size of the calculus increases, spontaneous passage becomes less likely. Unenhanced (noncontrast) helical CT (UHCT) has rapidly gained acceptance as the procedure of choice for patients presenting with acute renal colic. UHCT avoids the risk of contrast, which is particularly important for patients with renal insufficiency, diabetes, dehydration, or allergy to iodinated contrast agents; moreover, stone size can be accurately ascertained, and the correct diagnosis can be made in approximately 50% of patients whose symptoms are not caused by a renal stone.

Many calculi between 3 to 8 mm in size are followed conservatively in the hope of spontaneous passage, and patients may be managed on an outpatient basis. In spite of its advantages, UHCT cannot determine the functional status of the kidney. Larger stones (5–8 mm) may not be associated with high-grade obstruction and can be managed conservatively, whereas some small stones (3–5 mm) do result in high-grade obstruction and may need more aggressive management. The addition of a diuretic renal scan can determine the presence or absence of obstruction and has been shown to direct patient management; in one study, the scan changed the decision to admit or discharge the patient in 30% of cases.[160–162] Data have not yet been collected to determine if the addition of the scan and resulting change in practice leads to better patient outcomes and/or reduced medical costs.

Ultrasound or CT Show a Dilated Collecting System. Is There Ureteral Obstruction?

Dilatation of the urinary tract with no apparent cause may be incidentally detected by ultrasound, CT, or MRI in a patient with no symptoms of acute obstruction. If the dilated collecting system represents chronic obstruction, an intervention may be required to preserve renal function; if there is simply dilatation of a nonobstructed collecting system, no further workup is required. Diuresis renography is preferred in the evaluation of the nonacute dilated collecting system because it is noninvasive, relatively inexpensive compared to CT or MR urography, and allows the clinician to quantitate the physiologic significance of the anatomic abnormality by measuring both the relative renal function and the diuretic stimulated washout of the tracer from the dilated system. Contrast is avoided, and the gonadal radiation dose is substantially reduced compared to CT urography.

A Patient with Previous Obstruction has Recurrent Symptoms. Has Obstruction Recurred? Has Surgery Successfully Relieved a Documented Obstruction?

A patient with previous documented and treated obstruction may present with symptoms suggesting recurrent obstruction; sonography is often not helpful in this setting because the urinary tract can be dilated secondary to the previous episode of obstruction. Diuresis renography is the preferred examination.

Antenatal Sonography Shows a Dilated Pelvis or Ureter. Is There Obstruction or Loss of Renal Function?

The significance of an abnormal antenatal renal sonogram can be readily evaluated by MAG3 diuresis renal scintigraphy in

the newborn. Relative function can be quantitated and drainage assessed. If the diagnosis of obstruction is uncertain, sequential studies can be obtained with sonography to determine if the renal pelvis is enlarging or with sequential MAG3 scans to evaluate drainage and to determine if function is decreasing in the affected kidney.

Suspected Renovascular Hypertension (ACE Inhibition Renography)

The diagnostic and therapeutic approach to patients with suspected renal artery stenosis (RAS) remains uncertain and controversial. Atherosclerotic renal artery stenosis (ARAS) is the most common cause of secondary hypertension[163]; however, it may be present in as many as 25% of normotensive patients over age 50[164] and is often present as an incidental or secondary finding in hypertensive patients.[164,165] ARAS is far more common than renovascular hypertension (RVH), whose classical definition is based on cure or amelioration of the hypertension after revascularization. Consequently, it comes as no surprise that the Scottish, EMMA, and DRASTIC studies, and the more recent STAR and ASTRAL trials, indicate that the indiscriminate revascularization of an atherosclerotic RAS appears to have little advantage over optimal medical therapy and is no longer justified.[166–170] These five trials, however, did not distinguish between the impact of revascularization in a hypertensive patient with RAS and the impact of revascularization in a hypertensive patient with a functionally significant stenosis. Although indiscriminate revascularization of a stenotic renal artery in hypertensive patients is no longer justified, more focused selection criteria that evaluate the functional significance of the stenosis may lead to improved outcomes.

Spiral computed tomography (CTA) and magnetic resonance angiography (MRA) provide detailed images of the aorta and renal arteries and have high sensitivity and specificity for detecting RAS.[163,171] The main limitation of CTA and MRA is the lack of information on renal blood flow or pressure distal to the stenosis.[171] In azotemic patients, CTA carries the risk of contrast nephrotoxicity, and the use of MR contrast in patients with a low GFR is associated with nephrogenic systemic fibrosis.[172] Doppler ultrasound is reported to provide reliable hemodynamic assessment of renal artery lesions in selected centers but others have found it to be time consuming, operator dependent, lacking diagnostic uniformity, and too unreliable in obese individuals to be an efficient tool to screen hypertensive patients for a functionally significant RAS.[171,173] This introduction underscores the need for diagnostic procedures that can accurately select those hypertensive patients with RAS most likely to be cured or improved after revascularization.

ACE Inhibition Renography and Scan Interpretation

A functionally significant stenosis leads to a decrease in the perfusion pressure distal to the stenosis resulting in a decrease in the transglomerular pressure gradient and a decrease in GFR. The reduction in perfusion pressure stimulates the release of renin and increased production of angiotensin I which is converted to angiotensin II by ACE. Angiotensin II preferentially constricts the efferent arteriole of the glomerulus and raises the transglomerular pressure gradient—a process that maintains GFR in the face of a moderate reduction in perfusion pressure.

In patients with a functionally significant renal artery stenosis, the blockade of angiotensin II production by ACE inhibition leads to a reduction in GFR that can be detected by renography. ACE inhibitors also inhibit kininase II, a dipeptidyl carboxy-peptidase that inactivates bradykinin, a potent vasodilator that causes selective efferent arteriolar dilation; this mechanism also contributes to the ACE-induced reduction in GFR.[174–176]

The reduction in GFR in patients with a functionally significant RAS following ACE inhibition can be detected by a decrease in renal uptake of DTPA by the affected kidney compared to the baseline scan; tubular tracers such as MAG3 demonstrate cortical retention (Fig. 10.38, right kidney)[135,137,159,177,178] which is also secondary to the decrease in GFR and results from a decreased flow of primitive urine through the renal tubules.[179]

Consensus panels have recommended that the test be interpreted as high, low, or indeterminate probability for renovascular hypertension.[135,177] A normal or near normal renogram that is unchanged or improves following ACE inhibition is low probability for renovascular hypertension and indicates that revascularization is unlikely to ameliorate the hypertension (Fig. 10.38, left kidney). Abnormal baseline renograms that are unchanged following ACE inhibition are indeterminate and are not predictive of the response to revascularization. Unilateral deterioration of the renogram curve and/or of the relative function following ACE inhibition compared to the baseline study represents a high probability scan for renovascular hypertension and indicates a high likelihood that blood pressure will be normalized or ameliorated by revascularization.

Sensitivity and Specificity of ACE Inhibition Renography

ACE inhibition renography in an appropriately screened hypertensive patient with preserved renal function can detect renovascular hypertension with a sensitivity approximating 90%.[137,175,178,180–188] Using the criterion of ACE inhibition-induced changes between baseline and ACE inhibition scans to define a positive test, the test has a specificity ≥90% and consequently has a high positive predictive value.[178,187] The sensitivity and specificity of ACE inhibition renography, however, are affected by several factors that have contributed to confusion in the literature.

1. Use of the anatomic presence of a renal artery stenosis as a surrogate for renovascular hypertension. ACE inhibition renography is a test to detect a functionally significant RAS, NOT a test to detect the presence of RAS.[135,177] Nevertheless, many investigators have used the angiographic

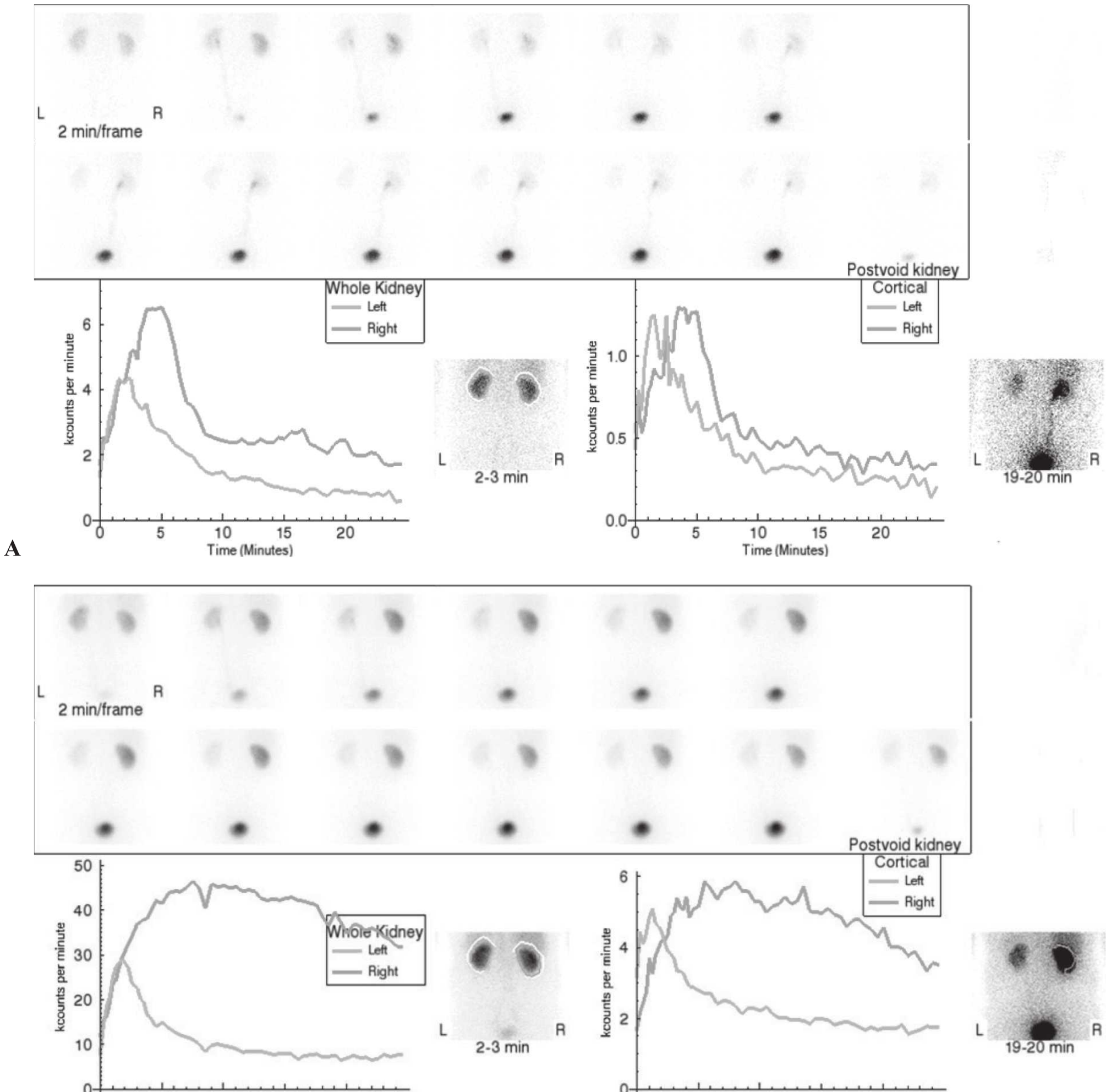


FIGURE 10.38 A 52-year-old patient with hypertension with a normal serum creatinine had a computed tomography (CT) scan for a possible incarcerated abdominal hernia. The CT scan revealed a heavily calcified right renal artery. A subsequent CT angiogram confirmed a renal artery stenosis and an angiotensin-converting enzyme (ACE) inhibition renal scan was requested to determine its functional significance. **A:** A baseline scan was obtained following the injection of 1.4 mCi of ^{99m}Tc MAG3 (52 MBq). The upper panel shows sequential 2-minute MAG3 images. The lower left panel shows the whole kidney renogram curves (*blue*, left kidney; *green*, right kidney); the lower right panel shows cortical renogram curves. The relative uptake was 51% (*left*) and 49% (*right*) with a MAG3 clearance of 295 mL/min/1.73 m^2. The images appear normal with time to maximum counts (T_{max}) and 20 min/max count ratios in the normal range for both kidneys although there was asymmetry with the T_{max} for the right kidney (4.8 min) more delayed than the T_{max} for the left kidney (1.8 minutes); in addition, the 20 min/max ratio for the right kidney, 0.30, was higher than that of the left (0.19). **B:** The patient received an intravenous injection of 2.5 mg of enalaprilat followed 20 minutes later with a second MAG3 injection of 9.0 mCi of MAG3 (333 MBq). The left kidney is normal. The relative function was essentially unchanged, 49% left, 51% right but the sequential 2-minute images show marked parenchymal retention of the tracer in the right kidney with a correspondingly abnormal whole kidney and cortical renogram curves. The marked change in the right kidney from the baseline to the ACE study indicates a high probability scan for renovascular hypertension. (See Color Plate.)

presence of a stenosis >50% as a surrogate for renovascular hypertension in spite of the fact that many of these stenotic lesions will not be hemodynamically significant.[106,177,178,189] In an attempt to circumvent this problem, other investigators have used the more stringent standard of >70% stenosis. Although good results have been obtained using RAS as the gold standard,[190] not surprisingly, the sensitivity and specificity of ACE inhibition renography is improved when the gold standard is the response to revascularization rather than the anatomic presence of RAS.[178,184,185,187,188]

2. Patient selection: test performance in azotemic and nonazotemic populations. Current data indicate that the test is not as accurate in azotemic patients with RAS. Although some studies report good results in azotemic patients,[183,191,192] most investigators have found ACE inhibition renography to be less accurate in azotemic patients.[135,137,177,178,181] In this population, a positive test retains a high specificity indicating a high likelihood that the hypertension will be ameliorated by revascularization,[193] but one study reported as many as 50% of tests in this population were intermediate probability.[137] Intermediate probability outcomes result from an abnormal baseline scan which is unchanged following ACE inhibition and are not predictive of the response to revascularization.
3. Analysis of the "intermediate probability" or indeterminate scan. To calculate sensitivity and specificity, indeterminate results must be placed with the high or low probability results. Intermediate probability studies placed in the "high probability" category increase sensitivity at the expense of specificity and, conversely, intermediate probability studies placed in the "low probability" category increase specificity at the expense of sensitivity.[194] The reported values for sensitivity and specificity vary depending on the frequency of azotemic patients in the study population and how intermediate probability results are handled in the data analysis.
4. Inconsistent use of recommended criteria for interpreting ACE inhibition renograms. Interpretative criteria for ACE inhibition renography have been published as an international consensus report in 1996; this report has subsequently been updated by the Society of Nuclear Medicine and published on its website.[135,177] Studies performed after 1996 should include an analysis based on these criteria especially because data show that strict adherence to these criteria improves the performance of ACE inhibition renography.[195]

Performance of the Examination

Detailed recommendations for performance of ACE inhibition renography are described in consensus reports[135,177] but several points should be emphasized.

1. Blood pressure must be monitored. Asymptomatic hypotension secondary to ACE inhibition can result in bilateral symmetrical abnormalities in the renogram curves.[194,196] This phenomenon is relatively uncommon but may occur in as many as 3% of patients referred for ACE inhibition renography, usually in patients who are volume or salt depleted.[196,197]
2. ACE inhibitors and angiotensin II receptor blockers (ARBs). The majority of ACE inhibition studies have been performed with captopril (25–50 mg) but enalaprilat (Vasotec, 40 μg per kg IV over 3–5 minutes, maximum dose of 2.5 mg) administered at least 15 minutes prior to tracer administration is an acceptable alternative.[177,183] Intravenous injection of enalaprilat avoids the possibility of a false-negative test due to delayed gastric emptying or poor absorption—a potential disadvantage is the possibility of a greater risk of a hypotensive response. Chronic ACE inhibition may reduce the sensitivity of the test[179,192] and, for this reason, guidelines recommend that captopril be discontinued for 4 days prior to the study and ACE inhibitors with a longer half-life be discontinued for 7 days. Data are limited regarding the impact of chronic ARB administration on the sensitivity and specificity of ACE inhibition renography.[175]
3. Diuretics. Chronic diuretic administration increases the likelihood of volume depletion which may lead to renal retention of the radiopharmaceutical, reduce the specificity of the test, and increase the risk of a hypotensive response. These concerns can be minimized if diuretics are discontinued for several days prior to the study.
4. Choice of radionuclide. In patients with azotemia, tubular agents such as MAG3 or I-123 are the agents of choice.[137,177] In patients with normal function, MAG3 and DTPA appear to give comparable results.
5. One-day versus two-day protocol. The traditional approach is a one-day protocol; a baseline study is performed, the ACE inhibitor is administered, and a second study is obtained allowing an immediate comparison between the baseline and ACE inhibition results. An alternative approach is to begin with ACE inhibition renography because a normal study is low probability for renovascular hypertension and obviates the need for a baseline study. If the ACE inhibition study is abnormal, the specificity can be improved by obtaining a baseline renogram; however, the patient will have to return for the baseline study on another day because of the earlier administration of the ACE inhibitor.

Does a Hypertensive Patient Have Renovascular Hypertension?

Risk factors for renovascular hypertension (RVH) include abrupt or severe hypertension, hypertension resistant to medical therapy, abdominal or flank bruits, unexplained azotemia in an elderly hypertensive patient, worsening renal function during ACE inhibition therapy, grade 3 or 4 hypertensive retinopathy, a history of heavy smoking, occlusive

disease in other vascular beds, and onset of hypertension under age 30 or over age 55. To determine the most appropriate test, patients need to be categorized into (1) those with low likelihood of RVH, (2) those with moderate to high likelihood of RVH and normal renal function, and (3) those with moderate to high likelihood of RVH and azotemia.

Technologies are evolving and multiple diagnostic imaging strategies have been proposed, but, to date, there is no generally accepted approach. Costs need to be considered in determining the clinical approach but costs are a moving target and hard to ascertain. Patients with a low likelihood of RVH can be treated medically without additional imaging studies. For the patient with one or more risk factors for RVH, normal renal function, and no known unilateral kidney disease, ACE inhibition renography provides a logical and cost-effective diagnostic approach. In this patient population, a recent analysis showed that ACE inhibition renogram as the first test was more cost effective than CT or MRA.[194]

The evaluation of the patient with azotemia or a patient known to have a small, poorly functioning kidney is more problematic. In this patient population, a positive ACE inhibition renogram still has a high predictive value for amelioration of the hypertension, but as many as half of the tests may be indeterminate for RVH, and the specificity of the test probably falls to about 80%. The advantages and disadvantages of other diagnostic approaches have been described, and test selection should be based on local expertise, cost, and how the test result will influence patient management.

Renal Transplant Scintigraphy

Donor Evaluation

Renal scintigraphy can evaluate global and individual renal function in potential donors as well as help determine which kidney to select for donation. Use of renal scintigraphy in donor evaluation, however, varies widely between centers.

Transplant Evaluation

Complications of renal transplantation can be divided into parenchymal failure (acute tubular necrosis [ATN], acute and chronic rejection, and calcineurin inhibitor toxicity) and mechanical failure (injury to the renal artery or vein, ureteral obstruction, and urine leak).[198] Sonography is usually the first approach for evaluation of renal graft dysfunction but a renal scan can provide complementary information. A normal scan immediately posttransplant excludes mechanical complications. Serial scans during the first 1 to 3 weeks posttransplantation may detect early rejection 24 to 48 hours before biochemical abnormalities occur and can be used to monitor recovery from posttransplantation ATN. Classically, ATN presents with relatively preserved perfusion accompanied by delayed uptake and excretion although severe ATN can also present with diminished flow. Rejection presents as diminished flow with delayed uptake and excretion of the tracer.[198]

Chronic transplant nephropathy represents cumulative and incremental damage to nephrons from both immunologic and nonimmunologic causes. Imaging studies are obtained if the clinician suspects complications relating to renal blood flow, urine leak, urinoma, obstruction, abscess, hematoma, or lymphoma. Sonography is typically the first approach. A renal scan may provide complementary information regarding obstruction or a urine leak and ACE inhibition renography can detect renovascular hypertension.[151,198] A renal scan cannot distinguish between rejection and calcineurin-inhibitor nephrotoxicity.

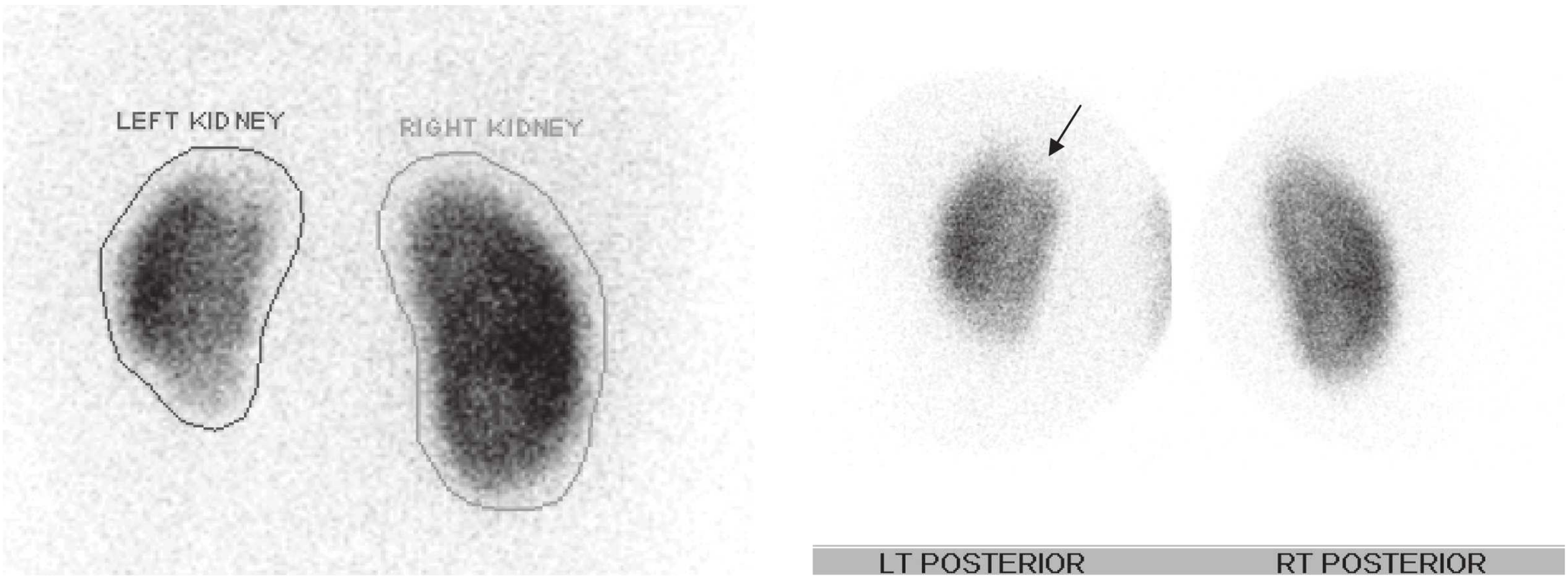
Renal Cortical Scintigraphy (Pyelonephritis and Scar)

DMSA is the radiopharmaceutical of choice for imaging the renal cortex.[128,129,151,199] Static images are obtained 2 to 4 hours after injection and sedation is rarely needed. A normal scan demonstrates homogeneous concentration of the radiotracer throughout the cortex except for a lower concentration in the region of the collecting system. DMSA scans can measure relative function and identify functioning renal tissue in patients with congenital abnormalities. The studies are most commonly obtained in patients with suspected pyelonephritis to distinguish upper from lower urinary tract infections and to detect the presence of scar following an episode of acute pyelonephritis. Pyelonephritis and scarring are recognized by focal areas of decreased DMSA uptake in the renal parenchyma; however, any process that replaces, injures, or destroys normal cortical parenchyma will result in an abnormal scan.

Does a Child Presenting with a Febrile Urinary Tract Infection Have Pyelonephritis?

Pyelonephritis is a serious illness in the pediatric population; renal scarring from recurring infection remains an important cause for substantial long-term morbidity.[200] Clinical and experimental studies have demonstrated that scarring can be prevented or diminished by early diagnosis and aggressive therapy. In infants and young children, pyelonephritis is not always accompanied by high fever, an elevated sedimentation rate, and leukocytosis. Furthermore, a normal voiding cystourethrogram does not exclude acute pyelonephritis, and it is increasingly recognized that sonography and excretory urography cannot be used to exclude acute pyelonephritis in infants and children. Renal cortical (DMSA) scintigraphy is more sensitive for the detection of pyelonephritis than sonography (Fig. 10.39), and many investigators recommend cortical scintigraphy in the initial evaluation of children with suspected pyelonephritis. MRI and CT with contrast are also sensitive tests for the detection of pyelonephritis, but MRI is expensive, and there is the possibility of an allergic reaction to iodinated contrast given during the CT scan.

There is no consensus on the use of DMSA scans in the evaluation and follow-up of patients with urinary tract infection. The diagnostic algorithm depends on how the clinician



A B

FIGURE 10.39. A 6-year-old boy presented with fever, vomiting, a urinary tract infection, and an erythrocyte sedimentation rate of 98. **A:** A dimercaptosuccinic acid (DMSA) scan obtained with a parallel hole collimator shows the left kidney to be smaller than the right kidney and also suggests an area of decreased DMSA activity in the left upper pole. **B:** Additional images of each kidney were obtained using a pinhole collimator which provides better resolution than the parallel hole collimator. Pinhole images clearly show an area of decreased DMSA uptake in the superior pole of the left kidney (*arrow*) consistent with pyelonephritis; the normal right kidney is shown for comparison.

will use the information. Some institutions treat pediatric patients with suspected pyelonephritis empirically and only pursue diagnostic studies if the patient does not respond. In other institutions, patients with pyelonephritis receive more aggressive therapy and/or follow-up in the hope of reducing the risk of scarring and recurrent infection and, thereby, avoiding the subsequent development of hypertension or renal failure.[128,129,151,199]

Radionuclide Cystography

Vesicoureteral reflux, urinary tract infections, and renal scarring can lead to hypertension and chronic kidney disease; however, a large percentage of patients with pyelonephritis do not have reflux. Furthermore, reflux often resolves spontaneously. Management of patients with urinary tract infection and/or reflux tends to be individualized and varies from center to center. Reflux may be suspected based on an antenatal ultrasound showing ureteral or calyceal dilatation, a urinary tract infection in infants or young children, acute pyelonephritis, or documented reflux in a sibling. A conventional voiding cystourethrogram (VCUG) with fluoroscopy is usually the first test to detect and grade the degree of reflux. If follow-up studies are required, the patient can be followed by radionuclide cystography. The technique is accurate for detecting reflux, and the radiation dose to the gonads is much less than with a VCUG.[128,129,151]

Direct Radionuclide Cystography

The bladder is catheterized, and the study is performed by instilling saline containing approximately 1 mCi (37 MBq) of a ^{99m}Tc radiopharmaceutical into the bladder. These radiopharmaceuticals are not absorbed into the blood from the urinary bladder. Images are continuously acquired during filling of the bladder and subsequent voiding. Reflux can be quantitated by analysis of data recorded on the computer during the study.

Indirect Radionuclide Cystography

Bladder catheterization is not required. The patient typically receives an intravenous injection of ^{99m}Tc-MAG3 for evaluation of individual kidney function, urine drainage, and reflux; dynamic images are obtained during bladder filling, voiding, and after voiding. This technique avoids catheterization, but it is not as sensitive as direct radionuclide cystography for detecting reflux.

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CHAPTER

11 Computed Tomography and Magnetic Resonance Imaging

Kyongtae T. Bae • Sonal Krishan

Cross-sectional imaging is essential for the diagnosis and clinical management of a number of renal pathologies. Although ultrasound (US) represents a first-line imaging modality in the assessment of the kidney because of its cost effectiveness, portability, and availability, the images are highly operator dependent and are limited in anatomic coverage as compared to computed tomography (CT) scans and magnetic resonance imaging (MRI). CT scans and MRIs offer images of superb anatomic detail and permit the accurate and noninvasive assessment of a wide range of renal and urologic pathologies including: congenital anomalies, obstructive disease, inflammatory lesions, vascular insufficiency, benign and malignant tumors, and trauma. Although CT scans remain far more commonly used in clinical settings, the role of MRI is growing because of its superior intrinsic tissue contrast and the absence of radiation exposure. Additionally, MRIs can be used as the problem-solving modality when US or CT findings are nondiagnostic.

TECHNICAL CONSIDERATIONS FOR COMPUTED TOMOGRAPHY SCANS AND MAGNETIC RESONANCE IMAGING

Principles of Computed Tomography Scans

Similar to conventional X-ray radiographic images, the physical basis for a CT scan is the attenuation of X-ray photons passing through the body. The basic hardware of a CT scanner system consists of an X-ray gantry (which supports a rotating X-ray tube and a set of X-ray detectors); a patient table that moves in and out of the gantry; and a computer system integrated with the gantry, data-storage hardware, and image-display console. A precisely collimated narrow X-ray beam (a fan-shaped beam) is generated, then transmitted through the patient's body and received and identified by the detectors on the opposite side of the gantry. The use of a rotating X-ray beam and detector arrays permits the detection and measurement of the X-rays attenuated by tissues from many different projections. It is from these measurements that the CT images are mathematically reconstructed.[1] The CT scan is operated at various user-selectable voltages (e.g., 80, 100, 120, 140 kVp) and currents (milliampere second [mAs]) that closely determine radiation dose and image quality.

Computed Tomography Attenuation or Computed Tomography Number (Hounsfield unit)

The spatial resolution of a CT image typically achieves 0.3 to 0.5 mm with an imaging matrix size of 512 × 512 pixels. Each pixel (picture element) value of a CT image represents the tissues' X-ray attenuation coefficient at that pixel, which is expressed in Hounsfield units (HU). The data size of each pixel is typically 2 bytes. Because a CT image consists of 512 × 512 pixels, the data size of a CT image is approximately 0.5 MB (512 × 512 × 2). The attenuation value assigned to each pixel is based on a reference scale in which −1,000 HU is the value assigned to air and 0 HU is assigned to water.[2] Fat is typically −30 HU or less, whereas calcification, bone, and iodinated contrast are usually greater than 100 HU. User selection of the number of shades of gray (window width) in the image and the central hue of gray (window level) permits the modification of displayed image contrast. By adjusting the window width and level, the image can be optimized for evaluating a wide range of tissues with varying attenuations. For example, bone and a contrast-filled bladder are typically presented as bright (high attenuation) structures, whereas the lungs are typically presented as dark (low attenuation) structures. Subtle structures such as a solid tumor within a normal parenchyma may be displayed more conspicuously in a specifically designed window setting (e.g., liver window).

Computed Tomography Technical Advances

CT technology has advanced from the traditional single-row detector array to a multiple-row detector array. Multidetector-row CT (MDCT) scans, which are now the standard method for performing CT examinations, allow

multiple channels of data to be acquired simultaneously. As a result, MDCT scans covers a large volume of area in a short scan time with thinner slices and an improved spatial resolution along the patient's craniocaudal axis. Presently, 64-detector MDCT systems are most commonly in use; however, the 320-detector MDCT has been recently introduced into clinical practice. Using MDCT, the entire abdomen can be imaged in a few seconds. MDCT scans offer a greater speed of acquisition and higher resolution images than single-detector CT scans and thus greatly facilitate multiplanar imaging and three-dimensional (3D) reconstruction (Fig. 11.1).

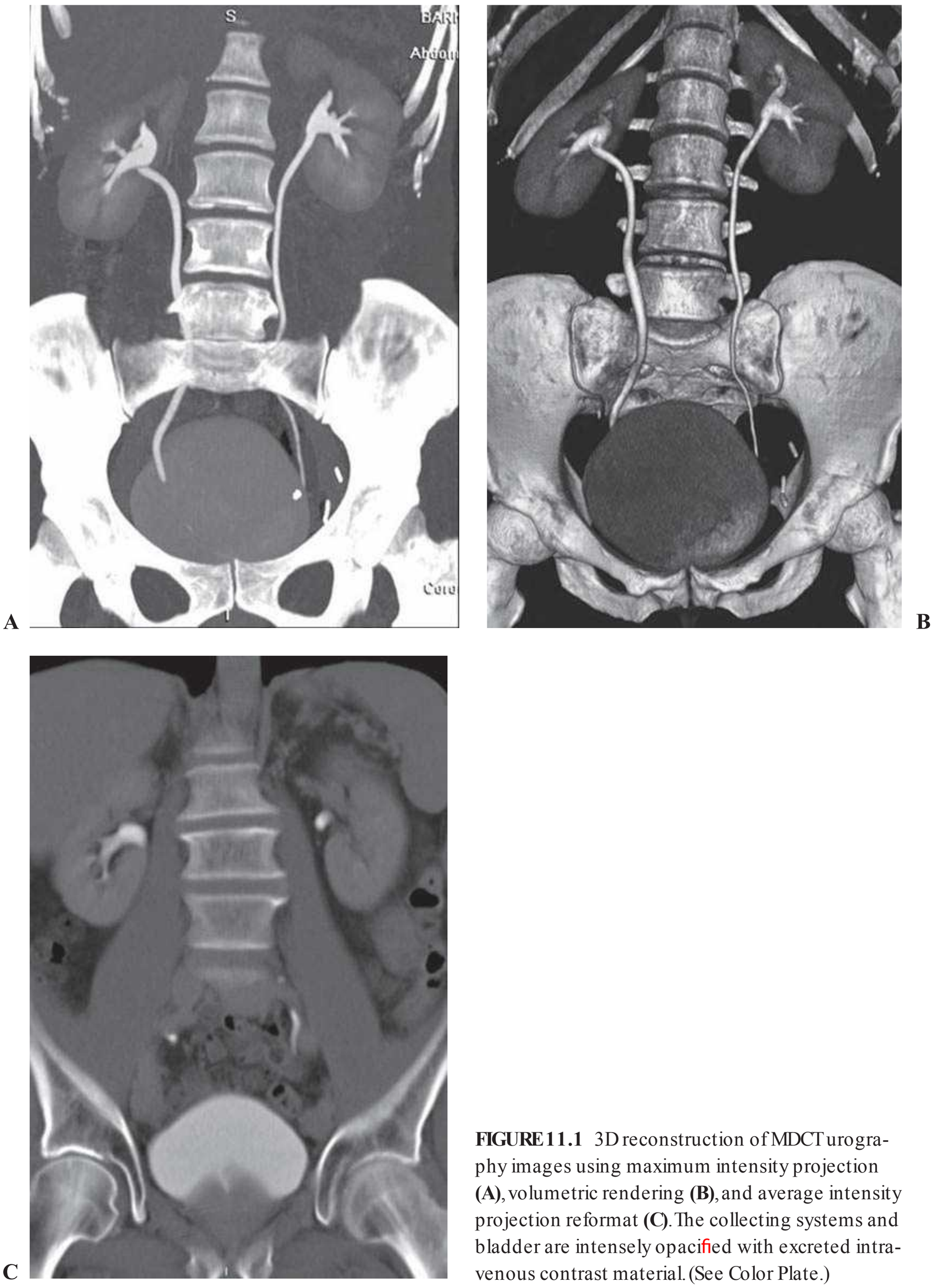


FIGURE 11.1 3D reconstruction of MDCT urography images using maximum intensity projection **(A)**, volumetric rendering **(B)**, and average intensity projection reformat **(C)**. The collecting systems and bladder are intensely opacified with excreted intravenous contrast material. (See Color Plate.)

The technical advances of MDCT scans have allowed highly technical and challenging clinical applications, such as CT angiography (CTA), to be practiced routinely. MDCT scans have essentially replaced the conventional catheter-based diagnostic renal angiography. In many institutions, MDCT urography is performed in place of the conventional intravenous urography.[3–5] MDCT renal imaging is widely used as the "one-stop shop" single imaging modality for the evaluation and surgical planning of many clinical conditions involving the kidney and urinary tract. In addition, the application of 3D techniques to CT scans allows for the accurate depiction of tumor depth, location, relationship of the tumor to adjacent structures, and the delineation of renal vascular anatomy as well as aid in preoperative planning (Fig. 11.2).

Recently, dual-energy MDCT scans were introduced, allowing the simultaneous acquisition of low- and high-voltage CT images during the same scanning phase. This technique has the potential to better characterize renal stone compositions and renal lesions through the assessment of their distinct CT attenuation profiles, thereby providing potentially improved physiologic and molecular information.[6]

Use of Contrast Media in Computed Tomography Scans

A CT evaluation of renal anatomy and pathology often requires the intravenous injection of iodinated contrast media. Intravenous contrast enhancement is useful for the depiction of small lesions by increasing their conspicuity, for the demonstration of vascular anatomy and vessel patency, and for the characterization of lesions through their patterns of contrast enhancement. On the other hand, an unenhanced CT scan is better suited to detect renal or urinary calcifications and intrarenal or perirenal hemorrhage because CT images obtained after the administration of contrast media may mask these abnormalities.[7–9] Unenhanced CT scans are also recommended for the quantification of tumor contrast enhancement on the postcontrast scans and for patients with poor renal function.

The commercially available radiographic contrast agents are tri-iodinated derivatives of benzoic acid. All currently available intravenous (IV) contrast media are excreted by the kidney through glomerular filtration, with no significant tubular excretion or resorption.[10,11] It is prudent that the physician inquire about the patient's history, particularly regarding the renal function and any history of allergies to the contrast material. A history of asthma and severe allergies increases the risk of subsequent reaction to contrast agent injection by a small percentage. The administration of corticosteroids, with or without antihistamines, 12 hours before a contrast injection to reduce the occurrence of adverse reactions in allergic patients has been well documented. Oral contrast is less critical in urologic imaging than in gastrointestinal imaging. In fact, the use of oral contrast media may be counterproductive for the evaluation of renal calculi or CTA.

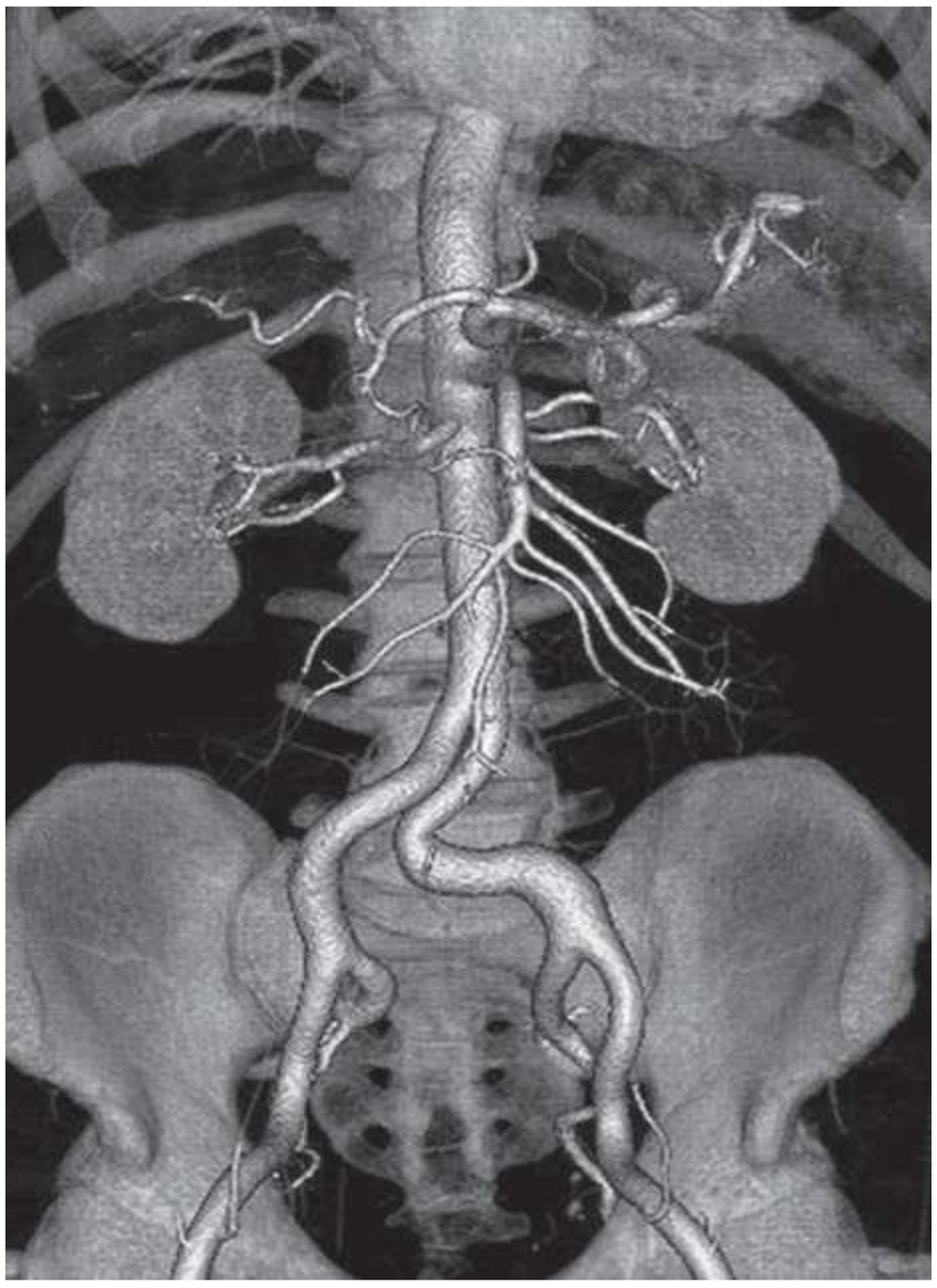
A contrast-enhanced CT scan is typically performed with 100 to 150 mL of 300 to 370 mg per milliliter of contrast medium injected at 2 to 3 mL per second. The amount of contrast medium is adjusted for the patient's size, clinical indications, and the CT scanner type. For CTA, fast injection rates (4 to 5 mL per second) are recommended. CT scan delays are determined by fixed delay or bolus tracking techniques.[12]

After the administration of an IV contrast agent, contrast-enhanced CT scans can be acquired at different contrast enhancement phases: early arterial, corticomedullary, nephrographic, and excretory phases (in the order of increasing CT scan delays) (Fig. 11.3). Although the kidney is normally imaged in routine abdominal CT scans at a single (nephrographic) phase of contrast enhancement, dedicated renal imaging protocols consist of scanning of the kidney at multiphases of contrast enhancement. Imaging phases must be selected in accordance with clinical indications because it is important to minimize the number of scanning phases to reduce the radiation exposure for a patient.

The early arterial phase begins with the arrival of the contrast medium in the renal artery and ends prior to the occurrence of intense renal venous return. This phase is primarily useful in arterial CTA (i.e., surgical planning and renal artery stenosis evaluation) and is very limited for the diagnostic imaging of the kidney and urinary tract. The corticomedullary phase of the kidney corresponds to an intense enhancement of the renal cortex prior to a substantial enhancement in the medulla. The depiction of a hypervascular renal mass and renal artery anatomy is maximized during the corticomedullary phase. The nephrographic phase of the kidney corresponds to homogeneous enhancement throughout the renal parenchyma with a loss of corticomedullary differentiation. The depiction of renal lesions in the cortex or medulla is maximized during this phase. The degree of contrast enhancement in a renal mass is evaluated by noting the difference in the CT scan attenuation of the mass between this phase and in the unenhanced CT images. The onset of the excretory or urographic phase is about 2 minutes after the start of contrast medium injection. Maximal opacification of the renal calyces, pelvises, and ureters occurs later, about 5 to 10 minutes after the injection of the contrast medium. This is the best enhancement phase for the assessment of (benign and malignant) conditions affecting the urinary tract.

Magnetic Resonance Imaging Principles

An MRI scan begins with placing the patient in the central bore of an electromagnet, which generates a static magnetic field. Nuclei with odd numbers of protons or neutrons (of which hydrogen is the most abundant in biologic tissue) align themselves with their magnetic moments, either parallel or antiparallel to the external field. A net magnetization vector lies in a direction parallel to the static magnetic field of the magnet bore of the MRI (called the z-axis by convention). A radio frequency (RF) transmission coil transmits RF pulses through the patient and energizes the protons in the z-axis. When the RF pulse is turned off, the protons give off



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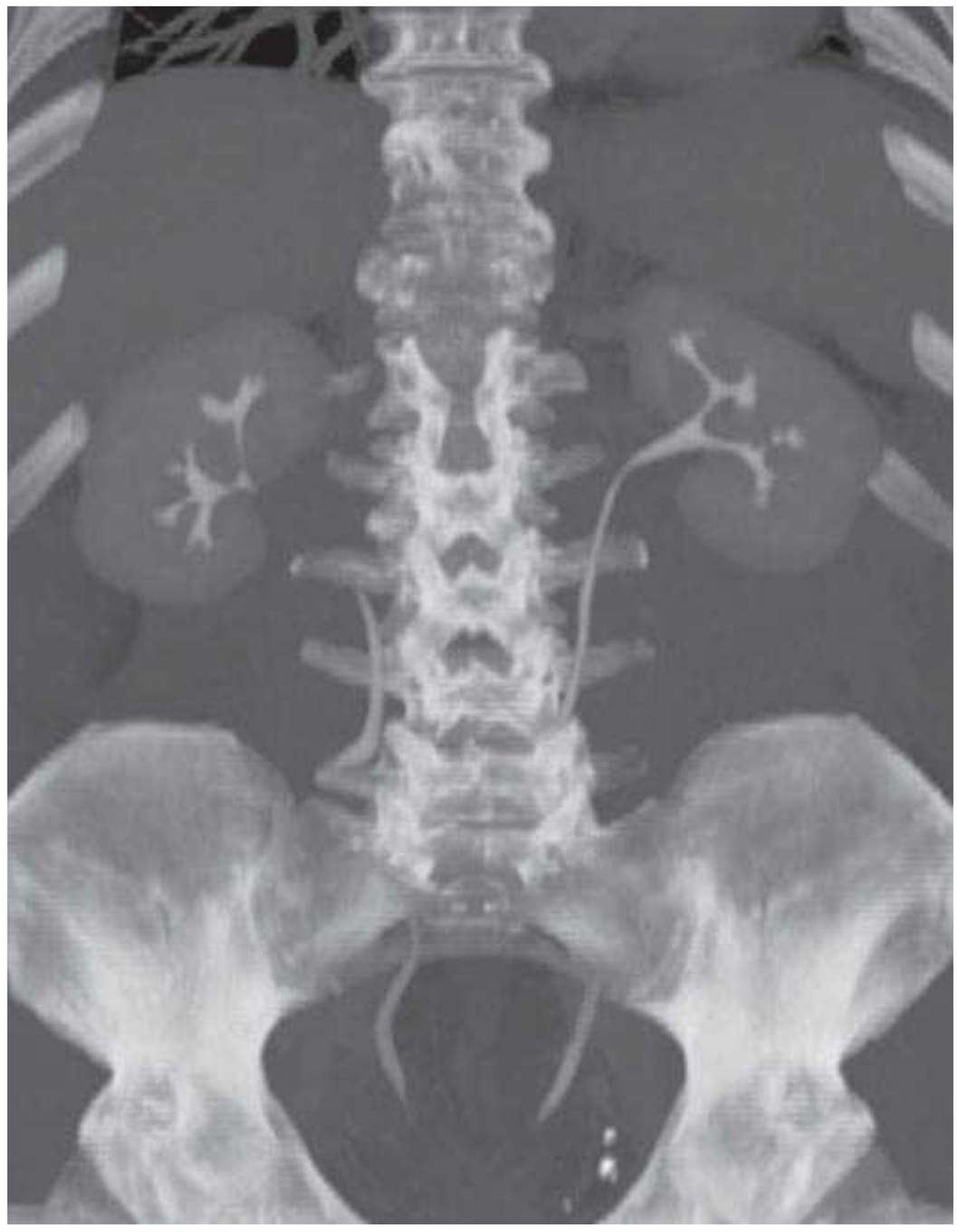

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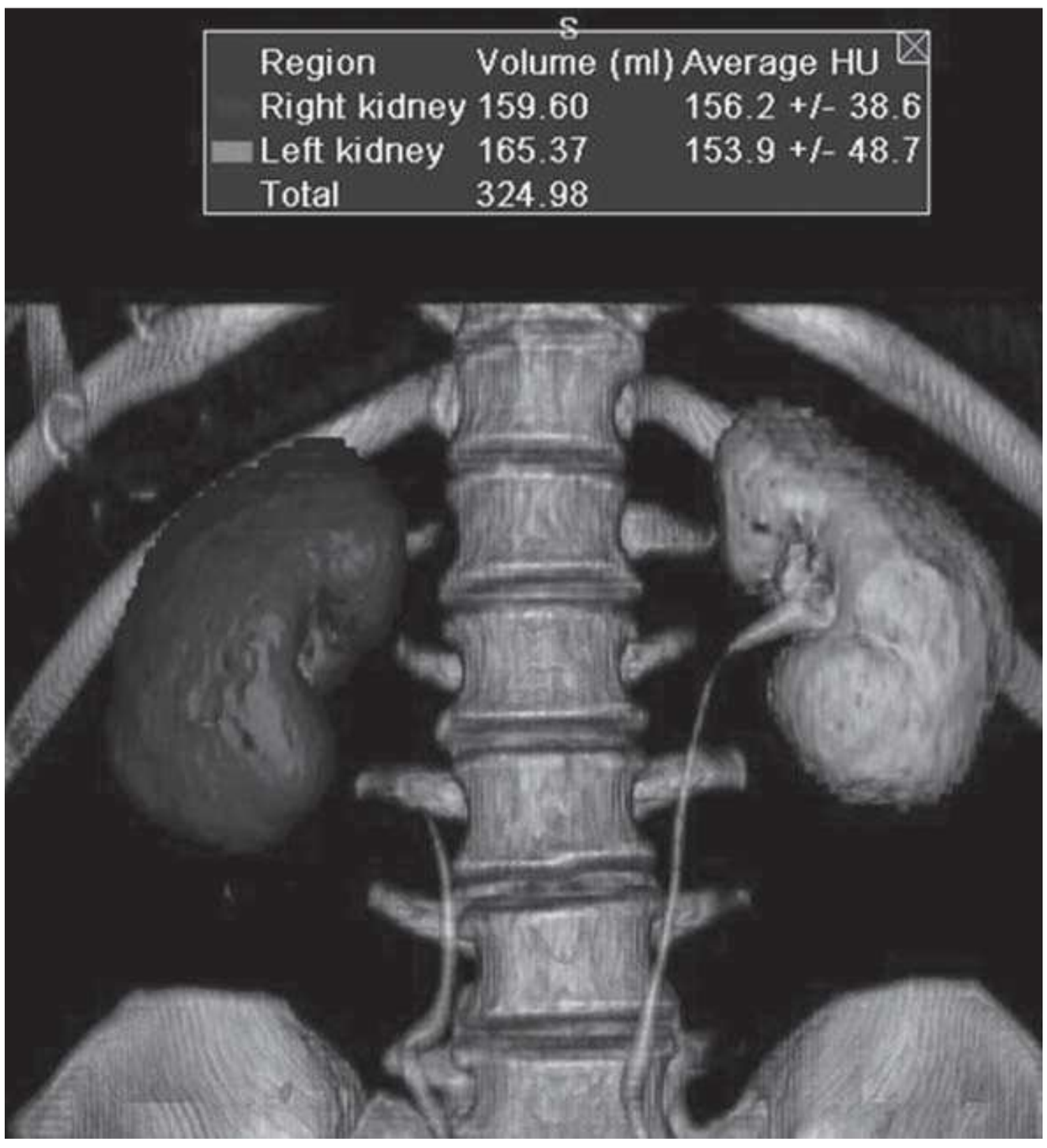

C

FIGURE 11.2 MDCT renal imaging performed as one-stop shop imaging study for renal donor work-up, including CT angiography **(A)**, urography **(B)**, and measurement of kidney volumes **(C)**. (See Color Plate.)

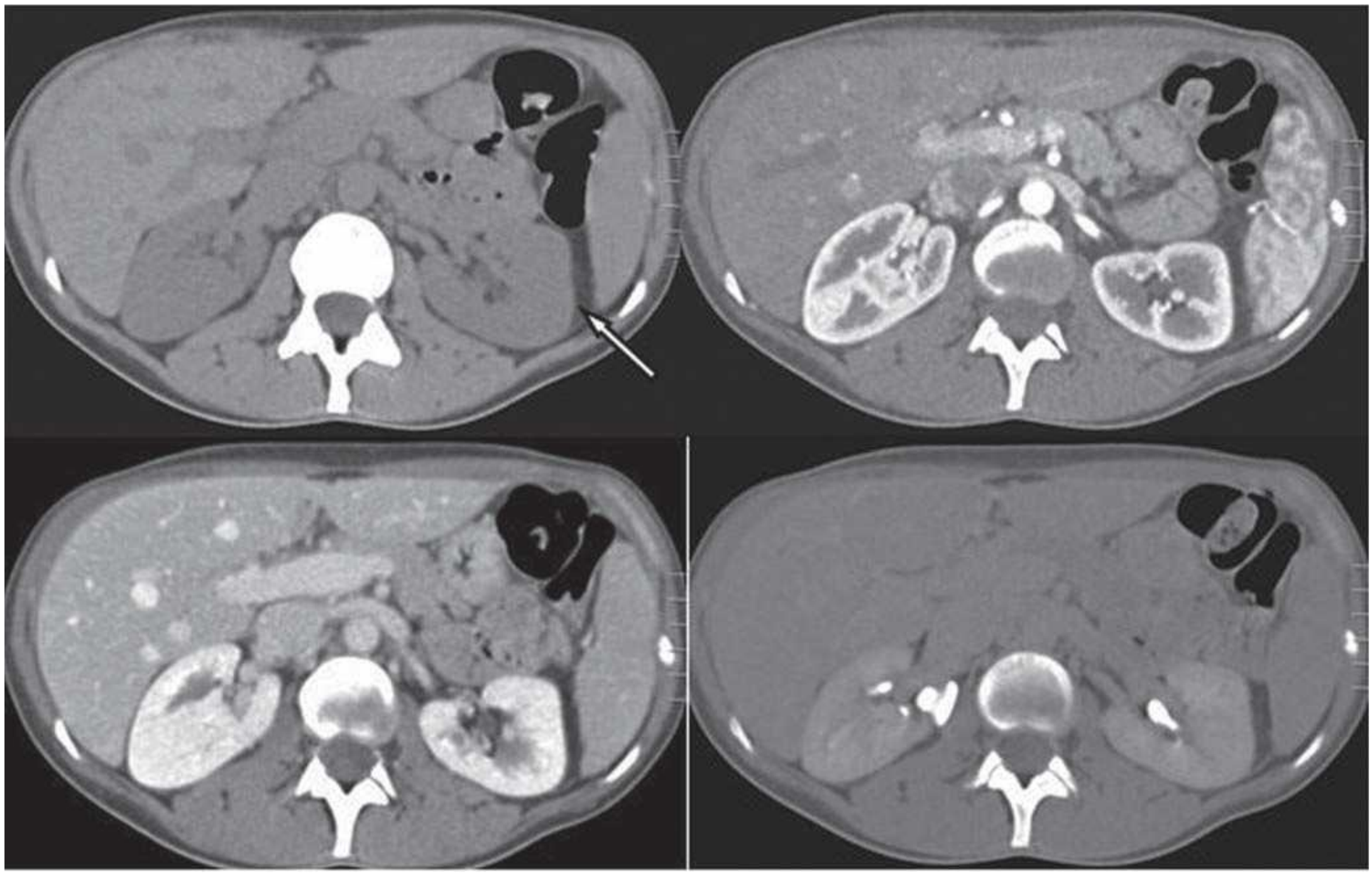

FIGURE 11.3 CT images of normal kidneys obtained without intravenous contrast (*left upper*), at corticomedullary phase with contrast (*right upper*), nephrographic phase (*left lower*), and delayed excretory phase (*right lower*). The arrow points to the peri-renal fat.

the energy (relaxation) that was imparted to them by the RF pulse.[13,14] This emitted energy is received by a receiver RF coil, and it is from this energy that the MR image is created. MRI pulse sequences determine the patterns of repetitive RF pulsations and the sampling of MR signals emitted during the intervals between RF pulses. Each MR sequence takes advantage of the intrinsic property of the body's tissues to absorb and release this energy. How this energy is imparted through the physics of the pulse sequences and whether energy is released quickly or slowly determines the weighting of an image. In general, an image is either T1 or T2 weighted. An MRI possesses exquisite contrast resolution, even without the use of IV contrast. On T1-weighted images, the fluid is generally dark (also called low signal) and on T2-weighted images, the fluid is bright (also called high signal).[13,14]

In order to map the anatomic distribution of different tissue signals, magnetic field gradients are established within the MR scanner along the x-, y-, and z-axes. Because the protons' resonance frequency depends on the magnetic field strength, controlled applications of magnetic field gradients induce protons to precess at different frequencies and phases according to their spatial distributions. The pattern of a received signal from a selected tissue volume can be converted to an image in which digital information related to protons' spatial distribution is reconstructed into images displayed over a gray scale.[13,14] Unlike CT attenuation values (HU), MR signal intensities are not directly specific to tissue compositions. Therefore, MR signal intensities between tissues are frequently compared in relative scales, such as a ratio to reference signal intensity.

Technical Advances in Magnetic Resonance Imaging

Because the respiratory motion of the kidney may significantly decrease the image quality, it is critical to perform an MRI scan with fast sequences within a breath hold. A variety of fast T1-weighted and T2-weighted sequences are available with the development of advanced coil and parallel imaging techniques to improve both temporal and spatial resolutions of an MRI. An MR angiography (MRA) is routinely performed when a diagnostic evaluation of the renal vasculature is requested. Although noncontrast sequences are available for an MRA, the dominant technique is the 3D gadolinium-enhanced MRA. For an evaluation of the urinary tract, an MR urography (MRU) has been developed. An MRU may be performed on T2-weighted sequences, exploiting the

long T2 of urine without gadolinium or on T1-weighted sequences after the administration of gadolinium contrast during the excretory phase.

Recent advances have resulted in new MR techniques for evaluating renal function such as perfusion, diffusion, oxygenation, and sodium concentration. A functional MRI of the kidney has not yet found broad clinical application, but it has great potential. Through the ongoing development of functional MRI techniques, we may expect an increasing role for functional MRIs in the management of patients with renal disease.[9,15,16]

The Use of Contrast Media in Magnetic Resonance Imaging

Gadolinium chelates are almost exclusively used for IV contrast materials for MRIs. Gadolinium agents have been shown to produce comparably lower incidences of allergic reactions than the iodinated contrast agents used in CT scans. The gadolinium compounds are filtered at the glomerulus and are neither reabsorbed nor secreted. They parallel iodinated contrast material in their pattern of initial intravascular and subsequent extracellular space distribution and in their pattern of excretion. Gadolinium-based contrast agents demonstrate a similar pattern of contrast enhancement as iodine-based agents (see Fig 11.4).[17,18]

For many years, gadolinium-based contrast-enhanced MRIs were believed to be safer and they were the preferred method of contrast (instead of iodine-based contrast material) for patients with renal impairments. However, since early 2006, evidence has been mounting that some gadolinium-based contrast agents may potentially cause the fibrosing sclerodermalike condition called nephrogenic systemic fibrosis (NSF) in patients with renal failure.[19–21] Patients at the highest risk of NSF include: (1) patients who have severe acute or chronic renal impairment with glomerular

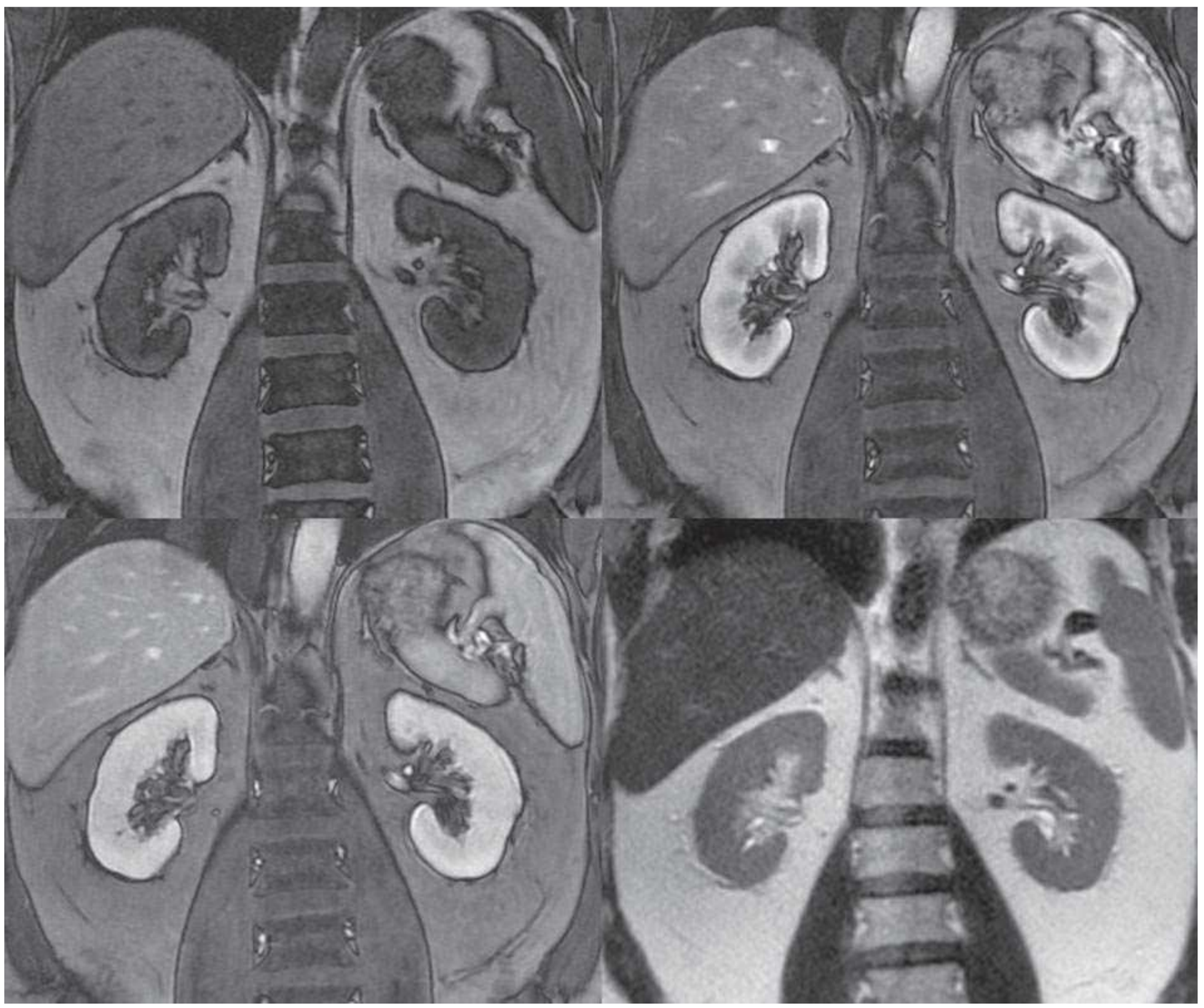

FIGURE 11.4 MR images of normal kidneys: T1-weighted without intravenous contrast (*left upper*), contrast-enhanced T1-weighted at corticomedullary phase (*right upper*), contrast-enhanced T1-weighted at nephrographic phase (*left lower*), and T2-weighted (*right lower*).

filtration rates (GFR) <30 mL per min/1.73m^2; (2) patients on dialysis (hemo or peritoneal); and (3) patients with reduced renal function awaiting liver transplantation. The recognition of this adverse reaction to gadolinium-based agents in renal-impaired patients emphasizes the need for an appropriate clinical indication for gadolinium-enhanced MRIs in this patient population. If an MRI is clearly indicated, the lowest dose of the agent that leaves the smallest amount of gadolinium in the body must be used and, in certain cases, immediate dialysis after the administration is recommended.[19–21]

MAGNETIC RESONANCE IMAGING VERSUS COMPUTED TOMOGRAPHY SCANS

CT scans and MRIs complement each other in providing diagnostic information for the detection and characterization of renal pathologies. Because CT scans are faster and easier to perform, it is more commonly used in the evaluation of renal and perirenal disease. The MRI remains primarily a problem solving modality adjunct to a CT scan or is used when a CT scan is contraindicated. The CT scan is superior for the reliable detection of calcified structures, whereas an MRI provides superb intrinsic tissue-contrast resolution and multiplanar imaging, which is particularly useful for the evaluation of renal vasculature.

If nephron sparing surgery is contemplated, an MRI is better able to differentiate a tumor from perinephric fat, the renal sinus, and the collecting system, thus helping the urologist decide if a partial nephrectomy is feasible. Staging renal lesions is more complete with an MRI than a CT scan, particularly when determining renal vein and inferior vena cava (IVC) involvement.[22,23] Patients who have genetic anomalies resulting in an increased risk for renal cell carcinoma (RCC) (e.g., von Hippel-Lindau), and those with conditions that produce other renal lesions that may mimic RCC (e.g., tuberous sclerosis), can be followed safely with a yearly MRI. This follow-up regimen will protect these high-risk patients from recurrent exposure to ionizing radiation from CT scans, with no loss in the ability to detect or characterize renal lesions.[24–37] Conversely, patients who are critically ill (e.g., intensive care unit patients) and those who have difficulty with the breath-holding requirements are not ideal candidates for renal MRIs. Patients unable to cooperate for other reasons (e.g., dementia, chronic obstructive pulmonary disease, morbidly obese) are also unsuitable for MRIs. Scanning these patients often results in a poor quality examination that may not be interpretable. Patients who have ferromagnetic implants (some neurovascular aneurysm clips, cochlear implants, pacemakers, defibrillators) are also contraindicated for MRIs. Furthermore, patients with claustrophobia may not be able to undergo MRIs.[24–37]

NORMAL ANATOMY

The kidneys lie within the retroperitoneum that is in turn divided by facial planes into three compartments centering the kidneys: the perirenal, the anterior pararenal, and the posterior pararenal spaces. The kidneys, the perirenal fat, and the adrenal gland are in the perirenal space enclosed by the anterior (Gerota) and posterior (Zuckerkandl) layers of the renal fascia. The perirenal space also includes the renal and adrenal vessels, the aorta, the inferior vena cava, and the perivascular lymph nodes. The kidneys lie lateral to and roughly parallel with the lateral border of the psoas muscle. The renal fossa is bounded medially by the psoas muscle, posteriorly by the quadratus lumborum muscle, laterally by the transversus abdominis muscles, and superiorly by the diaphragm. Anteromedially, the kidneys are covered by peritoneum; posteriorly, the 12th rib crosses the left kidney at a 45-degree angle, with approximately one-third or more of the left kidney superior to the inferior margin of the thoracic cage.[38–42] A fibrous envelope called the renal capsule covers the kidney and is firmly adherent to the renal substance. A potential space exists between the kidney and its capsule, which in abnormal situations (such as trauma and infection) may contain blood, pus, or urine.[38–42]

The renal sinus contains fat and fibrous tissue, renal vessels, nerves, and lymphatics. The sinus extends around the renal pelvis, the infundibula, and the calyces and is continuous with the perinephric fat. The renal arteries arise from the aorta and enter the renal hila. The renal veins lie anterior to the renal arteries, whereas the left renal vein usually runs between the aorta and the superior mesenteric artery.[38–42] The adrenal glands lie anterior and medial to the upper poles of kidneys and are bilobed V- or Y-shaped structures.

The anterior pararenal space contains the second and third parts of the duodenum, the pancreas, and the ascending and descending portions of the colon. It is limited anteriorly by the parietal peritoneum, and posteriorly by the renal fascia. The posterior pararenal space contains only fat and is bounded posteriorly by the transversalis fascia. The parietal peritoneum and transversalis fascia fuse laterally to form the lateroconal fascia. The anterior and posterior pararenal spaces communicate to a limited extent above and below the level of the renal vessels. Fascial planes are demonstrated as linear structures of soft tissue attenuation surrounded by low attenuation fat.[38–42] These fascial planes may unfuse and form a potential space between the perirenal and pararenal spaces to be filled with fluid; in particular, a sequelae of pancreatitis.

On unenhanced CT scans, the kidneys appear as oval structures with soft tissue (gray) attenuation surrounded by low attenuation (dark) perirenal fat (Fig. 11.3). They intensely (brightly) enhance with intravenous contrast. The appearance of the kidneys on MRIs depends on a number of factors, including the type of MR sequences, hydration, and contrast enhancement (Fig. 11.4). On T1-weighted images, the kidney presents with intermediate (gray) signal intensities similar to visceral organs and muscle. On T2-weighted images, the

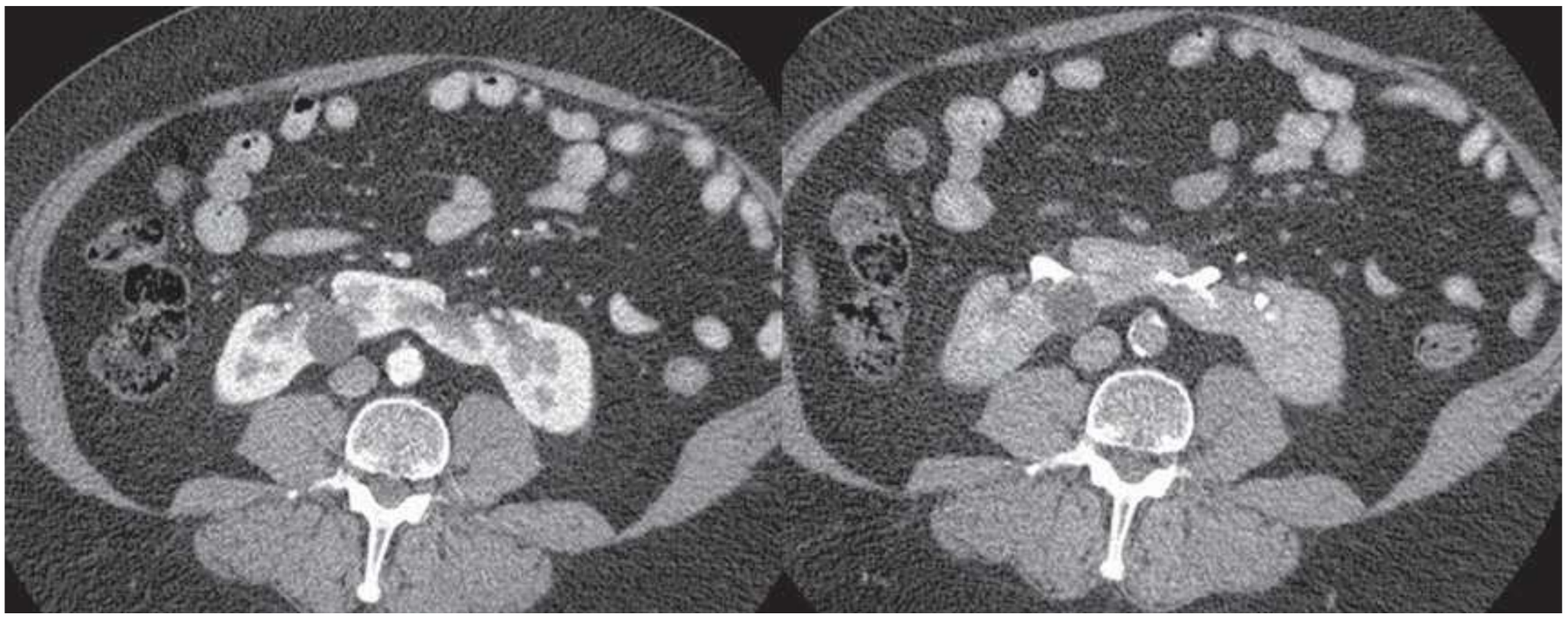

FIGURE 11.5 CT images of horseshoe kidney obtained at cortcomedullary (*left*) and delayed excretory phase (*right*). A small cyst is noted in the right kidney.

kidney is slightly hyperintense showing signal intensities similar to the spleen. The renal medulla is slighter darker on T1 images but is brighter on T2 images than the renal cortex. This is likely because of a greater unbound water (urine) content in the medulla.[25] Gadolinium contrast agents administered intravenously shorten T1 and enhance the renal parenchyma in T1-weighted images.[36,43–45] When lesion contrast enhancement is difficult to detect, a subtraction technique between unenhanced and enhanced T1-weighted images is required.

The calyces, renal pelvises, and ureters containing abundant water (urine) have low signals on T1-weighted images and high signals on T2-weighted images. The intraluminal signal intensities of the renal vessels vary widely depending on the MR sequences, imaging planes, and gadolinium contrast. The perirenal fat is bright on both T1- and T2-weighted images. The fat saturation technique suppresses the hyperintense perirenal fat signal and helps detect and characterize a renal lesion with fat, such as angiomyolipoma.

RENAL PATHOLOGIES

Congenital Variants of Renal Anatomy

Congenital and developmental variants of the kidney such as ectopic kidneys, horseshoe kidneys (Fig. 11.5), duplicated collecting systems, and various hypoplasia or dysplasia (Fig. 11.6) are routinely detected on cross-sectional imaging. An MRI is

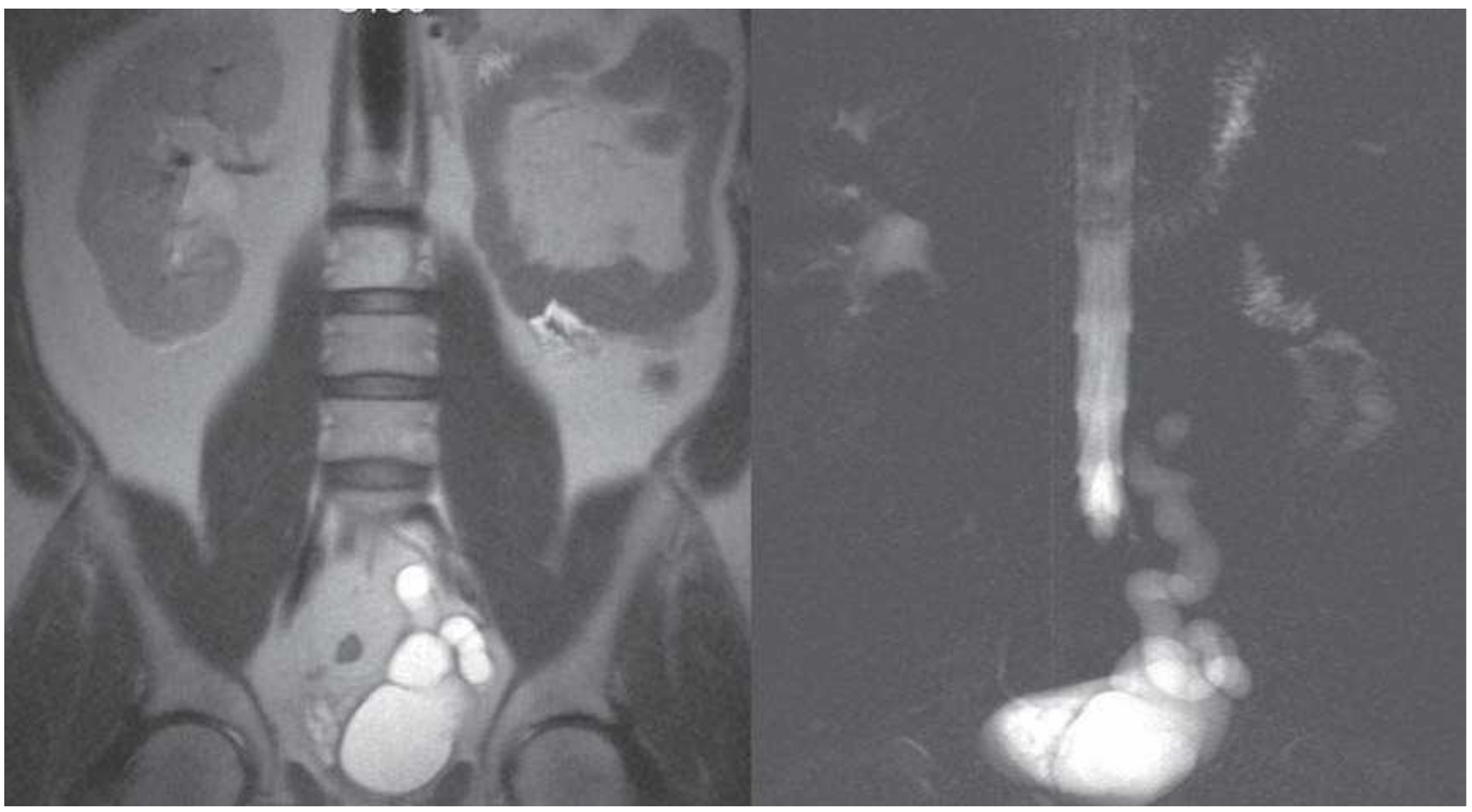

FIGURE 11.6 Left renal agenesis with dilated, tortuous ureteral remnant shown on T2-weighted MR (*left*) and MR urography (*right*). The right kidney shows compensatory hypertrophy.

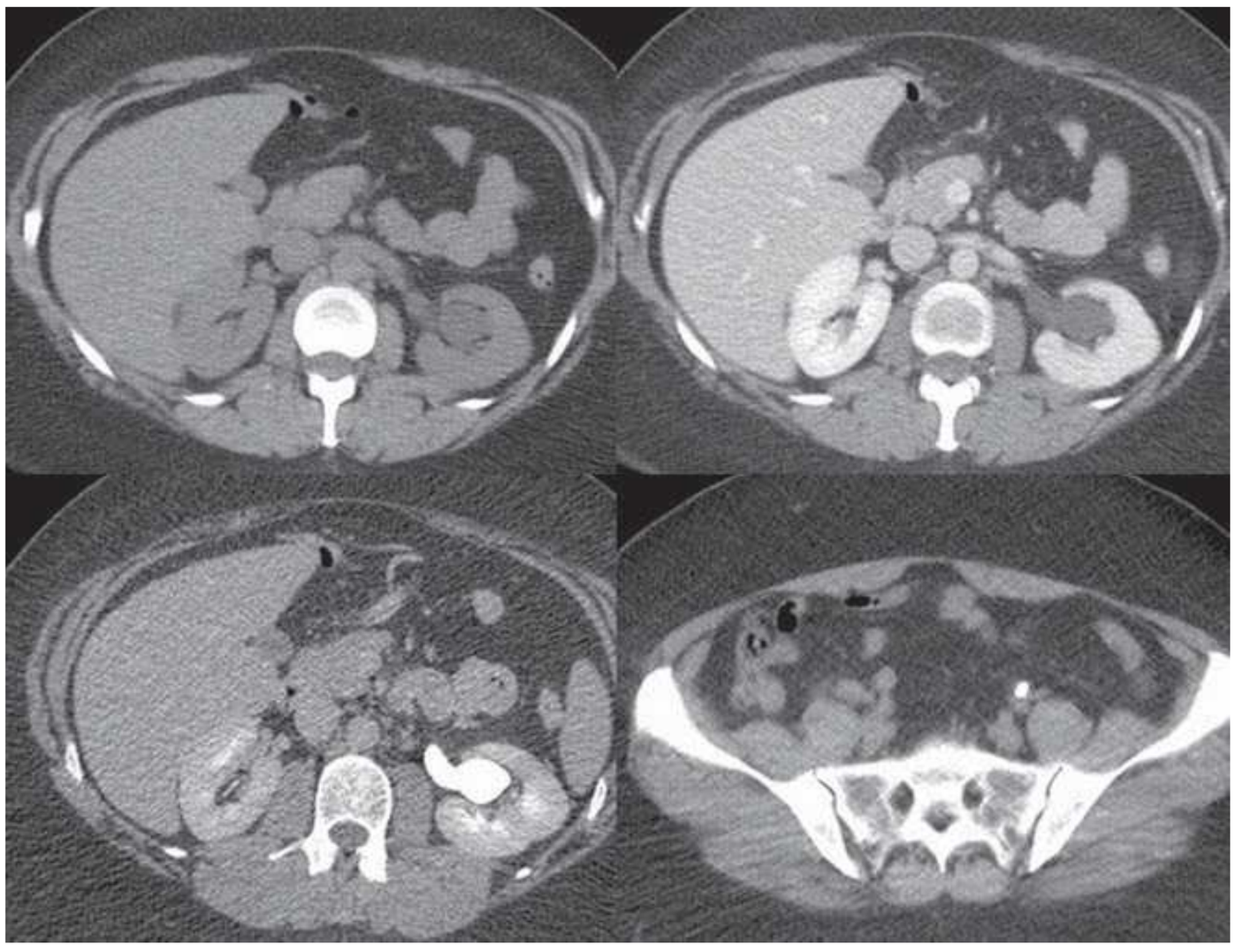

FIGURE 11.7 CT images of kidneys obtained without intravenous contrast (*left upper*), at nephrographic phase with contrast (*right upper*), and at delayed excretory phase (*left lower*). Mild hydronephrosis in the left kidney caused by a distal ureteral calculus (*right lower*) is conspicuously demonstrated by excreted contrast filling the dilated collecting system.

particularly attractive as an imaging modality for the serial follow-up of pediatric patients with suspected renal anomalies because it requires no ionizing radiation and offers superb intrinsic contrast without exogenous contrast agents.[46,47]

Obstructive Disease

Unenhanced renal CT scans have emerged as an attractive alternative to intravenous urography (IVU) and ultrasound (US) imaging in patients with suspected renal colic. A CT scan allows us to evaluate the abdomen and the retroperitoneum for other disorders that mimic renal colic, including diverticulitis, appendicitis, an aortic aneurysm, and retroperitoneal fibrosis. The dilated, fluid-filled collecting system and ureter, along with the anatomic site, degree, and cause of obstruction can be evaluated on unenhanced images (Fig. 11.7). The acutely obstructed kidney may be enlarged and edematous. The renal execretion of contrast can be assessed on contrast-enhanced CT scans. With an acute obstruction, the usual transient, early cortical–medullary phase contrast enhancement is prolonged (persistent nephrogram) with a delayed excretion of contrast into the collecting system (Fig. 11.8).[3,6,27,28,36,43,48–56] Chronic obstruction may cause a marked distention of the fluid-filled collection system and an atrophy of the renal parenchyma.[3,6,27,28,36,43,48–56]

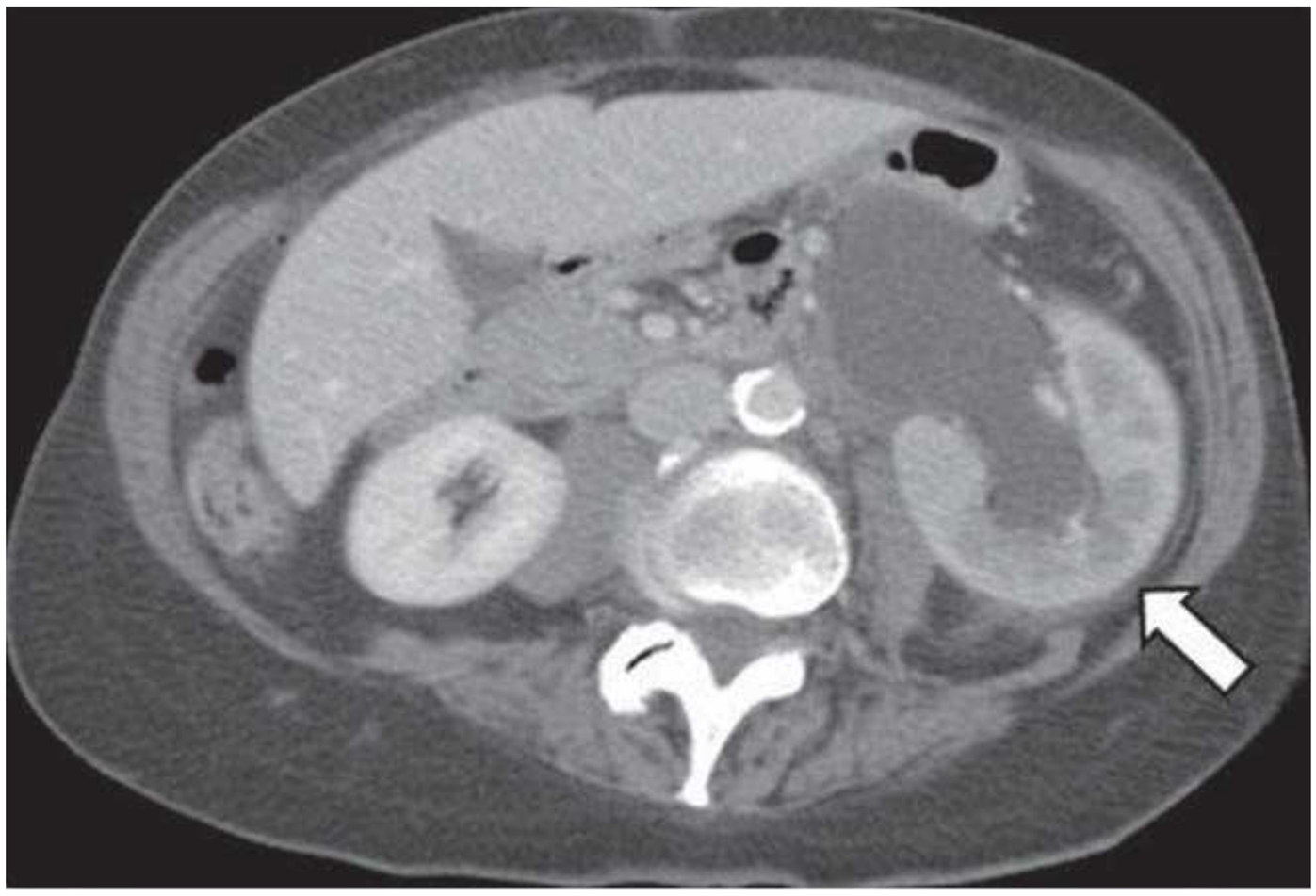

FIGURE 11.8 CT showing acute obstruction at the left uretero-pelvic junction with persistent cortico-medullary enhancement (compare the pattern of enhancement to the right kidney).

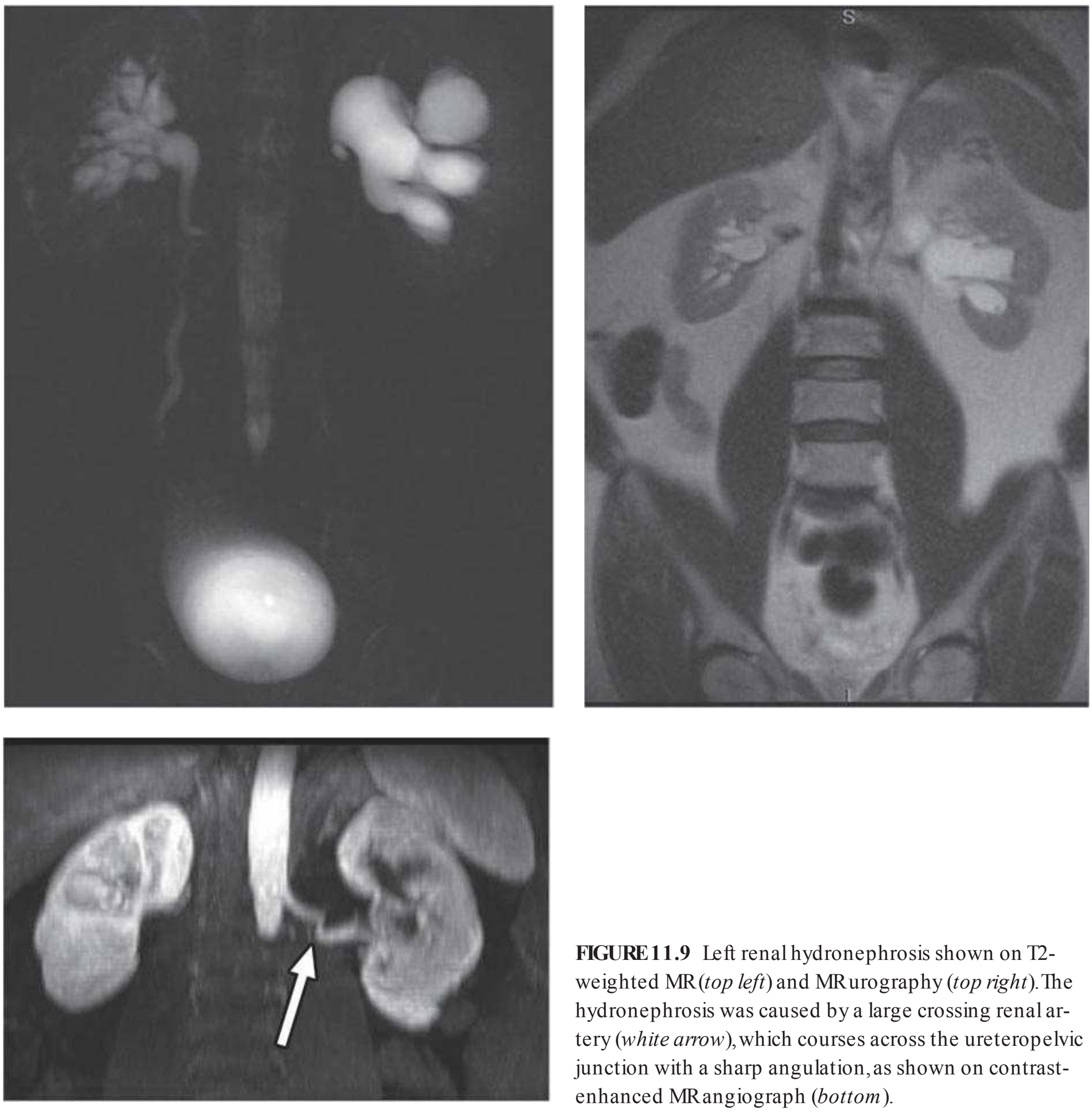

FIGURE 11.9 Left renal hydronephrosis shown on T2-weighted MR (*top left*) and MR urography (*top right*). The hydronephrosis was caused by a large crossing renal artery (*white arrow*), which courses across the ureteropelvic junction with a sharp angulation, as shown on contrast-enhanced MR angiograph (*bottom*).

Although an MRI is not commonly used as the first-line diagnostic imaging modality for the evaluation of suspected obstructive disease, the ureteral, pelvic, and calyceal dilatation caused by a distal obstruction can be readily delineated on transaxial, sagittal, or coronal MRIs. Gadolinium contrast is not necessary to determine the presence of an obstruction. In particular, an MRI that uses heavily T2-weighted images without gadolinium contrast has been shown to be accurate in detecting an obstruction (Fig. 11.9).[3,6,27,28,36,43,48–56]

Nephrolithiasis

Because CT scans provide an accurate, rapid, and safe evaluation of suspected renal calculi without contrast, it has replaced IVU for this application. Unenhanced CT scans have been reported to have sensitivities ranging from 96% to 100% and specificities ranging from 92% to 100% for the diagnosis of nephrolithiasis or urolithiasis.[57–63]

Regardless of the chemical composition, renal and ureteral calculi are generally radiodense on CT scans (with the rare exception of indinavir-induced stones). Thus, cystine and urate calculi that are difficult to detect on conventional abdominal radiographs can be readily diagnosed on unenhanced CT scans. Crixivan (indinavir sulfate), a protease inhibitor used to treat patients with HIV, can precipitate in the urinary system forming radiolucent stones that may not be directly visualized on CT scans. When nephrolithiasis is suspected, the initial CT scan should be obtained before the administration of contrast agents because the high-attenuation contrast excreted into the collecting system may obscure underlying calculi.

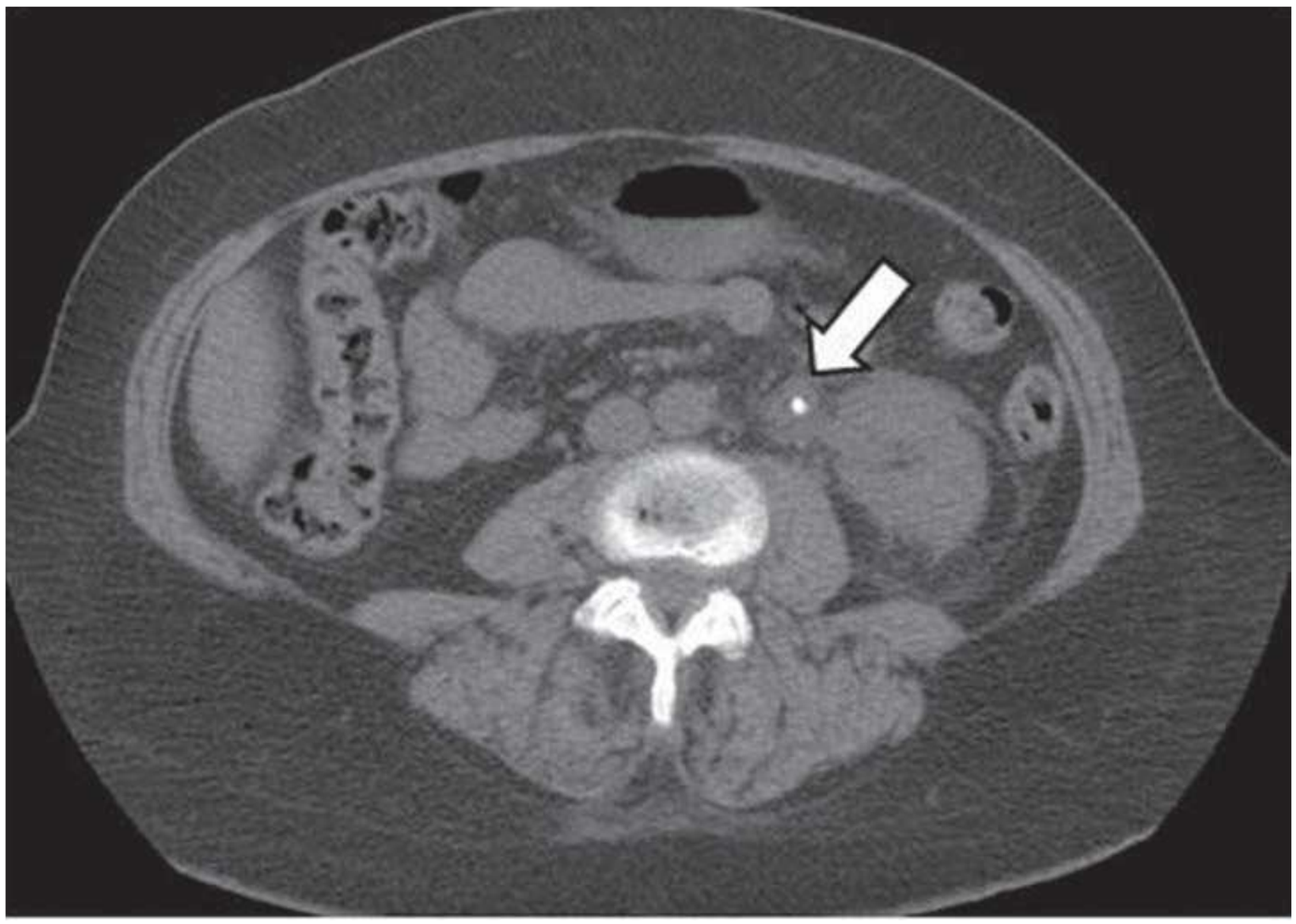

FIGURE 11.10 Unenhanced CT showing a hyperattenuating calculus at the left uretero-pelvic junction with peri-nephric and peri-ureteric soft tissue stranding consistent with acute left renal colic due to nephrolithiasis.

The most common locations for an obstruction by a stone are the natural anatomic points of narrowing: the ureteropelvic junction (Fig. 11.10), the pelvic brim where the ureter crosses the iliac vessels, and the ureterovesical junction. The most obvious sign of a ureteral stone on a CT scan is a focus of high attenuation (similar to bone) within the ureter.[57–63] Secondary signs of an obstruction include ureteral dilatation, asymmetric inflammatory change of the perinephric fat, hydronephrosis, and nephromegaly. An obstructing stone at the ureterovesical junction may be difficult to differentiate from a stone that recently passed into the bladder. In such a case, scan the patient in a prone position and the stone that falls to the anterior portion of the bladder is a stone that has passed.[57–63]

Renal or ureteral calculi are not directly visible on an MRI because they do not produce MR signals. They may be indirectly identified as foci of a signal void within the renal parenchyma, the collecting system, or ureters (Fig. 11.11). However, other objects including air, metal, and sutures also present as sites of signal void on an MRI.

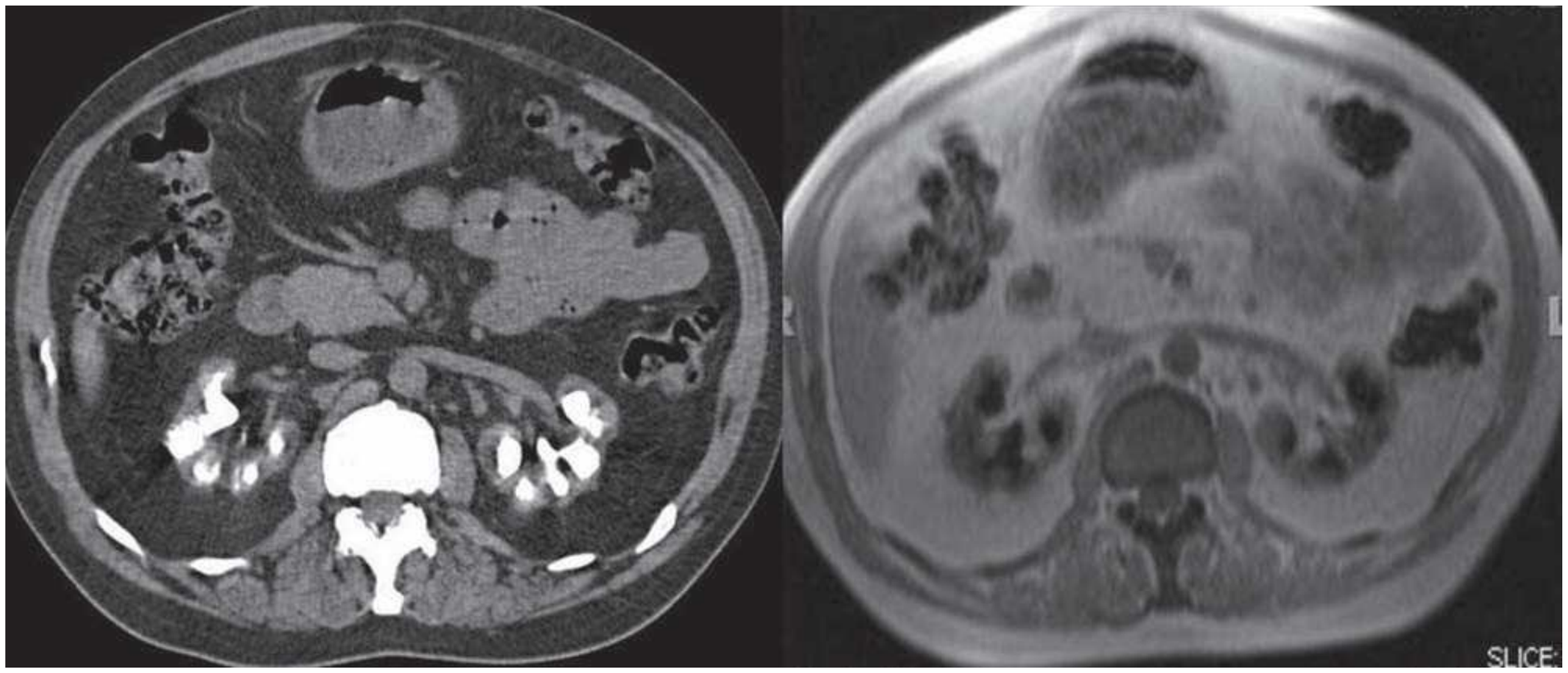

FIGURE 11.11 Extensive medullary calcinosis is shown as multiple hyperattenuating foci in the bilateral kidneys on unenhanced CT (*left*) but only as signal voids on T1-weighted MRI (*right*), undifferentiated from gas in the gastrointestinal tract.

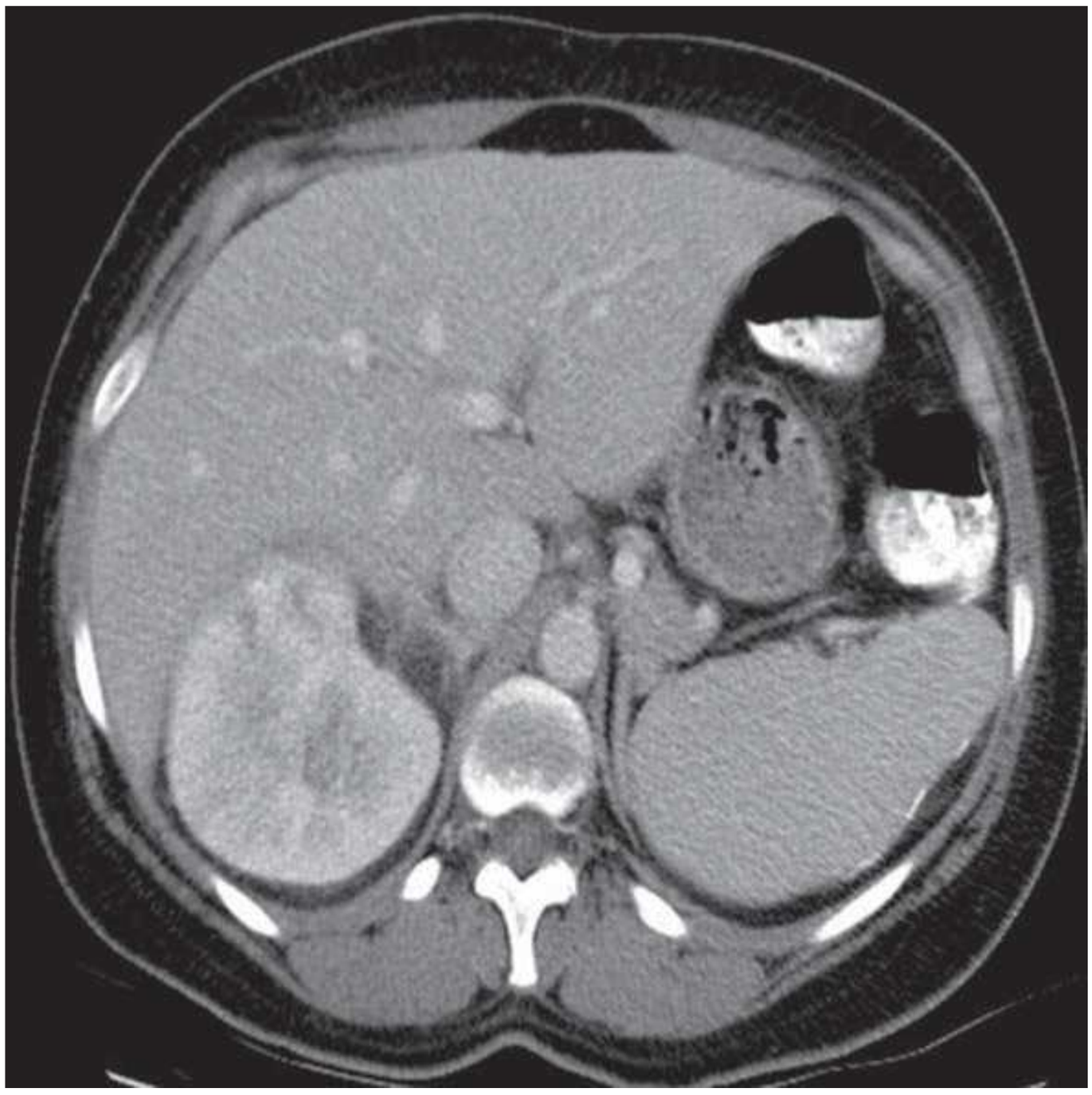

FIGURE 11.12 Contrast-enhanced CT in a patient with clinical symptoms of acute pyelonephritis showing striated nephrogram in an edematous right kidney with multiple linear non-enhancing areas.

Inflammatory Disease

Acute Pyelonephritis

Acutely inflamed kidneys may present with a range of findings on a CT scan. These include renal enlargement, heterogeneous patterns of contrast enhancement or striated nephrograms (Fig. 11.12), and pelvicaliceal air. Perirenal effusions and a thickening of the perirenal fascia may be seen with severe inflammation. A CT scan is also useful in depicting long-term sequelae of renal infections, renal parenchymal scarring and atrophy, and deformities of the collecting system (Fig. 11.13).[30,40,64–66] A CT scan is also superior to ultrasonography in detecting and delineating renal and perirenal abnormalities that are associated with pyelonephritis and abscesses.

Acute pyelonephritic kidneys may show loss of corticomedullary distinction on T1-weighted MRIs. Perirenal edemas may produce a decrease in the signal intensity of the surrounding fat and the thickening of renal fascia. Areas of inflammation and abscess cavity walls demonstrate contrast

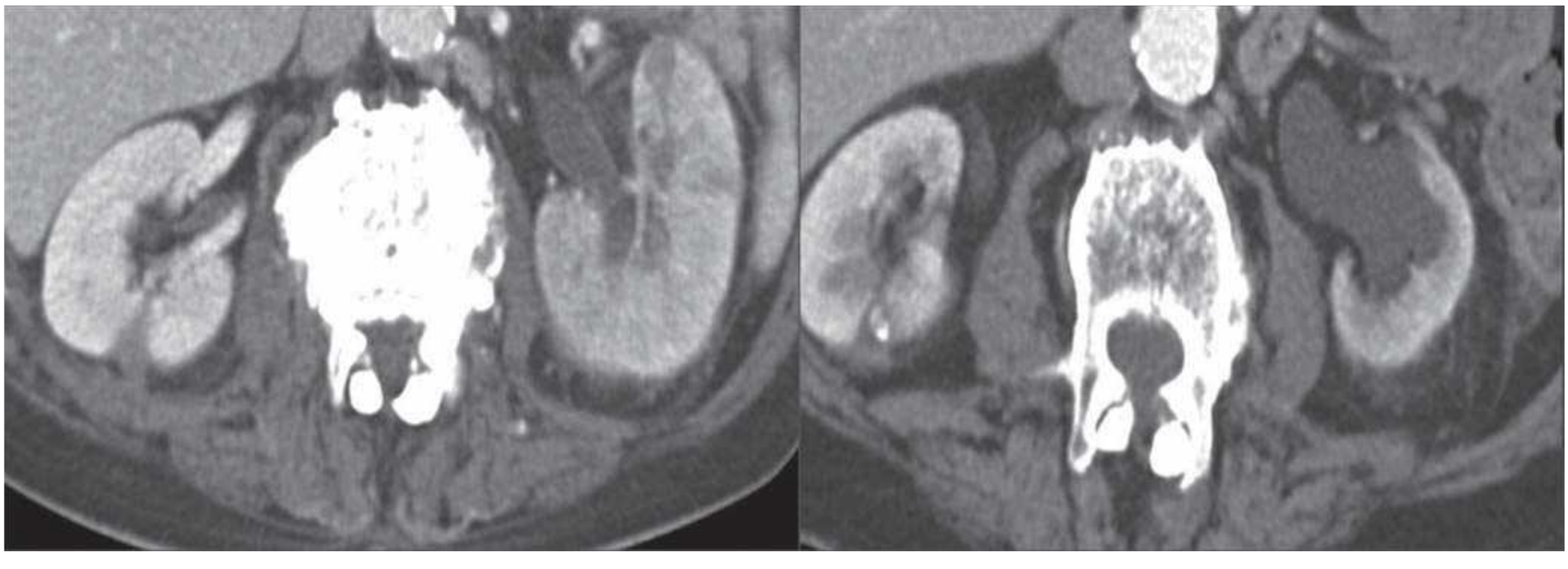

Figure 11.13 Left kidney with recurrent acute pyelonephritis (*left*) resulting in atrophy shown in follow-up CT (*right*).

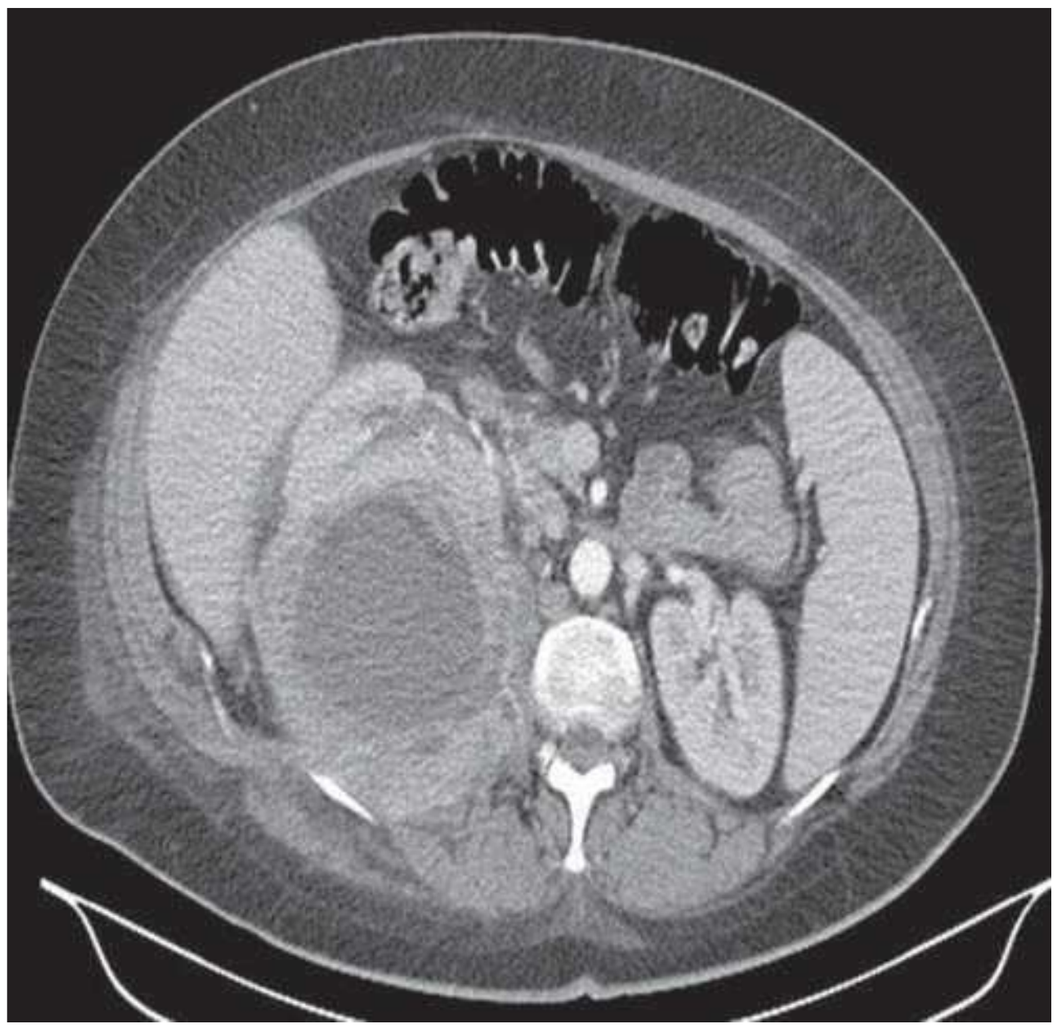

FIGURE 11.14 Contrast-enhanced CT showing a large, thick-walled abscess arising from the posterior cortex of the right kidney and extending into the adjacent posterior pararenal space.

enhancement after the administration of gadolinium contrast agents. On the T2-weighted images with fat saturation, edema and an inflammatory reaction may present with areas of an increased signal in the renal parenchyma and perirenal space.[30,41,66]

Renal Abscesses

Renal abscesses appear as well-defined collections of fluid in nonlobar distributions. An abscess may present with a thick wall that enhances after the administration of contrast agents in CT scans or MRIs and may contain air (Fig. 11.14). Abscesses may extend or rupture into the perirenal fat. A CT scan is superior to sonography in delineating abscesses and the pararenal extent of inflammation and is preferred when intervention is planned.[67,68] Abscesses may show varied signal intensity on MRIs depending on the content of fluid within the abscesses.

Fungal Infections

Fungal infections of the kidney are rare and are seen in immunocompromised patients. CT findings include a focal or global lack of contrast excretion, renal mass, renal enlargement, and filling defects of soft-tissue masses within the renal collecting system.[68]

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is an uncommon inflammatory condition that often follows chronic renal obstruction. It produces intrapelvic and intracalyceal collections of fluid or fatty material. The renal parenchyma is often atrophic and is replaced by accumulated fat, pus, and cellular debris and calcification.[69] Calcification in the collecting system and perirenal abscesses are frequently present.

Cystic Disease

Cysts in the kidneys are extremely common and an uncomplicated renal cyst can be diagnosed reliably by a CT scan or an MRI. Simple renal cysts are variable in size and number. On a CT scan, a simple cyst usually appears as a well-defined rounded mass of water attenuation (0 to 20 HU), with an imperceptible wall and no enhancement after the administration of contrast agents. The MRI appearance of a simple renal cyst is characterized by a sharply demarcated, homogeneous, and hypointense mass on T1-weighted images. The simple cyst becomes uniformly hyperintense on T2-weighted images and shows no enhancement following contrast medium administration.[70–73] Complex renal cysts may be irregular in shape and have thicker or calcified walls. Fluid within complex cysts may present with high attenuation (hyperdense cysts) on a CT scan or complex signal intensity on an MRI (bright T1 and dark T2 or bright T1 and T2 images); in fact, they may simulate solid tumors. Such lesions require a further diagnostic evaluation with a dedicated renal CT scan or an MRI protocol, including scanning before and after the administration of a contrast agent. The degree of contrast enhancement within a lesion is critical in the characterization of the renal lesion.

Cystic renal lesions are often characterized according to the Bosniak classification system.[71,72] Class I cysts are simple benign cysts. Class II cysts have one or more thin (<1 mm) septa running through them, thin areas of mural calcification, or fluid contents of increased attenuation; they do not enhance after the administration of contrast medium and are benign (Fig. 11.15). Class III cysts are more complicated and contain thickened septa, nodular areas of calcification, or solid nonenhancing areas. Such lesions are suggestive of malignancy and should be biopsied or surgically explored, although fewer than half will turn out to be malignant. Class IV cystic masses are clearly malignant, with solid enhancing nodules or irregular walls, and should be treated accordingly (Fig. 11.16). A subcategory, IIF, has been suggested for lesions with multiple class II features, and these require follow-up.[65,70,74–81]

Parapelvic Cysts

Extraparenchymal cysts commonly occur in the renal sinus (parapelvic or peripelvic cysts). These cysts are often discovered incidentally, are frequently multiloculated, and may be large enough to displace hilar fat and compress adjacent renal parenchymas.[65,70,74–81] Although they do not communicate with the collecting system, they may simulate hydronephrosis because of their proximity to

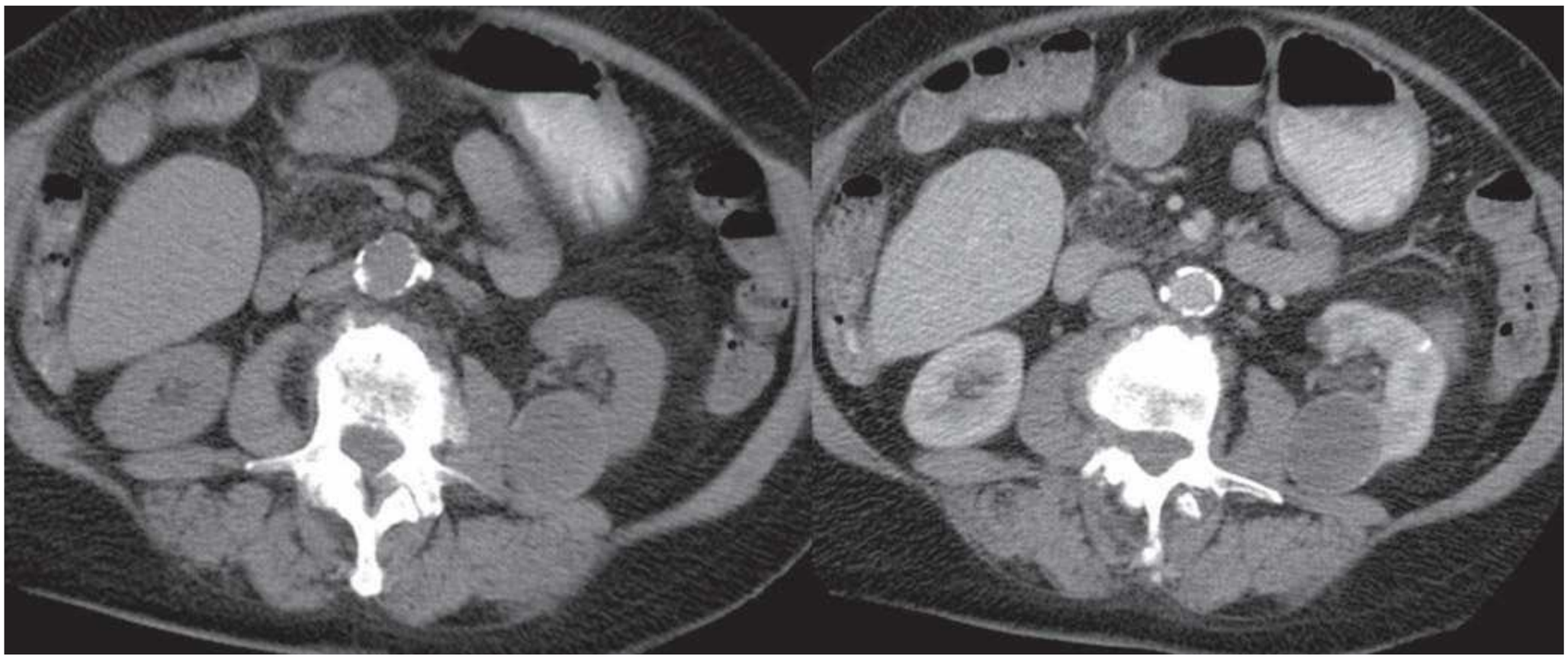

FIGURE 11.15 Bosniak class II cyst in the left kidney with slightly higher attenuation contents and subtle peripheral hyperdense rim on the pre-contrast CT but no contrast enhancement on the post-contrast CT.

the collecting system. When the diagnosis is uncertain, a delayed CT scan or an MRI after the administration of contrast agents can help differentiate unenhanced parapelvic cysts from a contrast-enhanced dilated collecting system (Fig. 11.17).

Autosomal Dominant Polycystic Kidney Disease

In individuals with a positive family history, the diagnosis of autosomal dominant polycystic kidney disease (ADPKD) can be established by radiologic imaging. The reported sonographic criteria[82] cannot be used with a CT scan or MRI because these imaging modalities have a higher sensitivity than US for the detection of renal cysts, particularly small cysts.[83,84] The kidneys are affected bilaterally in almost all instances, but may be quite asymmetric. Early in the disease, the kidney is close to normal in size with a substantial amount of normal renal parenchyma. However, with disease progression, the kidneys gradually enlarge as the cysts increase in number and size and replace normal parenchymas (Fig. 11.18).[85]

Most ADPKD renal cysts are simple cysts but increase with complex cysts as the disease progresses. The CT attenuation and MRI signal intensity of complex cysts affected by hemorrhage or infection may vary. ADPKD patients with

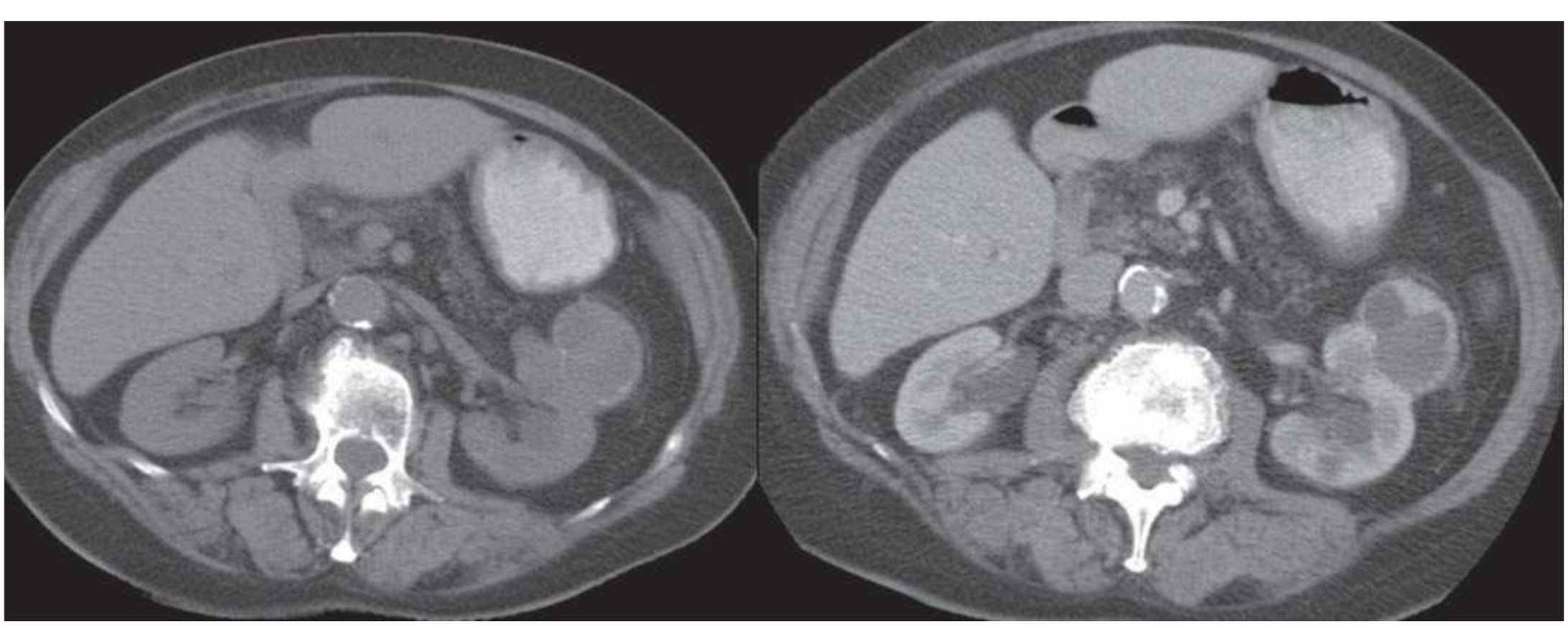

FIGURE 11.16 Pre- and post-contrast CT of Bosniak class IV cyst in the left kidney demonstrating peripheral nodular enhancement.

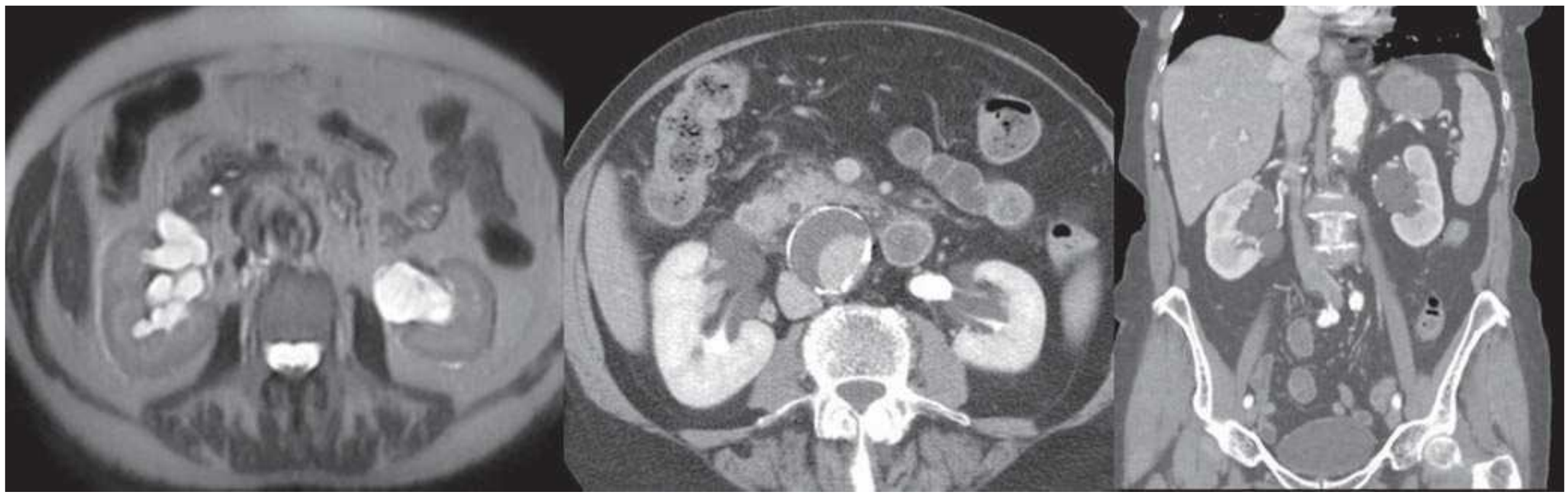

FIGURE 11.17 Bilateral parapelvic cysts are shown as bright structures on axial T2 MRI (*left*) and water-attenuation cystic structures on contrast-enhanced axial CT (*middle*) and coronal CT (*right*). The parapelvic cysts on axial CT do not enhance but are surrounded by delayed excretion of contrast medium within the collecting system. An incidental large abdominal aortic aneurysm is noted.

clinically suspected renal infection or pain are referred for a CT scan or an MRI evaluation of intracystic infection or hemorrhage.[65,70,74–81,85,86] But this is often difficult because the attenuation and signal intensity values of these cysts vary depending on the presence of blood products, proteinaceous mucoid material, or simple cyst fluid. Sequential studies allow for the evaluation of a fresh hemorrhage and the resolution of hematomas.

Acquired Cystic Disease Associated with Chronic Dialysis

Patients with acquired cystic disease associated with hemodialysis typically present with multiple bilateral cysts of varying size in small kidneys (Fig. 11.19). Although sonography is usually used for a diagnostic evaluation, small fibrotic end-stage kidneys may be difficult to image with sonography.[87] A contrast-enhanced CT scan or MRI is more sensitive

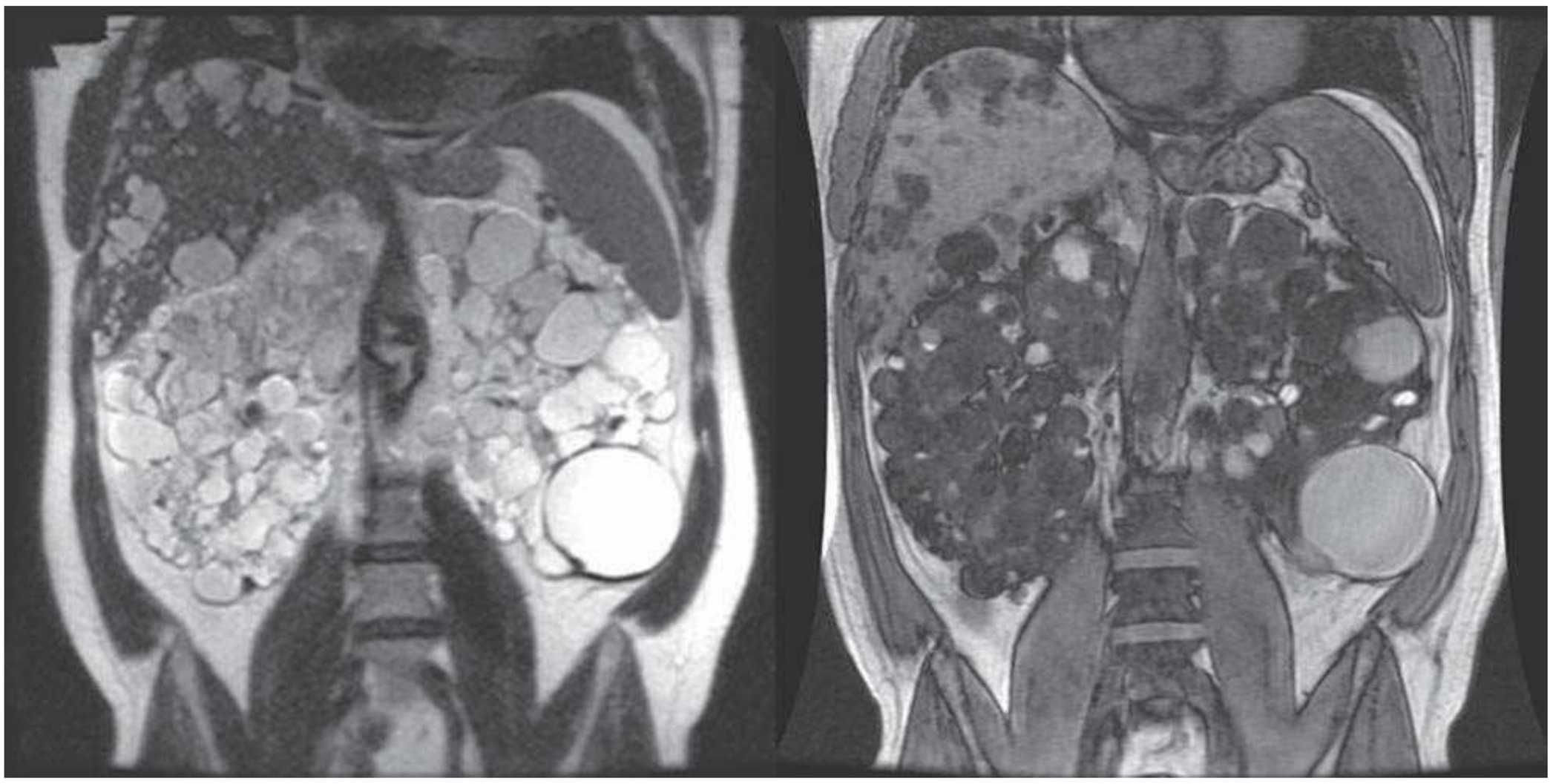

FIGURE 11.18 ADPKD on coronal MR images. Innumerable cysts occupying markedly enlarged bilateral kidneys on T2 (*left*) and T1 (*right*) MR images. Simple cysts are of bright T2 and dark T1 signal intensity, whereas complex cysts present with intermediate to bright T1 signal intensities. Extensive hepatic cysts are also present.

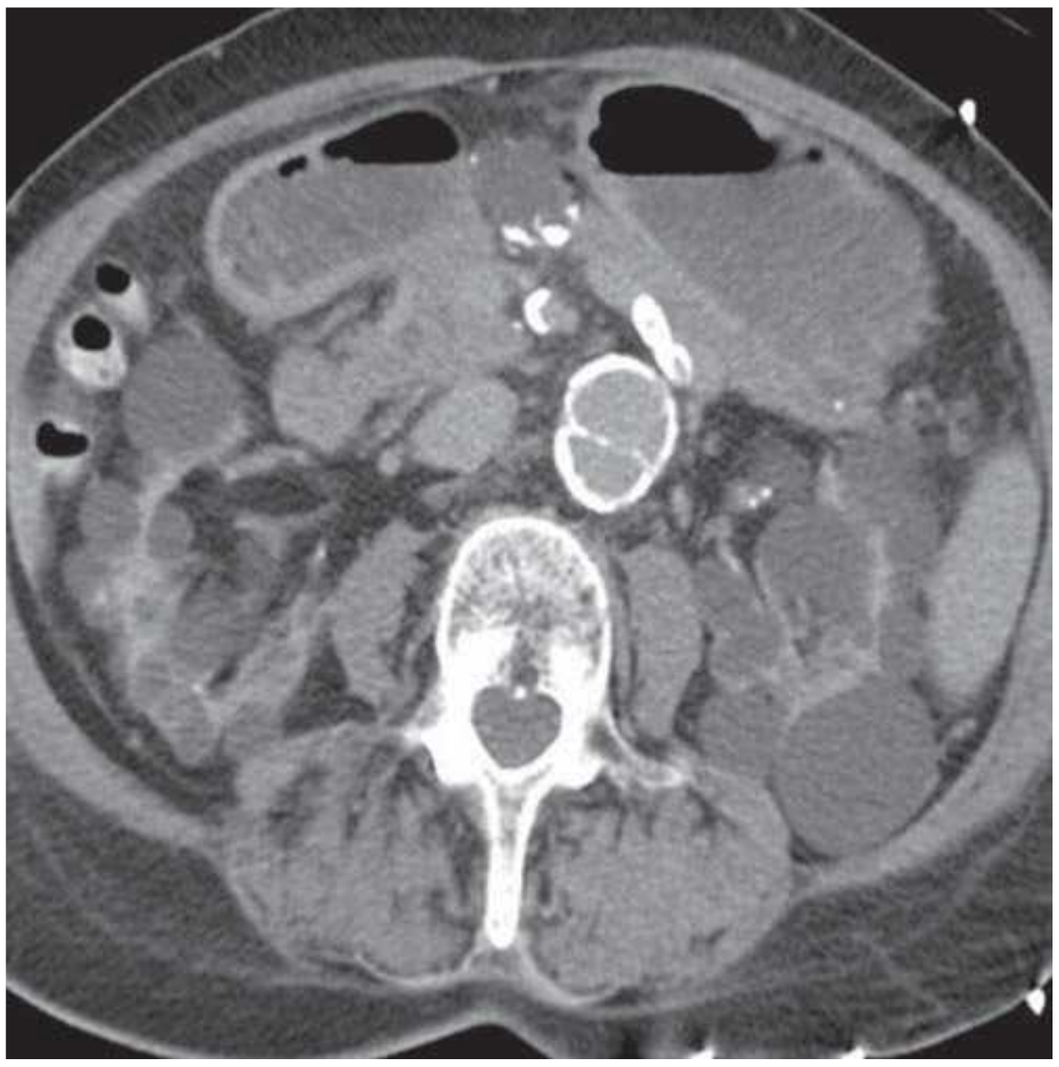

FIGURE 11.19 Contrast-enhanced CT showing multiple cysts of varying sizes in both kidneys that are markedly atrophied.

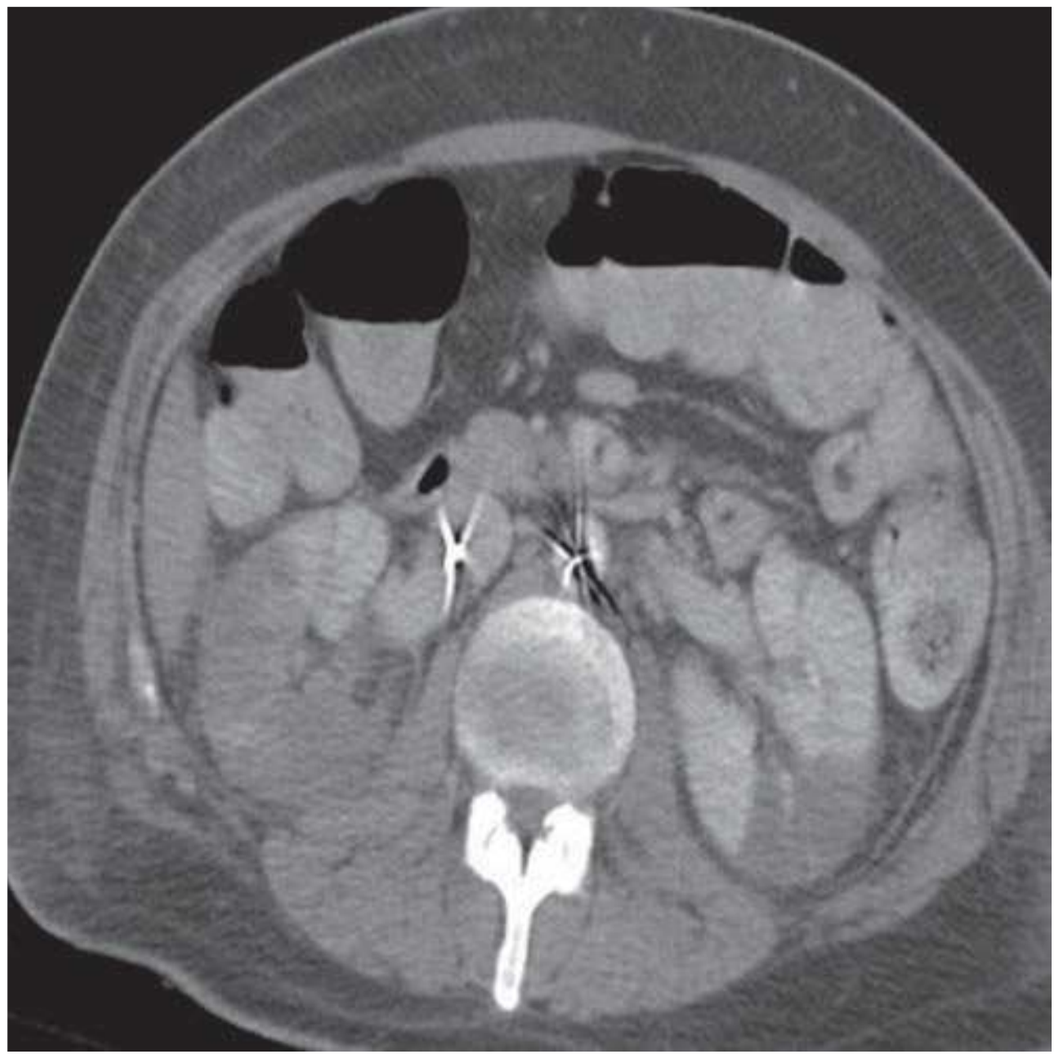

FIGURE 11.20 Contrast-enhanced CT demonstrating bilateral multiple renal infarcts as wedge-shaped nonenhancing low-attenuation areas.

to determine the extent of the disease, cyst complications, and renal carcinomas.[88–90] Because of limited or an absent renal function of these individuals and the presence of numerous simple and complex cysts, an MRI is the preferred modality for the evaluation of potential renal malignancies. Nevertheless, a contrast-enhanced CT scan may also be used if patients are receiving dialysis.[90]

Vascular Pathologies

Renal infarcts typically present as clearly marginated wedge-shaped peripheral areas of low attenuation on contrast-enhanced CT scan (Fig. 11.20) or low signal intensity on contrast-enhanced MRI. A parenchymal atrophy caused by chronic vascular insufficiency may be detected on a CT scan or MRI.

A CT scan or an MRA is used to assess the number and size of the renal arteries, which is crucial for the evaluation of a renal donor for transplant. A CTA and an MRA have largely replaced conventional catheter angiography for this application. A CTA and an MRA can also demonstrate renal artery aneurysms or stenosis (Fig. 11.21), arteriovenous malformations, and focal or diffuse stenoses caused by atherosclerosis, connective tissue disease, or fibromuscular dysplasia. Although the 3D gadolinium-enhanced protocol is the preferred technique for an MRA, new time of flight MR protocols have been introduced to image renal vessels without gadolinium for patients with a severely compromised renal function.

Renal vein thrombosis typically presents with intraluminal filling defects and vein enlargement (Fig. 11.22). Acute venous occlusion may result in renal enlargement and parenchymal edema and, occasionally, hemorrhagic infarction. A contrast-enhanced CT scan or an MRI may show a persistent nephrogram and delayed contrast excretion. A parenchymal atrophy and the development of venous collaterals may be followed with chronic renal vein thrombosis.[3,91–98]

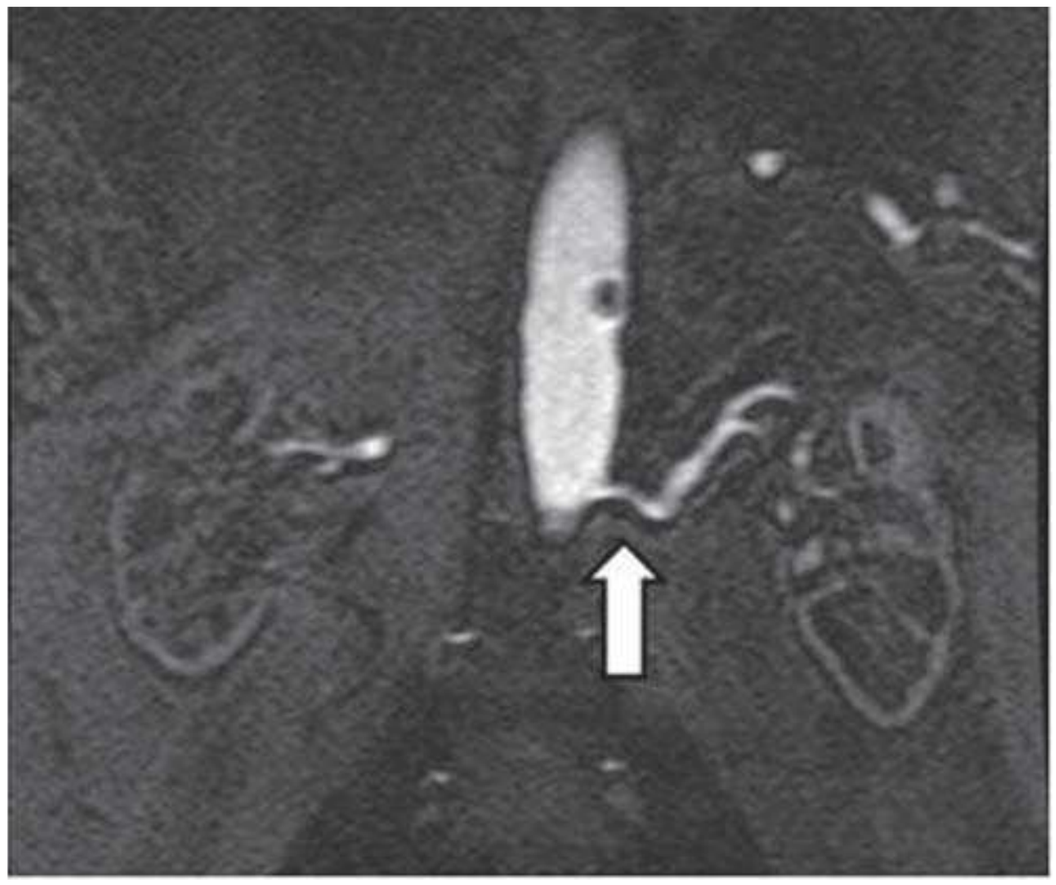

FIGURE 11.21 Contrast-enhanced coronal MR angiography showing a focal stenosis near the origin of left renal artery (*arrow*).

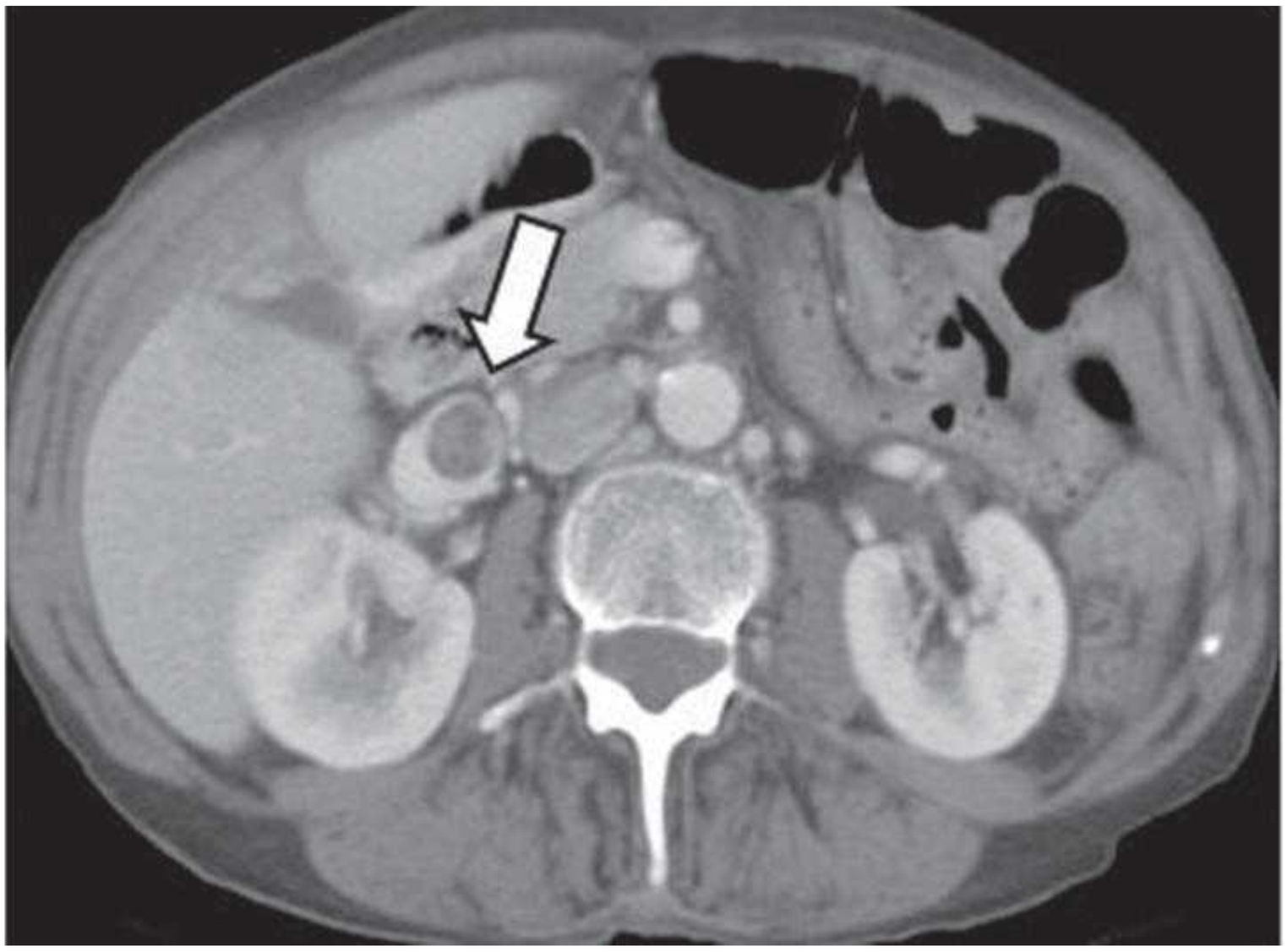

FIGURE 11.22 Contrast-enhanced CT showing a low-attenuation filling defect (*arrow*) in an expanded right renal vein consistent with renal vein thrombosis.

Renal Transplant Evaluation

The structural integrity of renal allografts and common peritransplant complications (hematomas, urinomas, lymphoceles, and abscesses) can be readily assessed with a CT scan or an MRI. However, the characterization of graft dysfunction, such as the differentiation of rejection and acute tubular necrosis (ATN), remains challenging by any radiologic imaging method. Recent applications of functional MRI techniques including diffusion, blood oxygen level dependent (BOLD), and sodium MRI are promising for the noninvasive evaluation of graft dysfunction[16] but are not ready for routine clinical application. A CTA or an MRA are very useful for the evaluation of the allograft vasculature including arterial or venous stenosis and aneurysm.[99,100]

Renal Tumors

The evaluation of hematuria is a common reason for a urology referral. Historically, the workup included IVU, urine cytology, and cystoscopy. Other imaging modalities available for hematuria workup include a CT scan, ultrasonography, an MRI, and a retrograde pyelography. In particular, MDCT is increasingly used as a single-imaging comprehensive evaluation of a patient with hematuria. The unenhanced phase of the CT scan is highly reliable for diagnosing urolithiasis. The enhanced nephrographic phase aids in the detection of renal parenchymal masses. The excretory phase with 3D reformation allows for the evaluation of the entire urothelium. MDCT has been shown to have high sensitivity in detecting upper tract urothelial cancers. Some investigators add a corticomedullary phase to characterize the renal artery and vascularity of parenchymal renal masses. One concern about this comprehensive CT technique is the radiation dose to the patient, and some investigators advocate not covering the entire abdomen and pelvis in all phases of the examination in order to limit the radiation dose.[32,37,52,56]

An MRI is equivalent to a CT scan in its ability to detect renal lesions of approximately 1 cm[56] and in detecting a lymphadenopathy.[56] An MRI detects polar lesions better than a CT scan and has up to 100% sensitivity in detecting a renal vein invasion. Most renal tumors, including benign lesions, enhance on CT scans and MRIs. T1-weighted chemical-shift MRIs are highly reliable for the detection of fat content within renal lesions. With fat saturation sequences, the fatty portions of a mass drop in signal, which is diagnostic of a fat-containing renal mass such as angiomyolipoma. After the exclusion of angiomyelolipomas and in the absence of lymphoma and metastatic disease, all other enhancing renal lesions represent surgical lesions. Unfortunately, neither CT scans nor MRIs can reliably differentiate some benign enhancing tumors such as oncocytomas from renal cell carcinomas.[32,37,56,65,71,94,101–109]

Benign Tumors

Angiomyolipoma

Angiomyolipomas are benign lesions composed of variable amounts of fat, smooth muscle, and abnormal blood vessels. They occur spontaneously in the general population, mainly in women in the fifth decade of life. In patients with tuberous sclerosis, they occur at a much younger age and are frequently multiple, with an incidence of 50% to 80%.[110,111] A confident diagnosis can be made with a CT scan if the fatty tissue predominates. The tumor may grow very large in size and may extend into the perinephric space. An intralesional hemorrhage often occurs. The MRI appearance of angiomyolipomas depends on the amount of fat, smooth muscle, and vessels within the lesion.

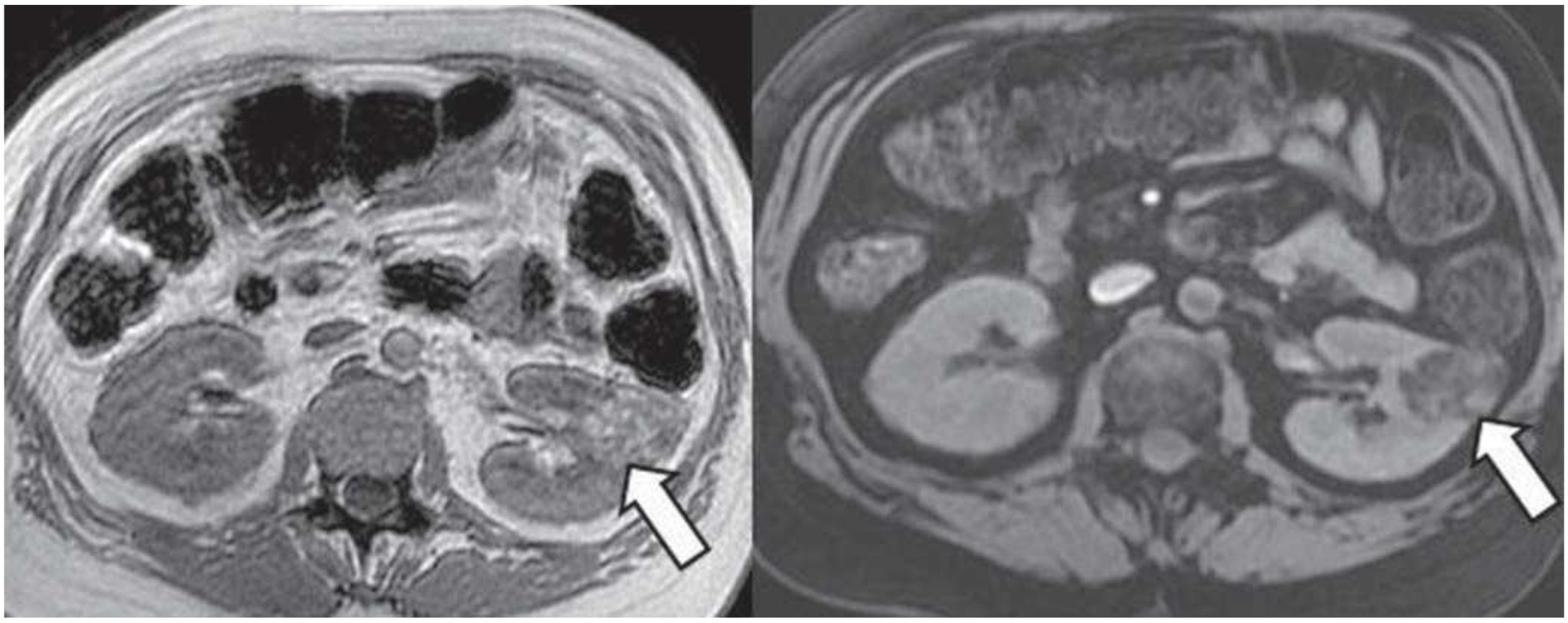

FIGURE 11.23 Angiomyolipoma (*arrow*) in the left kidney with bright signal intensity similar to the surrounding body fat on T1-weighted MRI without fat saturation (*left*) but showing a signal drop on T1-weighted with fat saturation (*right*).

Angiomyolipomas composed predominantly of fat present with signal intensity on T1- and T2-weighted images similar to the surrounding fatty structures and demonstrate a signal drop on the fat suppression sequences (Fig. 11.23).[65,102,110,112]

Renal Oncocytomas

Renal oncocytomas are rare tumors that typically appear as smoothly marginated, homogeneously enhancing solid masses. These masses may demonstrate a characteristic central linear area of lower attenuation (stellate central scar) on a contrast-enhanced CT scan. But, this appearance on CT is found in only a small proportion of these tumors and is not specific, and the diagnosis of these benign masses necessitates an operative biopsy/resection (Fig. 11.24).[7,65,76,105,107] A recent CT study reported that the segmental enhancement inversion between two phases of a contrast-enhanced CT scan was significantly more frequent in oncocytomas than in renal cell carcinomas and could be diagnostically useful in differentiating the two.[113]

Renal Adenomas

Renal adenomas are indistinguishable from other solid renal masses on CT scans or MRIs. They may show variable contrast enhancement and may present as a contour abnormality or distortion of intrarenal anatomy, necessitating further workup and intervention.[114]

Malignant Tumors

Renal Cell Carcinoma

Renal cell carcinomas are a relatively common malignancy. Their appearance on CT scans and MRIs varies with the size and tumor vascularity. Tumors may distort the renal contours or alter intrarenal architecture (Fig. 11.25). On a CT scan, their attenuations are similar to those of the surrounding renal parenchyma in unenhanced images. Tumors often enhance heterogeneously on enhanced CT scans, with central areas of low attenuation (Fig. 11.26). On an MRI, tumors demonstrate a variable signal on both T1- and T2-weighted images. The most common appearance is a heterogeneous mass of low-to-intermediate signal on T1-weighted images that increases in signal intensity on T2-weighted images. Dynamic CT scans or MR scanning after a bolus of contrast may demonstrate an intense enhancement of relatively vascular tumors and retroperitoneal feeding of collateral vessels.[93,115–117] An extension of the tumor into the perirenal

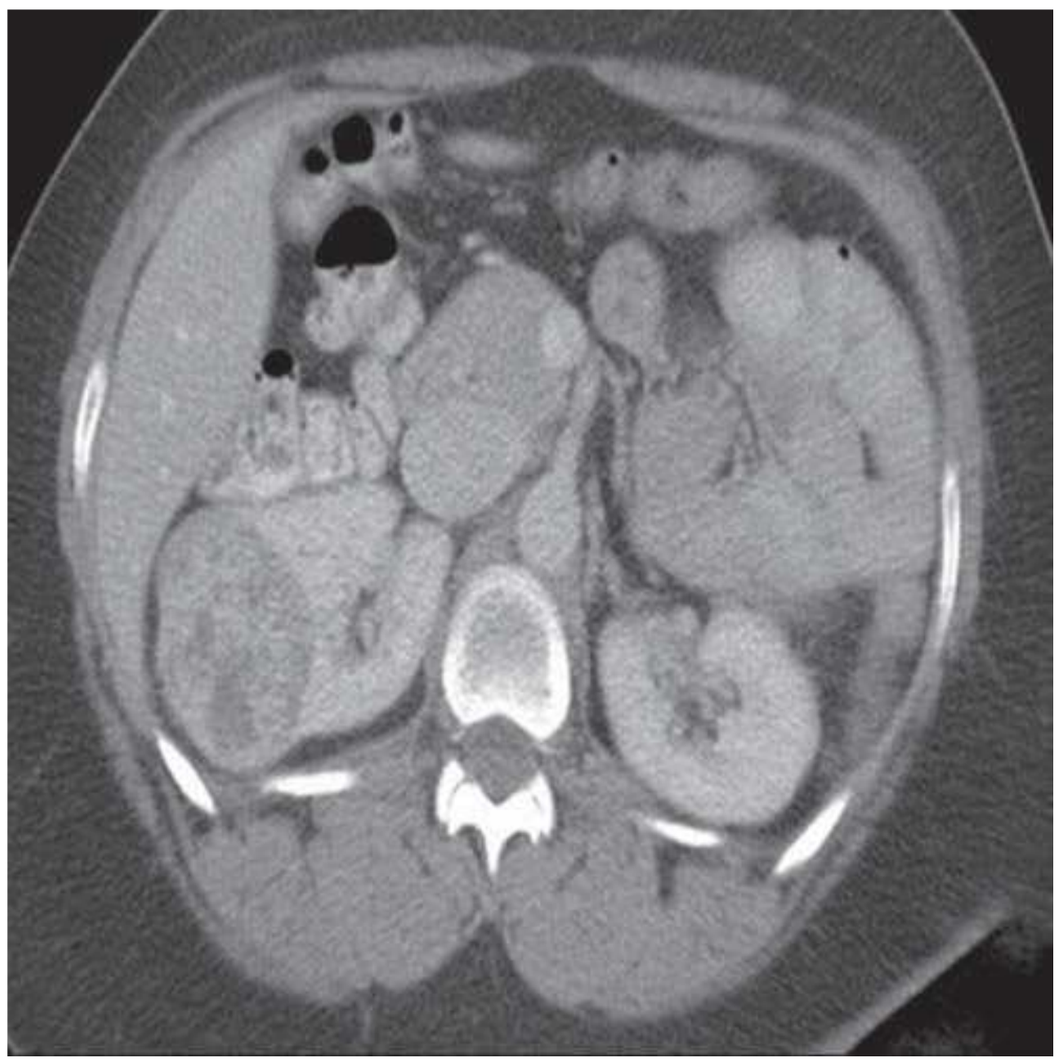

FIGURE 11.24 Contrast-enhanced CT showing an oncocytoma in the right kidney with mixed areas of enhancement and low attenuation. This is difficult to differentiate from renal cell cancer based on imaging alone.

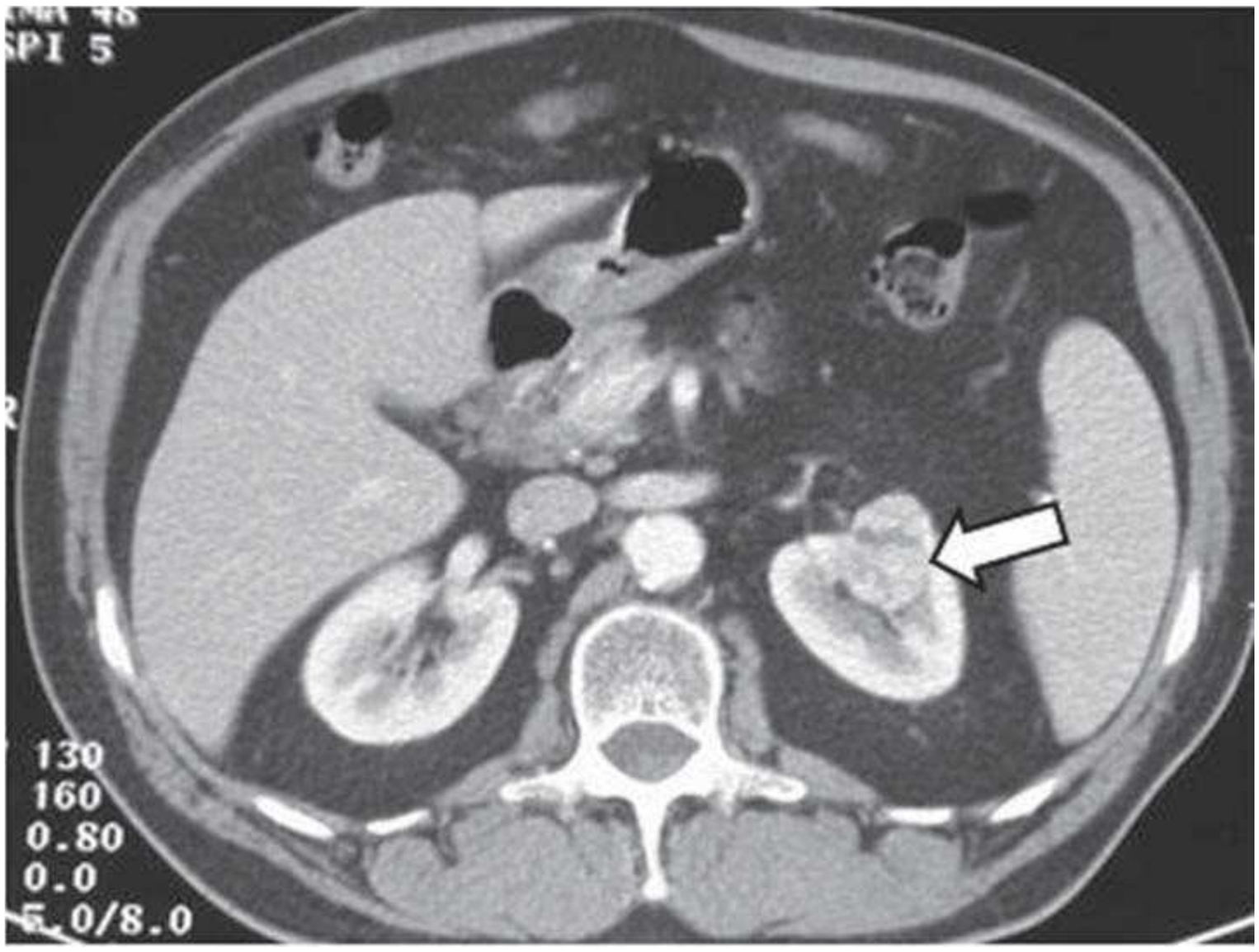

FIGURE 11.25 Contrast-enhanced CT showing a subtle left renal cell cancer replacing the left renal sinus fat.

fat or the adjacent liver, spleen, or paraspinous musculature; an interruption of the perirenal fascial planes, renal veins, and inferior vena cava; and perihilar and perivascular adenopathy can be assessed accurately by CT scans or MRIs. MRIs and CT scans have been shown to be comparable in the diagnosis and the staging of renal cell carcinomas. Because of greater spatial resolution, a CT scan is superior to an MRI in detecting small lesions, and an MRI has not been used as a screening tool in patients with hematuria. However, MRIs have been shown to be more accurate than CT scans in tumor staging. MRIs are particularly beneficial for the evaluation of the patency of the renal veins and inferior vena cava, the delineation of perivascular lymphadenopathy, and the extension of the tumor into adjacent organs.[93,115–117] Three-dimensional image reconstructions of renal tumors can be obtained with MDCT.[56,118] This information can help the surgeon, especially if a partial nephrectomy is being considered. CT-guided radiofrequency ablations of renal cell carcinomas are an alternative treatment option, particularly in patients that are not surgical candidates. CT scans are also used to follow these patients to assess for residual disease or recurrence.[9,40,56,93,102,106,108,119–124] In many institutions, a CT scan is the technique of choice for the diagnosis and staging of renal cell cancer; an MRI is used when a contrast-enhanced CT scan is contraindicated, or if frequent follow-up is required in high-risk patients.[93,115–117]

Renal Lymphoma or Leukemia

Primary lymphoma of the kidney is rare because there is no lymphatic tissue within the kidneys. Renal involvement may be due to hematogenous spread or contiguous invasion from an adjacent retroperitoneal lymphadenopathy. The kidneys are more commonly involved in non-Hodgkin lymphoma than in Hodgkin lymphoma, particularly when the disease has relapsed.[125–127]

Renal lymphoma has a variable radiologic appearance on CT scans and MRIs. The most common pattern is multiple masses that are of the same or slightly higher attenuations than normal renal parenchyma on an unenhanced CT scan and are low to intermediate in signal intensity on T1- and T2-weighted MRIs. Lymphomas are hypovascular tumors and demonstrate lower contrast enhancement than normal renal parenchymas[125–127] on contrast-enhanced CT scans or MRIs

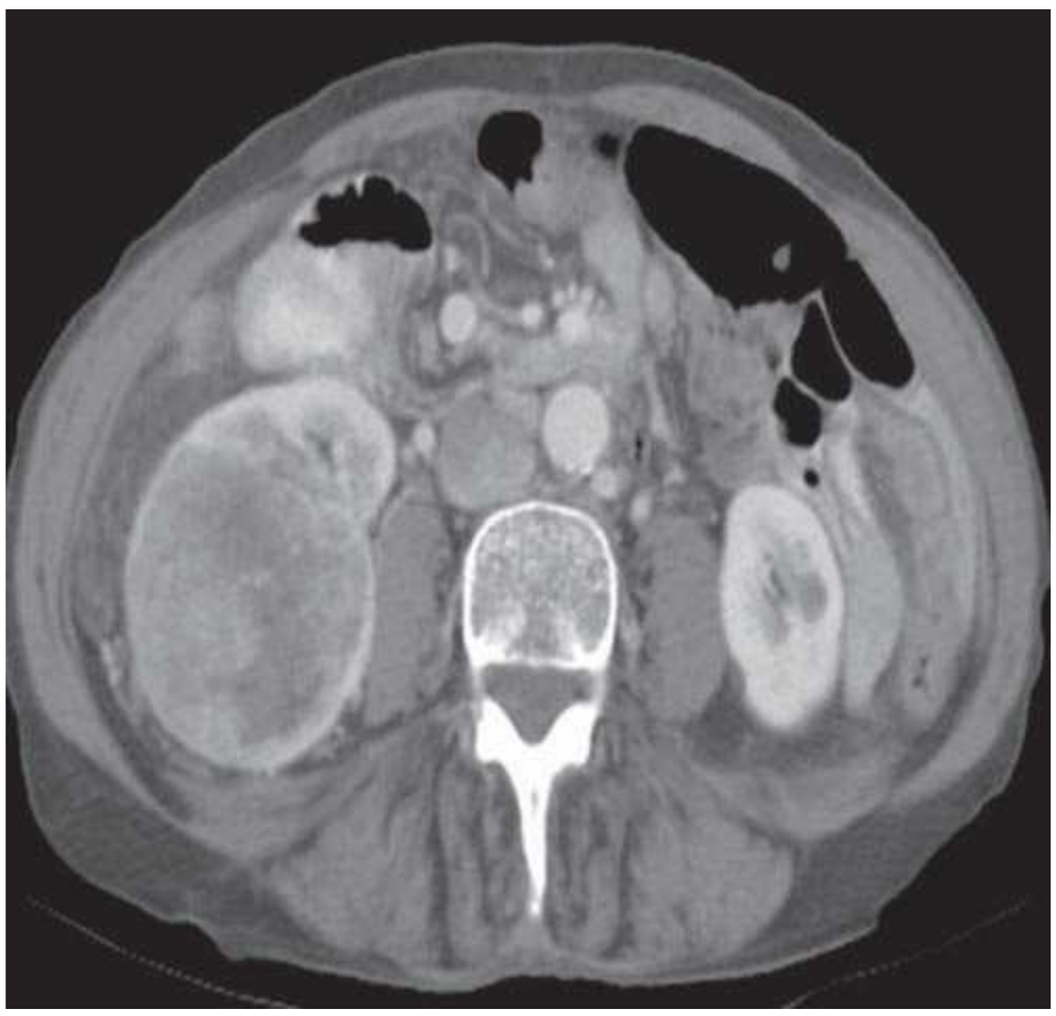

FIGURE 11.26 Contrast-enhanced CT showing a large right renal carcinoma with heterogeneously enhancing solid areas and necrosis.

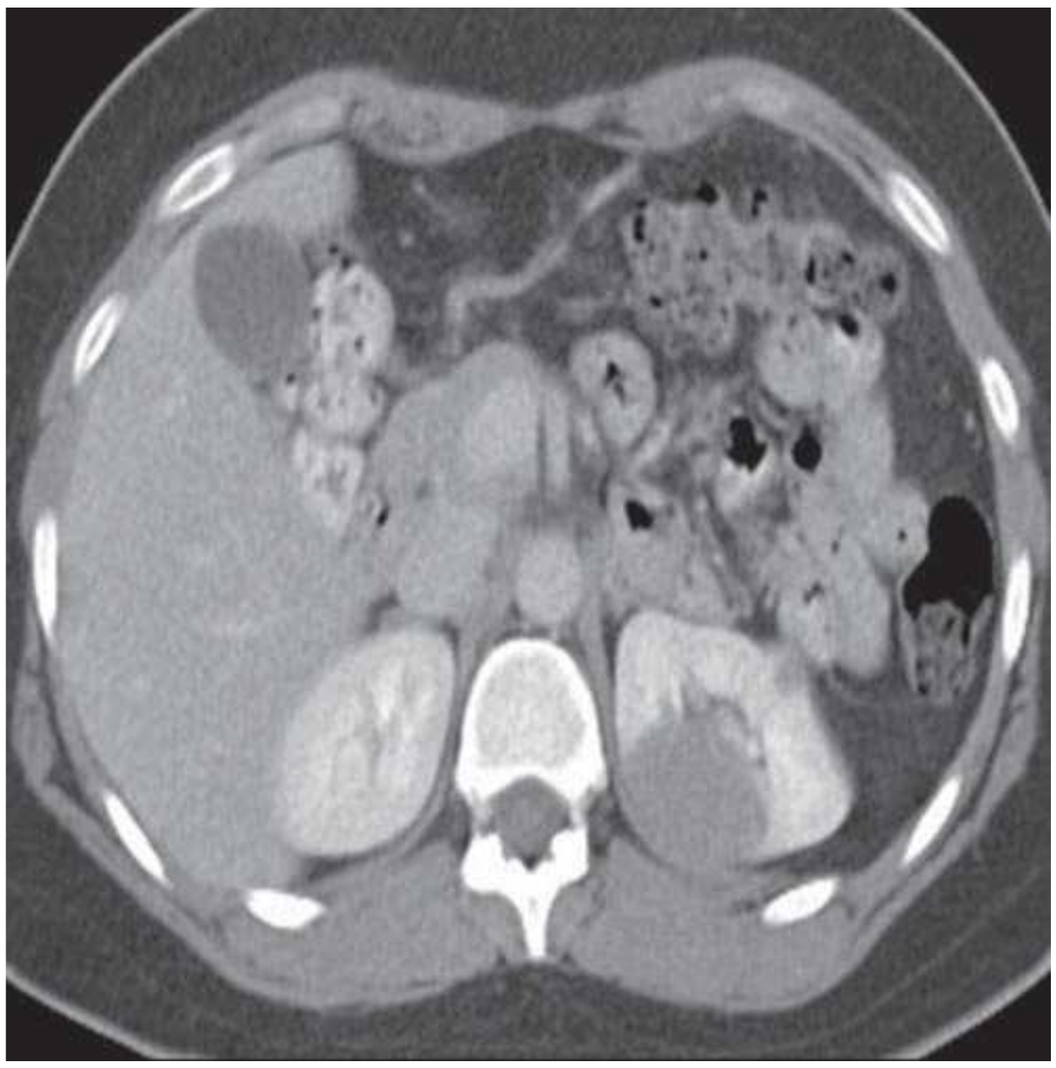

FIGURE 11.27 Contrast-enhanced CT showing a low-attenuation mass in the left kidney consistent with lymphoma.

(Fig 11.27). Other appearances include a direct invasion from adjacent retroperitoneal lymphadenopathy, an infiltrative pattern with renal enlargement, or a solitary mass.[125–127]

Leukemic renal infiltration is frequently seen at a postmortem examination and can be associated with renal impairment. It may present as unilateral or bilateral renal enlargement or focal mass or masses.[128]

Metastatic Disease

Primary tumors that may metastasize to the kidney include carcinomas of the lung, breast, adrenal gland, and colon; malignant melanoma; and non-Hodgkin lymphoma.[41,43] These tumors may present as either a solitary or multiple focal mass that demonstrates less enhancement than normal renal parenchyma on a contrast-enhanced CT scan or an MRI. The direct extension of extrarenal retroperitoneal tumors may lead to renal obstruction and a loss of function.[41,43]

Renal Sarcomas

Sarcomas of the kidney are rare. They present as solid masses that may grow very large in size and vary in their degree of vascularity.[129] Large tumors often contain central areas of necrosis. The imaging characteristics are nonspecific, making it difficult to distinguish a renal sarcoma from a renal cell carcinoma.

Transitional Cell Carcinoma

Transitional cell carcinomas of the renal collecting system and ureter present with three characteristic appearances on a CT scan or an MRI: sessile soft-tissue filling defects in the lumen of the enhanced collecting system (Fig. 11.28) or ureter; thickening of uroepithelial wall; or infiltrating nonenhancing renal masses (Fig. 11.29). Tumors may be smooth or papillary in contour and may cause ureteral obstruction and renal functional impairment. In addition to assessing the collecting system and ureter, CT urography allows us to detect and characterize renal lesions and extrarenal masses and is more sensitive in detecting renal calculi.[28,32,43,52,54,56,93,130] A CT urography is also the imaging of choice for tumor staging, demonstrating the invasion of perihilar fat and associated lymph node enlargement. Alternatively, an MR urography can be used to assess the collecting system in patients with a contraindication to iodinated contrast CT scans.

Renal Trauma

Renal injury is common, occurring in 8% to 10% of the cases of blunt and penetrating abdominal trauma. About 90% of renal injuries result from blunt force injury and 10% from penetrating

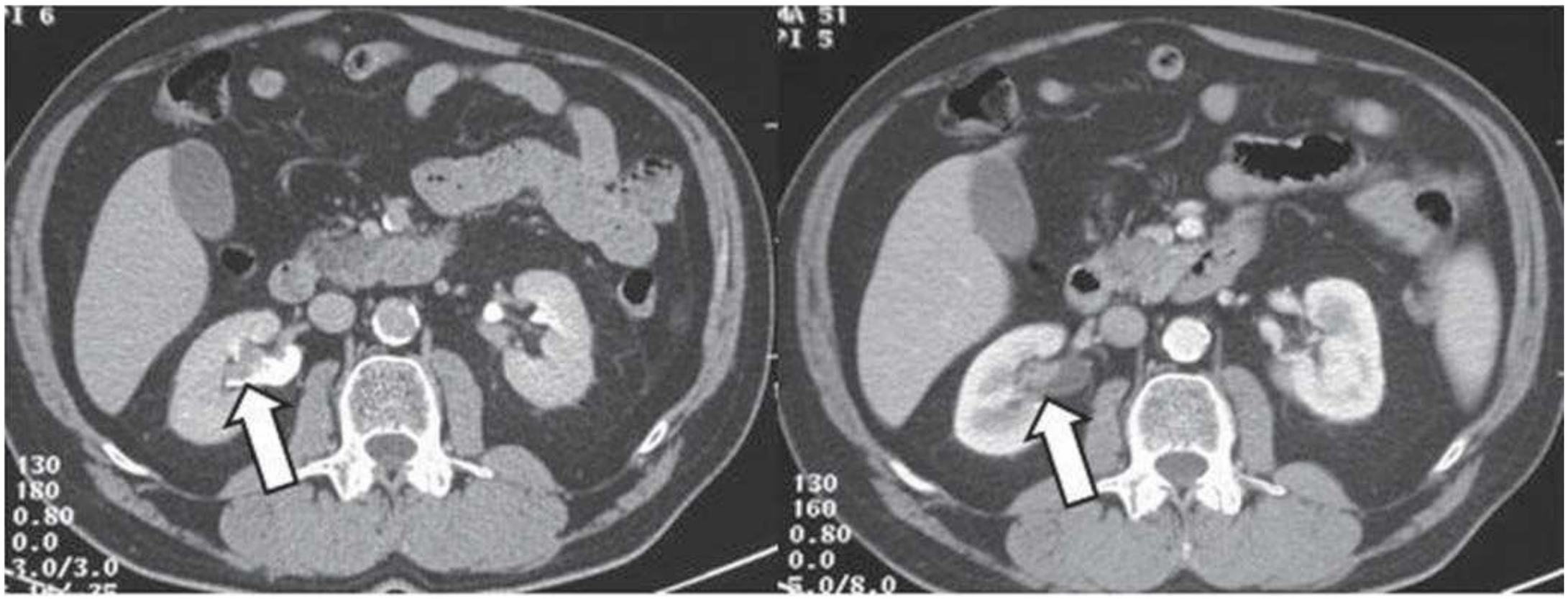

FIGURE 11.28 TCC in right kidney presenting as a filling defect on the excretory phase (*left*) and demonstrating subtle enhancement on an earlier enhancement phase (*right*).

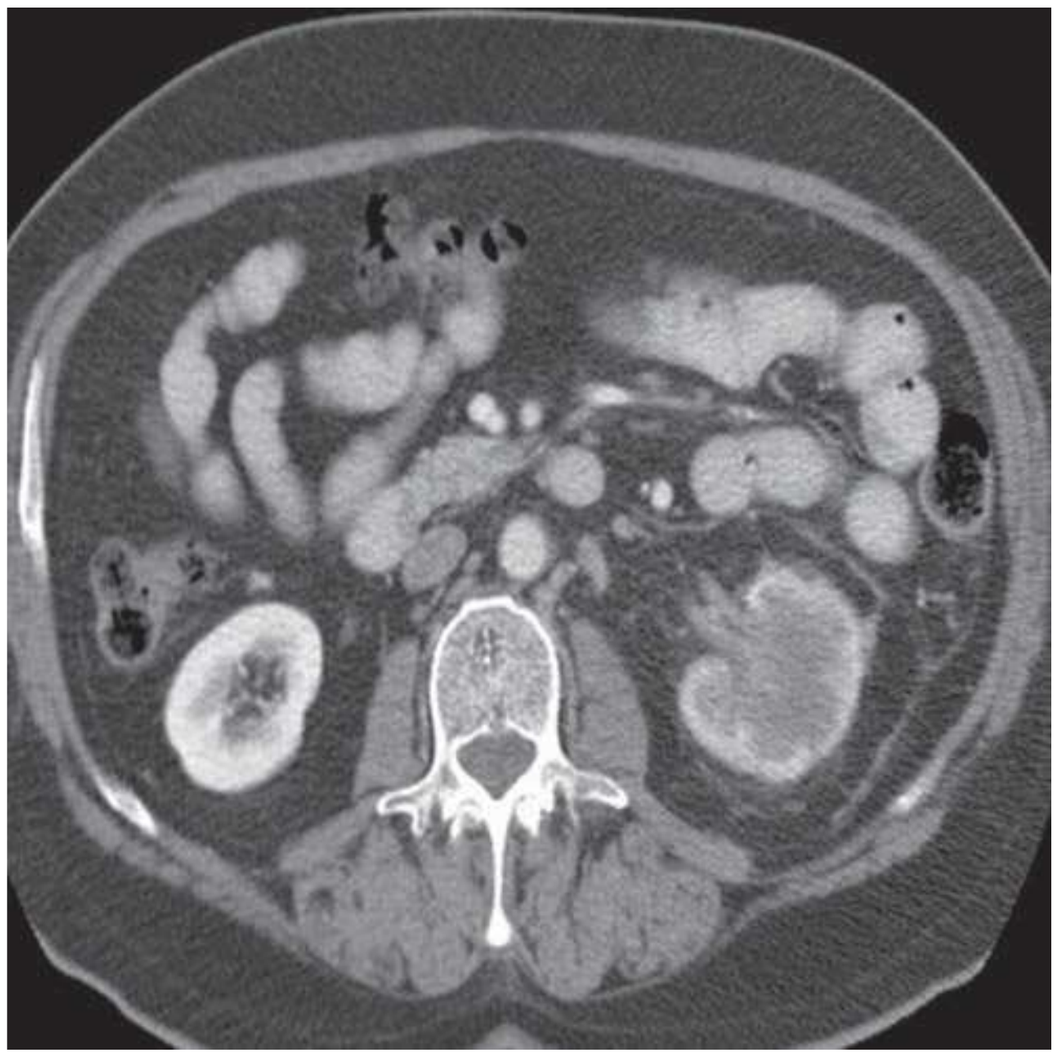

FIGURE 11.29 TCC in left kidney presenting as diffuse infiltrating mass that effaces the normal corticomedullary differentiation, resulting in a faceless kidney.

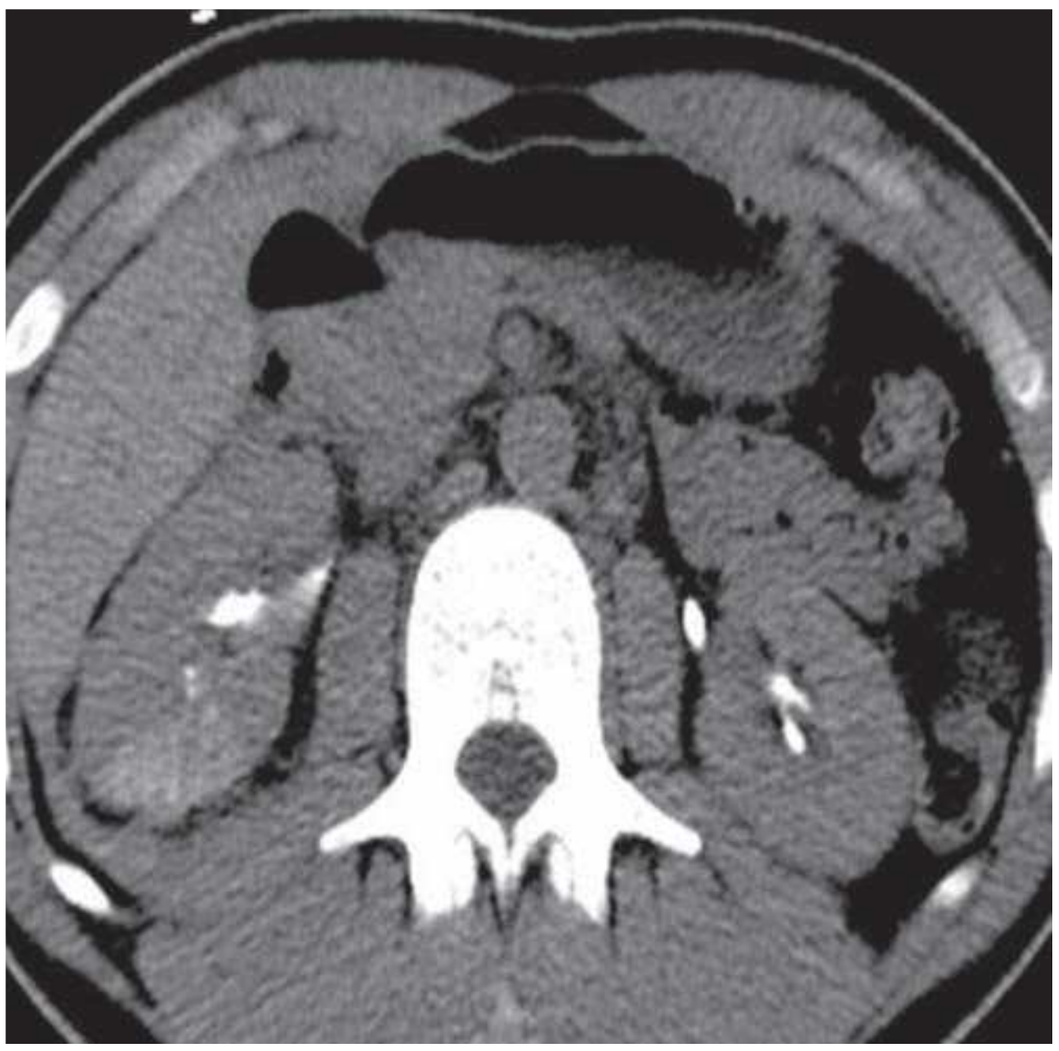

FIGURE 11.30 Unenhanced CT showing a focal hyperdense area in the right kidney consistent with contusion. Contrast in the collecting system is from contrast-enhanced CT performed a day prior.

trauma, but vary somewhat with location. The clinical indications for the imaging evaluation of the genitourinary system depend on several factors, including the overall hemodynamic status of the patient, other injuries sustained, the site of blunt or penetrating trauma, and the presence or absence of gross hematuria.[37,56,131] The American Association for the Surgery of Trauma (AAST) has devised a renal injury severity score based on surgical observations. A CT grading scale following the surgical management is classified as grade I, a minor contusion with or without concomitant subcapsular hematoma; grade II, a superficial laceration without the involvement of the collecting system; grade III, a deep parenchymal laceration with or without urinary extravasation; and grade IV, a renal pedicle injury.

The majority of blunt renal injuries are CT grades I and II and are managed conservatively without intervention.[37,56,131] Contusions are visualized as ill-defined low attenuation areas with irregular margins in the renal parenchyma. They may appear as regions with a striated nephrogram due to differential blood flow through the contused parenchyma or as focal areas of renal parenchymal extravasation on delayed CT studies (Fig. 11.30). These lesions usually resolve during follow-up imaging.[37,56,131] More significant renal trauma may or may not require intervention by angiography or surgery. Controversy exists over the management of grade III injury, with the most recent trend favoring conservative therapy. Grade IV and penetrating renal injuries are surgically explored.[132–134]

Patients with suspected renal injuries who are clinically stable can benefit greatly from a CT assessment. A contrast enhanced CT scan can detect renal contusions and lacerations. Lacerations present with irregular streaks of low attenuation within the contrast-enhanced renal parenchyma and are classified as incomplete lacerations (affecting the renal parenchyma without communicating with pelvicalyceal structures) and complete lacerations (interrupting the collecting system). Subcapsular hematomas are shown as a lower density intracapsular hemorrhage compressing the enhanced kidney (Fig. 11.31).

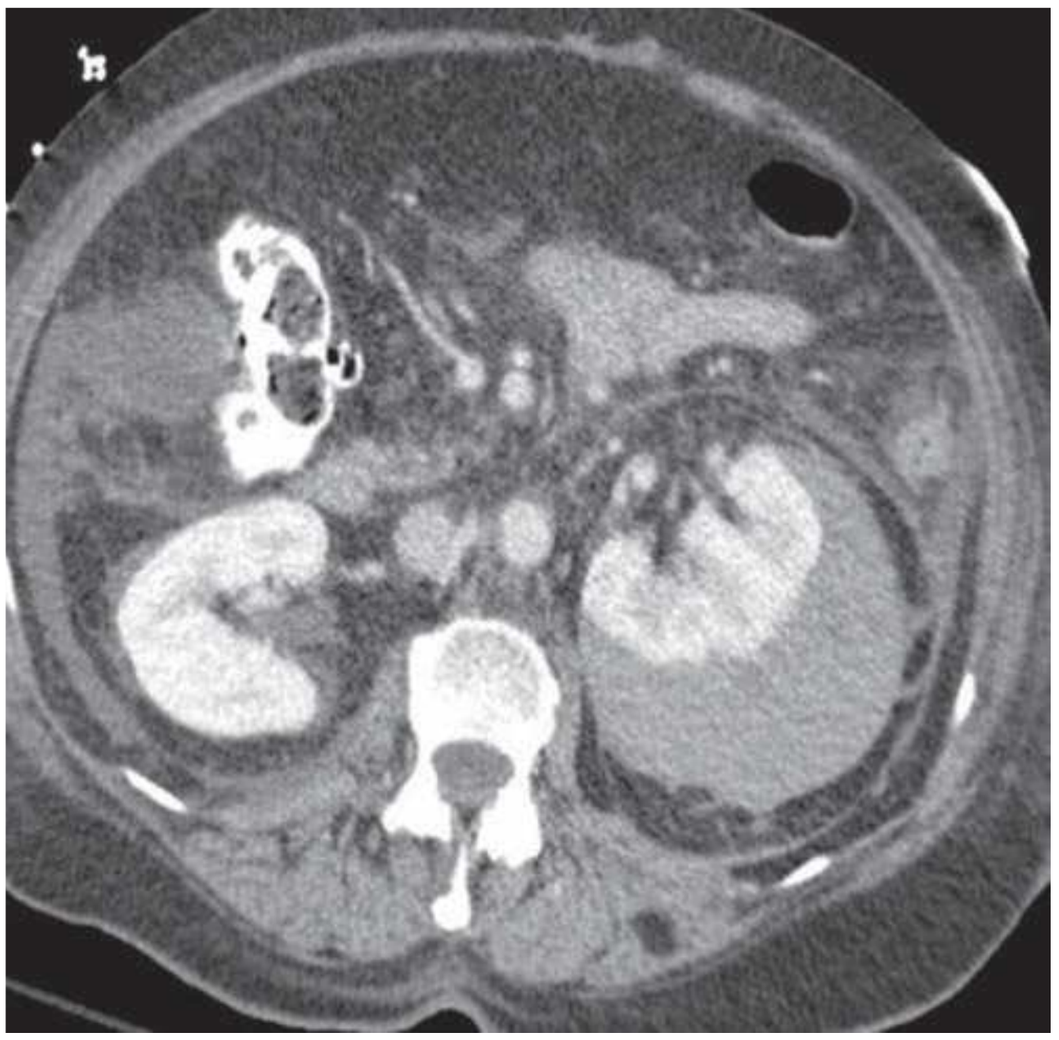

FIGURE 11.31 Contrast-enhanced CT showing a large left subcapsular hematoma displacing the left kidney.

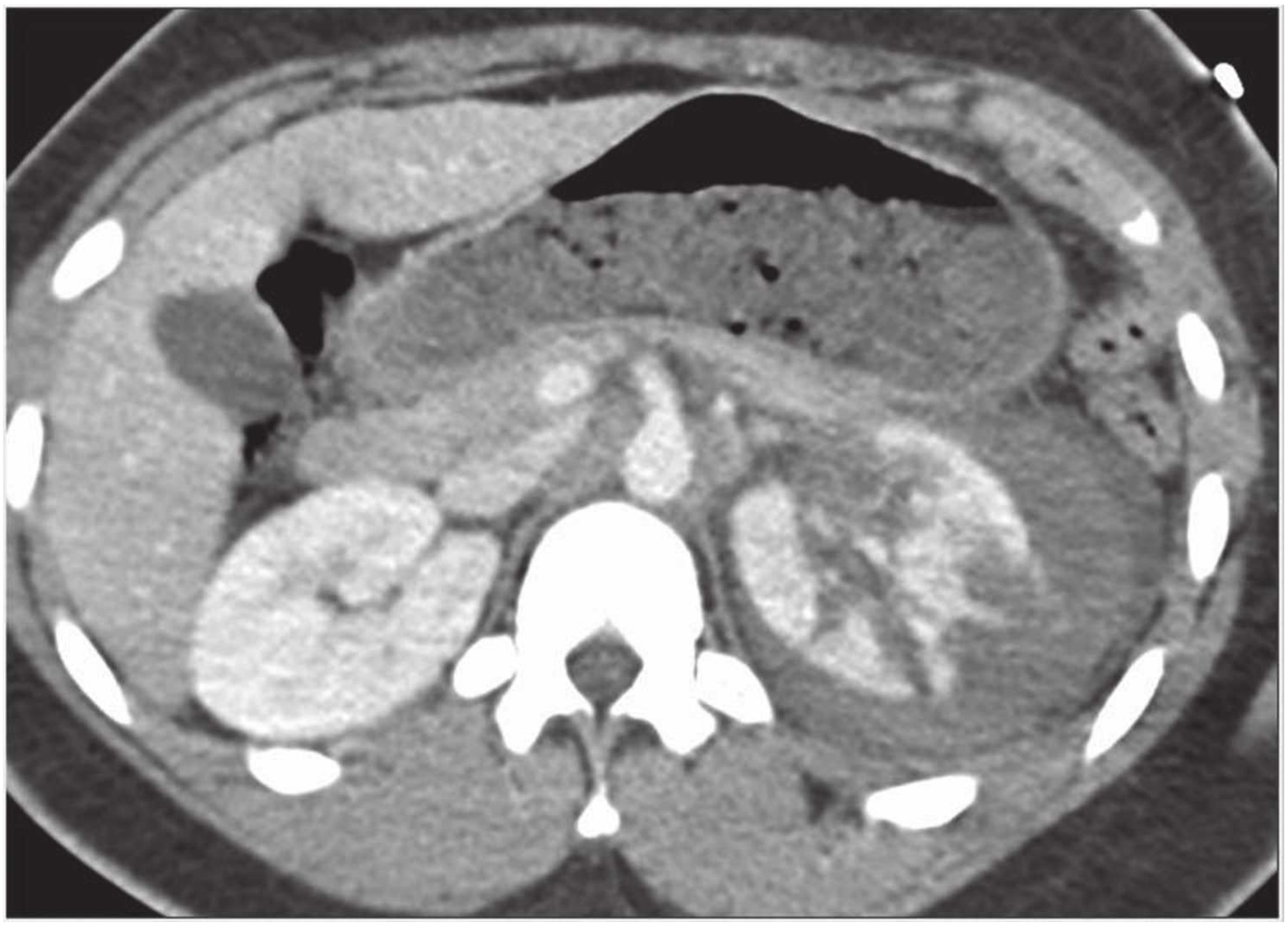

FIGURE 11.32 Contrast-enhanced CT showing shattered left kidney with perirenal hematoma.

Extravasated blood varies in its CT scan attenuation value, depending on the age of the hematoma and the hemoglobin content. With fluid collections filling the perirenal or pararenal spaces and displacing the kidney, a perirenal hemorrhage or urine extravasation (best seen on delayed images) should be considered. Fresh blood may have a higher attenuation value than extravasated urine in an unenhanced CT scan. Renal infarction may occur with a segmental occlusion of polar arteries due to trauma.[132–134] On a CT scan, catastrophic renal injuries, such as shattered kidneys, can be easily detected (Fig. 11.32). Concomitant injuries to other visceral organs may be present.[132–134] When there are underlying renal abnormalities including renal cysts, tumors, and hydronephrosis, relatively minor trauma may result in major injuries to kidneys, thereby confounding the diagnosis.

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CHAPTER

12 Diagnostic Angiography and Therapeutic Endovascular Intervention of the Renal Circulation

Alex Perino • Rajan Gupta • Ivan P. Casserly

The last decade has seen a dramatic paradigm shift in the diagnostic evaluation of pathology of the renal arterial and venous circulations. Noninvasive angiographic techniques, notably computed tomography (CT) and magnetic resonance (MR) angiography, have advanced to the degree that the diagnostic accuracy of these noninvasive imaging modalities rivals that of invasive angiography.[1,2] In fact, in the renal venous circulation, these modalities are typically superior to invasive angiography. Hence, invasive angiography of the renal circulation is now generally performed to confirm the findings of noninvasive studies of the renal artery for the purpose of vascular intervention. The result is a more targeted and individualized approach to angiography that answers a clinical question or helps direct an endovascular therapy.

In contemporary practice, the majority of pathologies involving the renal circulation that require intervention are treated using an endovascular as opposed to a surgical approach. The majority of procedures are performed in the renal artery, with the primary pathologies being treated including atherosclerosis and fibromuscular dysplasia (FMD). Novel endovascular treatments are also emerging for the treatment of resistant hypertension (HTN). Renal venous interventions are rare.

This chapter outlines the angiographic anatomy of the renal arterial and venous circulations, describes the technique of renal artery angiography, and provides an overview of the most commonly performed interventions in the renal circulation.

ANGIOGRAPHIC ANATOMY

Renal Artery

In most individuals, a single right and left renal artery arises from the abdominal aorta at the level of the L2 vertebra (Fig. 12.1). Although both renal arteries arise at a similar level from the abdominal aorta, the right renal artery typically has an anterior origin, whereas the left renal artery has a posterior origin (Fig. 12.2).[3] In addition, owing to the inferior location of the right kidney, the right renal artery has a downward course, whereas the left renal artery has a more horizontal course (Fig. 12.1B). Each renal artery divides at the hilum of the kidney into a posterior branch that runs posterior to the renal pelvis and supplies the majority of the posterior portion of the kidney and an anterior branch that typically divides into four segmental branches (apical, upper, middle, and lower) (Fig. 12.3).[4–6] The upper and lower poles of the kidney are supplied by the apical and lower anterior segmental branches, with the upper and middle segmental branches supplying the remainder of the anterior surface of the kidney. Each segmental branch divides into lobar and subsequently into interlobar branches that course between the renal pyramids. At the corticomedullary junction, each interlobar artery divides into two arcuate arteries that terminate in interlobular arteries, which supply the inflow to the afferent arterioles in the glomeruli.

Anomalous anatomy of the renal arteries is common. The most common variations include[4,6]:

1. Variation in number of renal arteries (Fig. 12.4): Multiple renal arteries are very common, with 30% of patients having multiple renal arteries to a single kidney, and 10% of patients with multiple renal arteries to both kidneys. Some authors divide these "extra" renal arteries into two groups based on the course of the artery: accessory arteries course along the route of the main renal artery and enter the kidney at the hilum, whereas aberrant renal arteries have a distinct course to the main renal artery and enter the kidney from the renal capsule away from the hilum. Accessory and aberrant renal arteries typically arise from the lower thoracic and abdominal aorta, or iliac arteries. Less common sites of origin include the lumbar or mesenteric vessels. Accessory or aberrant renal arteries most commonly supply the upper or lower poles of the kidney.
2. Variation in branching pattern (Fig. 12.5): Prehilar branching of the main renal artery is seen in approximately 12% of individuals. This anatomic variant may complicate efforts to provide embolic protection during interventional procedures at the ostium of the main renal artery.

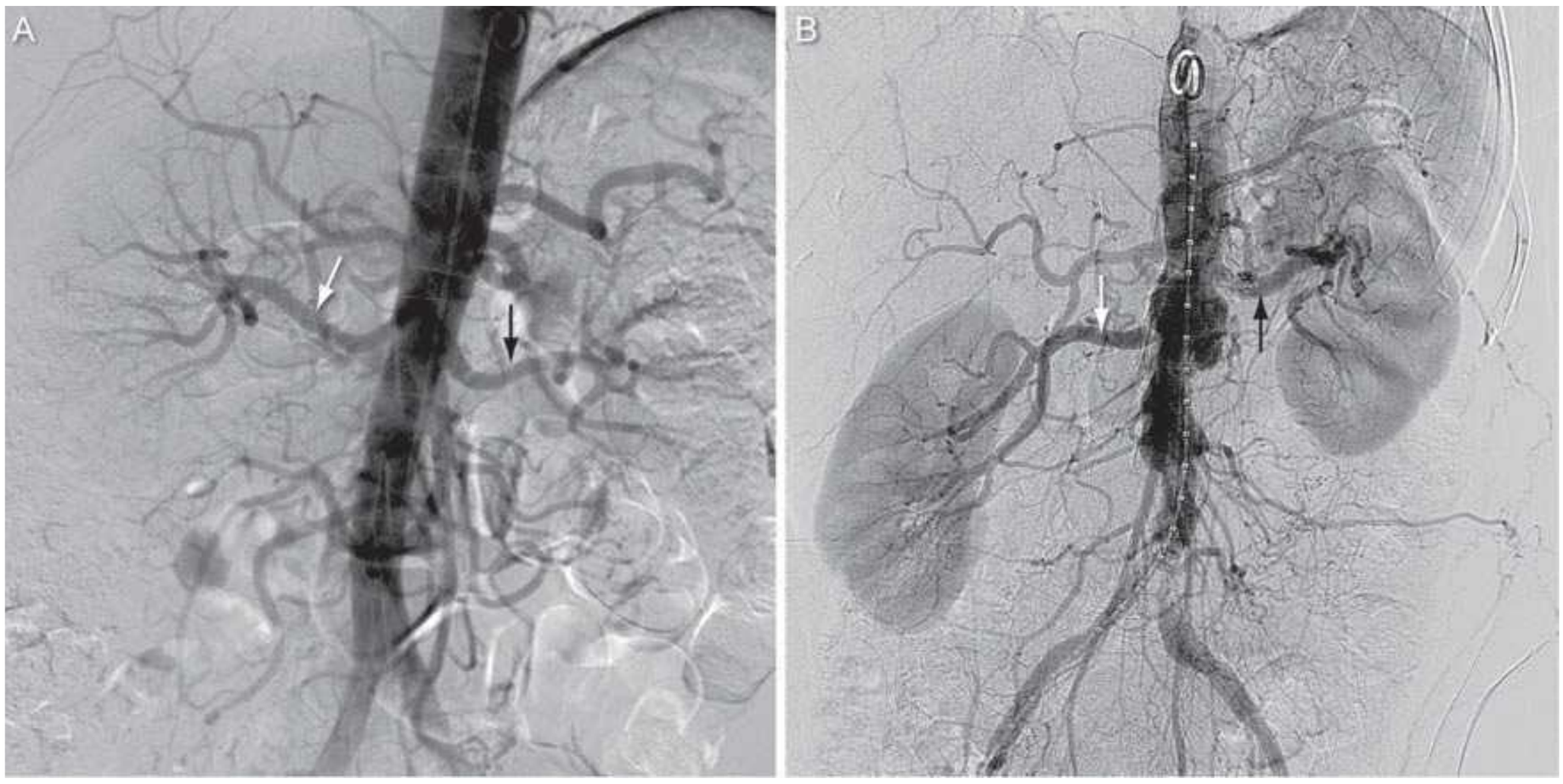


FIGURE 12.1 A,B: Abdominal aortograms from two separate patients showing origin of renal arteries from L2 level of abdominal aorta. Typical inferior course of right renal artery shown in Figure 12.1B. *White arrow*, right renal artery; *black arrow*, left renal artery.

Renal Vein

The venous drainage of each kidney generally parallels the arterial pattern. A single renal vein drains blood from each kidney in most individuals (Fig. 12.6). The renal vein lies anterior to the renal artery at the hilum and drains to the inferior vena cava (IVC), which is located to the right of the abdominal aorta. As a result, the length of the left renal vein is significantly longer than the right renal vein (6–10 cm versus 2–4 cm). In addition, the left renal vein must course between the superior mesenteric artery and the abdominal aorta in order to reach the IVC (Fig. 12.6). In contrast to the right renal vein, the left renal vein receives several tributaries, including the adrenal, gonadal, and lumbar veins.

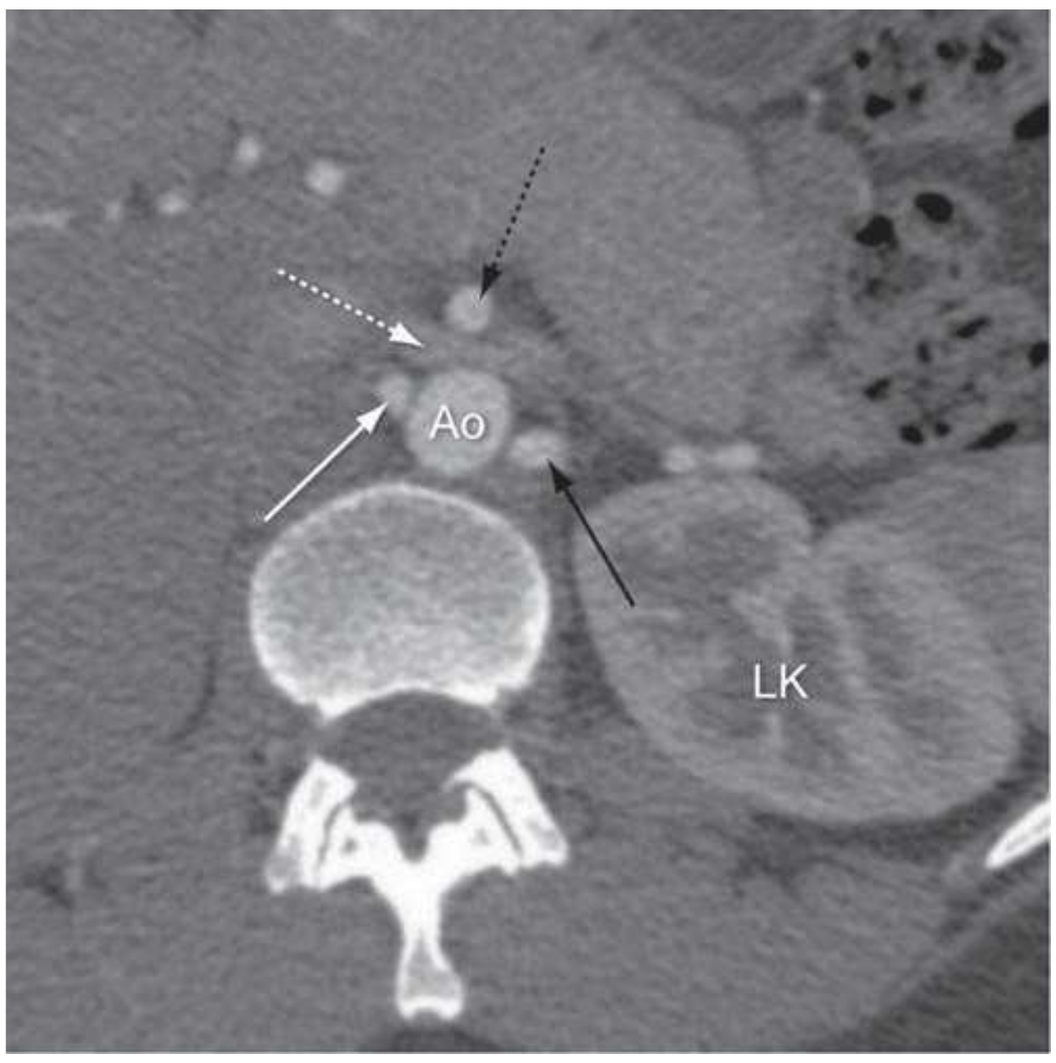


FIGURE 12.2 Axial (i.e., cross-sectional) computed tomography image at level of L2 showing anterior (ventral) origin of right renal artery (*white arrow*) relative to the left renal artery (*black arrow*). *Interrupted black arrow*, superior mesenteric artery; *interrupted white arrow*, left renal vein; *Ao*, aorta; *LK*, left kidney.

Anomalous anatomy of the renal veins is common. The most common variations include:

1. Variation in number of renal veins: Multiple right renal veins have been reported in 15% to 30% of individuals.
2. Variation in course of renal veins: This variation is typically seen in the left renal vein. The most common variation is a circumaortic course, where the left renal vein divides into anterior and posterior limbs that encircle the abdominal aorta, and is estimated to occur in ~15% of individuals. Less commonly, the left renal vein has a completely retroaortic course, which is seen in 3% of individuals.

INVASIVE ANGIOGRAPHY OF THE RENAL CIRCULATION

Prior to reviewing the specifics of the technique of renal artery angiography, it is worth discussing the essential elements that are common to all renal angiographic procedures including the use of contrast agents and contemporary imaging systems for peripheral angiography.

Contrast Agents

The contrast agent most commonly used in current practice for renal artery angiography is Iodixanol (Visipaque, GE Healthcare). This agent has an osmolality that is similar to that of blood

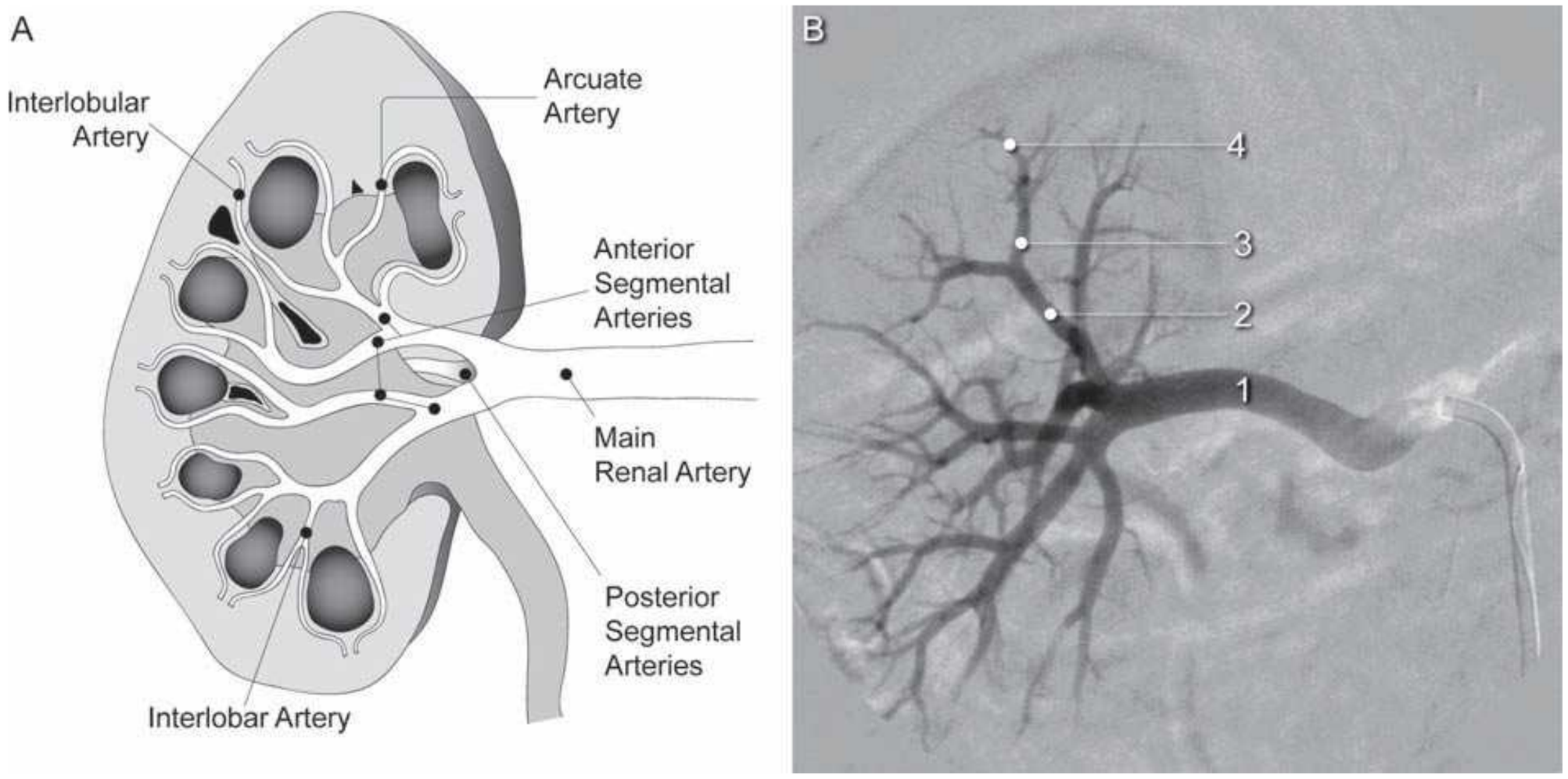


FIGURE 12.3 Renal artery anatomy. **A:** Schematic diagram of renal artery and its named subdivisions. **B:** Angiographic image of right renal artery. *1*, Main renal artery; *2*, segmental artery; *3*, interlobar artery; *4*, arcuate artery.

(i.e., 300 mOsm per kg H_2O), which differentiates it from low (620–790 mOsm per kg H_2O) and high (>1,700 mOsm per kg H_2O) osmolar agents and results in significantly improved tolerance by patients.[7] Although Iodixanol has a lower iodine concentration compared to low and high osmolar contrast agents, the impact on the imaging characteristics achieved with this agent is negligible, especially when contemporary imaging equipment is utilized. Iodixanol has the highest viscosity of all the available contrast agents which can make intravascular injections difficult, especially when performing hand injections using small caliber catheters. This impact can be minimized by warming the agent to 37°C prior to injection, which is standard practice in most angiographic laboratories, and using automated systems for contrast injection

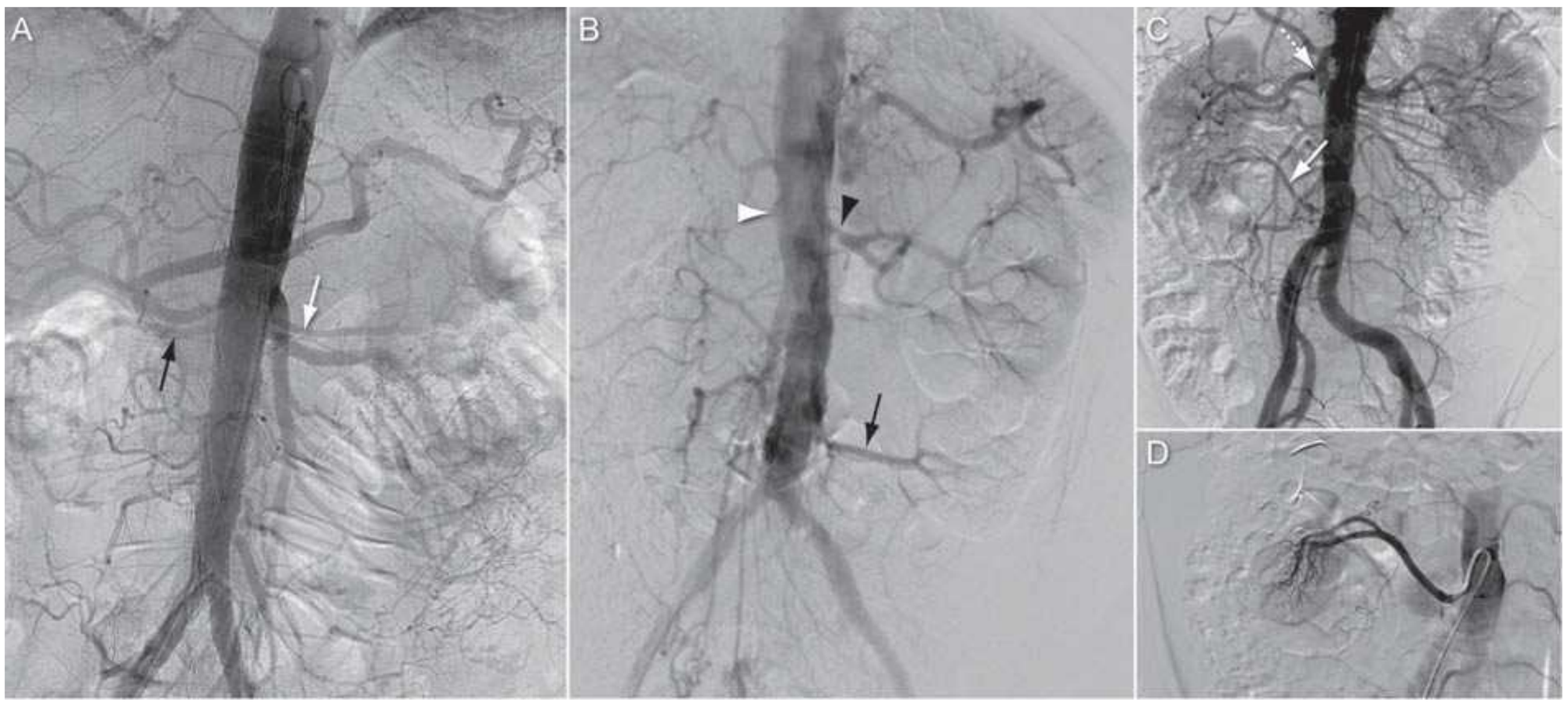


FIGURE 12.4 Multiple renal arteries. **A:** Abdominal aortogram from patient with bilateral accessory renal arteries. *Black arrow*, right lower accessory renal artery; *white arrow*, left upper accessory renal artery. **B:** Abdominal aortogram from patient with aberrant left renal artery arising from lower segment of abdominal aorta just above the aortoiliac bifurcation (*black arrow*). *White arrowhead*, occluded right renal artery; *black arrowhead*, severe stenosis at origin of left main renal artery. **C:** Abdominal aortogram from patient with aberrant right renal artery arising from lower segment of abdominal aorta just above the aortoiliac bifurcation (*white arrow*). *Interrupted white arrow*, right main renal artery. **D:** Selective angiogram of aberrant right renal artery from patient shown in Figure 12.4C.

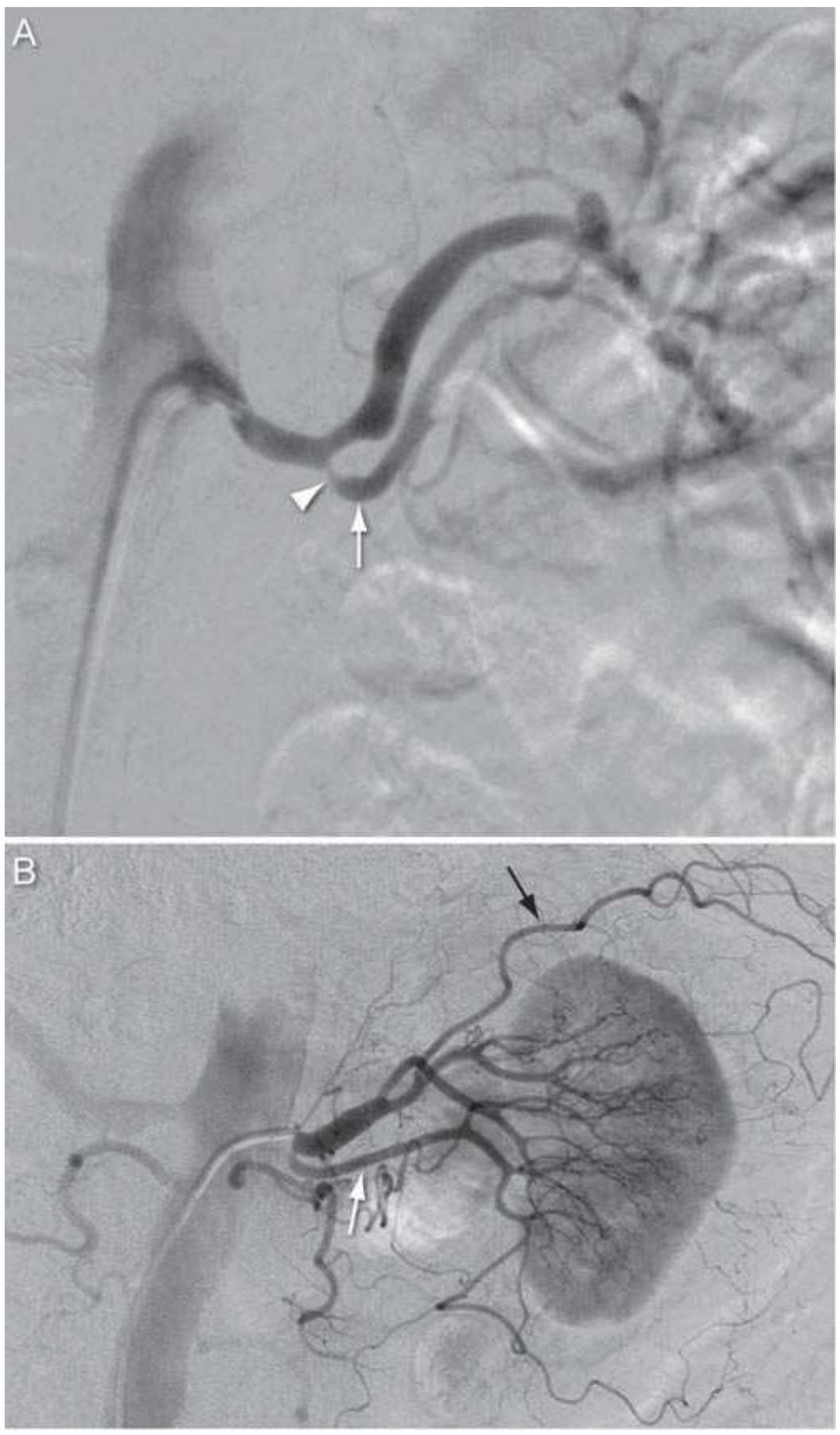


FIGURE 12.5 A,B: Angiograms from two patients showing prepolar branching of the left main renal artery (inferior division of main renal artery indicated by *white arrow*, stenosis at origin of inferior division of main renal artery in Fig. 12.5A indicated by *arrowhead*, extrarenal branch of left main renal artery in Fig. 12.5A indicated by *black arrow*).

(e.g., ACIST CVi, ACIST Medical Systems, Eden Prairie, MN; and Avanta, MEDRAD Inc., Warrendale, PA).

Alternate contrast agents have been used for patients with severe renal insufficiency in whom the risk of contrast-induced nephropathy is significant and in those with an allergy to iodinated contrast agents, Carbon dioxide (CO_2) has been used as an intra-arterial contrast agent since the early 1970s. However, it wasn't until the introduction of digital subtraction angiography (DSA) (see later text) in 1980 that CO_2 angiography became a useful diagnostic tool (Fig. 12.7).[8] In general, CO_2 angiography is well suited to imaging of large caliber vessels below the level of the diaphragm such as the abdominal aorta, aortoiliac segment, and proximal segments of major branches from the abdominal aorta (including renal arteries).[9] Imaging of smaller caliber vessels is problematic, and images must be acquired at a high frame rate to ensure acquisition of the image before dissipation of the gas.

The use of gadolinium-based contrast agents for intra-arterial angiography has largely been abandoned in current practice due to the emergence of nephrogenic systemic fibrosis as a potential complication of exposure to this agent in patients with significant renal insufficiency.[10–12] Similar to CO_2 angiography, angiography with gadolinium provides reasonable diagnostic data in larger caliber vessels and requires a high frame rate for image acquisition.

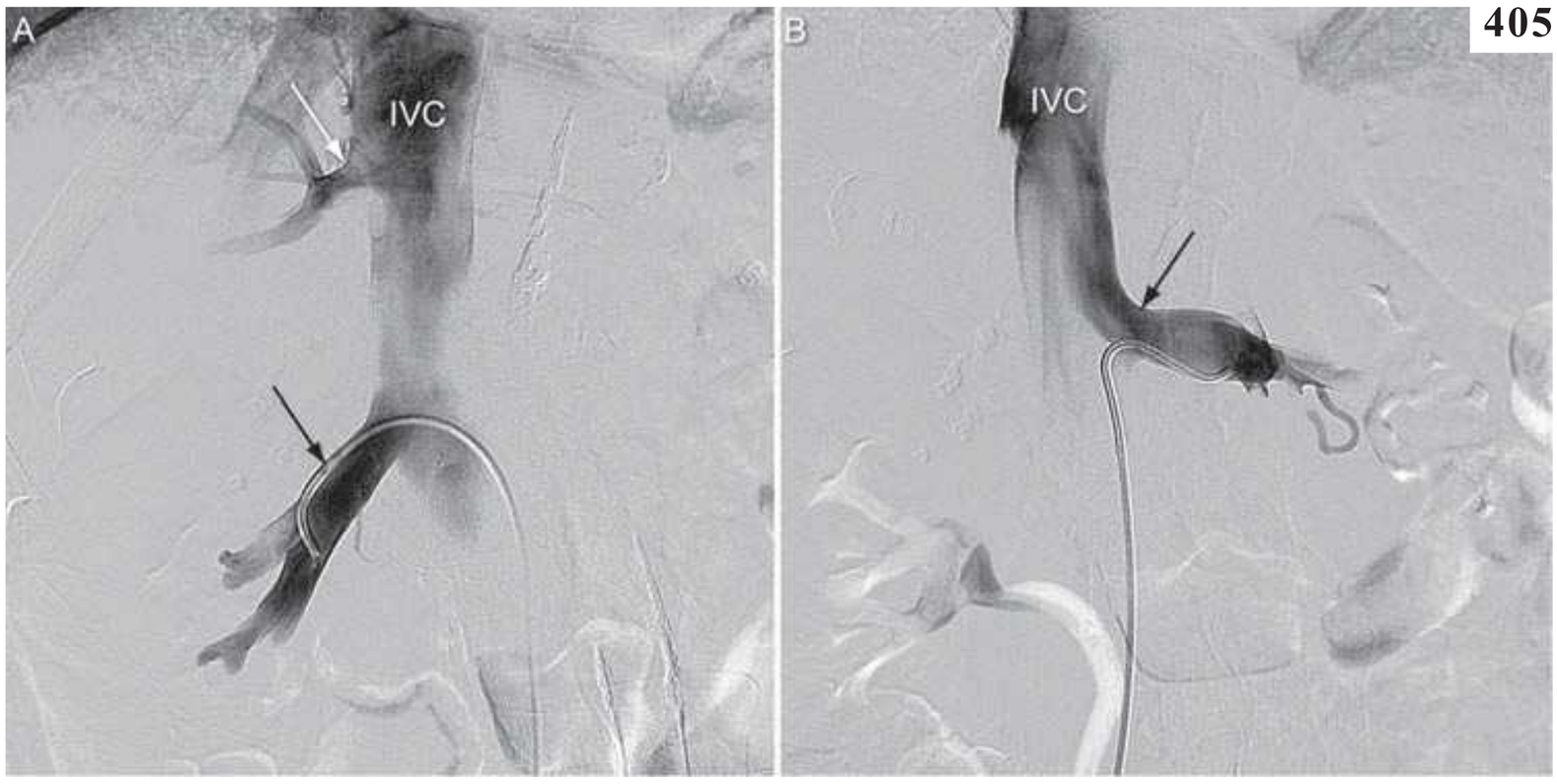
Imaging Systems

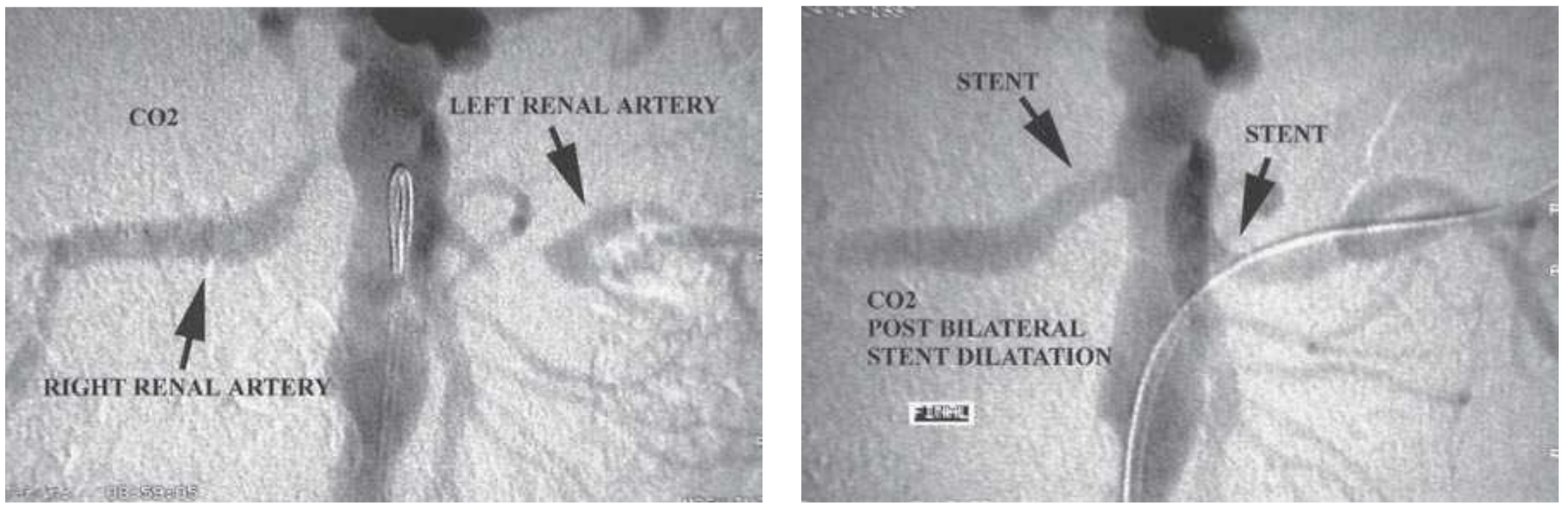
The basic image equipment requirements for acquisition of angiographic images of the peripheral circulation (including renal circulation) includes an image chain that consists of a generator and X-ray tube located beneath the table on which the patient is positioned, and an image intensifier, optical distributor, and camera located above the patient (Fig. 12.8). Real-time presentation of the angiographic image is generated on a monitor located within view of the operator. Over the last decade, a number of advancements in the imaging chain, most notably the introduction of flat panel technology in image intensifiers and the digitization of image processing, have enhanced the quality of the images generated.

In addition to this basic imaging chain, the technology of digital subtraction angiography (DSA) is fundamental to imaging of the cardiovascular system. Although this technique was developed in the 1970s, continued refinement of the technology used to generate DSA imaging has occurred providing additional improvements in image quality and functionality. In essence, the technique of DSA involves the acquisition of X-ray images before the administration of the contrast agent and during opacification of the vessel of interest following the intra-arterial injection of contrast proximal to the vessel segment of interest.[9,13] Processing of the images is then performed that involves subtracting the background X-ray image (referred to as the "mask" image) that displays bony and soft tissue structures from the X-ray image during vessel opacification, resulting in an image that displays vessel opacification alone (Fig. 12.9). The technique requires that no significant motion (e.g., patient motion, respiratory motion, peristalsis) occurs between the acquisition of the background image and vessel opacification. DSA represents the gold standard for imaging of the vascular tree, including the renal circulation.

Renal Artery Angiography Technique

With the advances in CT and MR angiography, diagnostic angiography of the renal arteries is typically performed to confirm the diagnostic findings of these noninvasive anatomic imaging modalities prior to planned interventional procedures.[1,2] However, in clinical practice, there remains a subset of patients with significant renal insufficiency who require





A B

FIGURE 12.6 Venography of right and left renal veins. **A:** Right renal vein (*black arrow*). Hepatic vein indicated by *white arrow*. *IVC*, inferior vena cava. **B:** Left renal vein.

FIGURE 12.7 Aortography at baseline **(A)** and following stenting **(B)** of the right and left renal arteries using CO_2 as contrast agent. (Reproduced with permission from Morris CS, Rimmer JM. Diagnostic and therapeutic angiography of the renal circulation. In: Schrier RW. *Diseases of the Kidney and Urinary Tract*, 8th ed. Philadelpha: Lippincott Williams and Wilkins; 2007:420–449.)

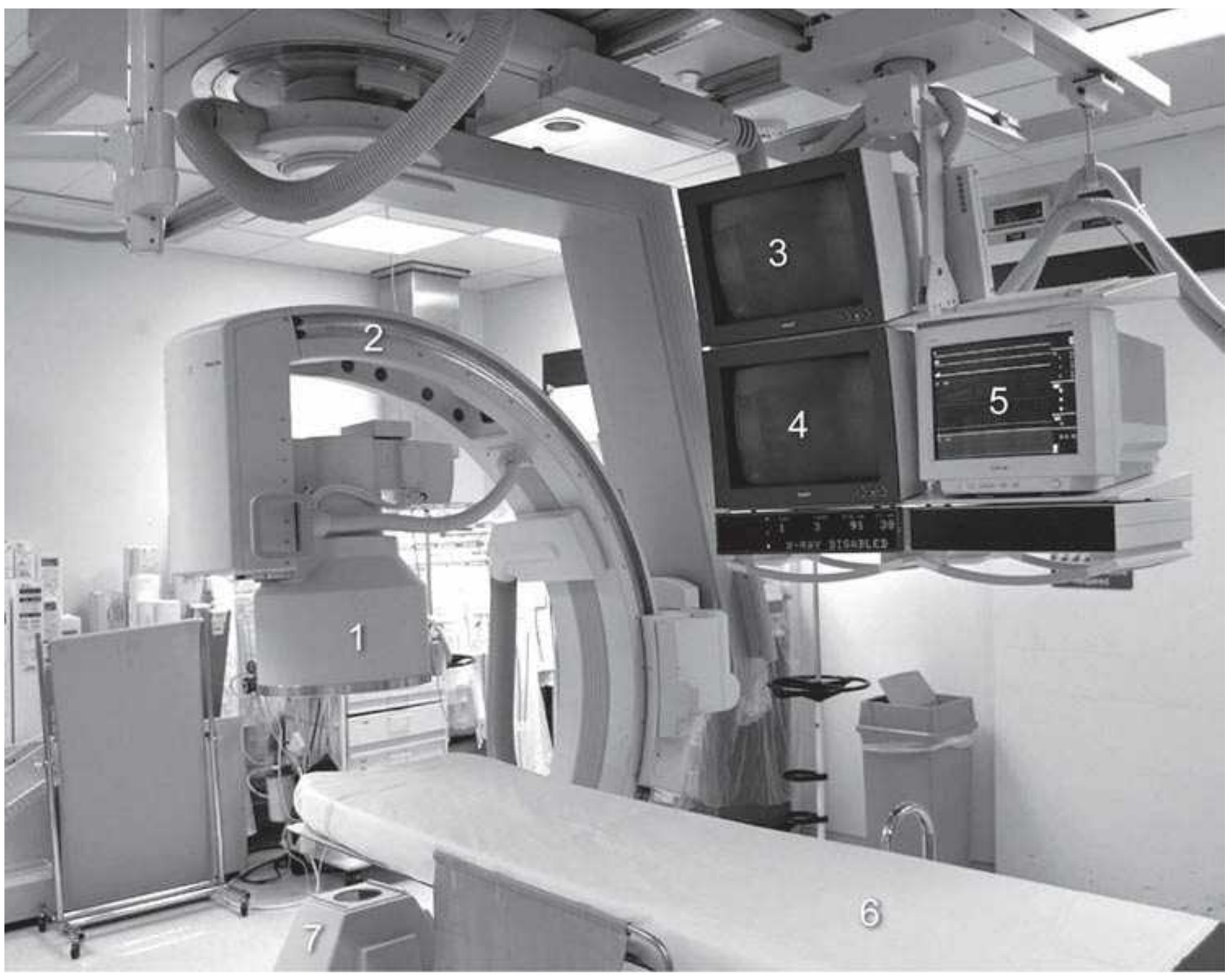


FIGURE 12.8 Picture of contemporary angiographic suite. *1*. Image intensifier. *2*. C-arm that allows movement of the image intensifier relative to the patient. *3*. TV monitor. *4*. TV monitor to display live fluoroscopic image. *5*. Monitor to display invasive and noninvasive blood pressure recordings, oximetry, electrocardiographic tracing, and heart rate. *6*. Patient table. *7*. X-ray tube.

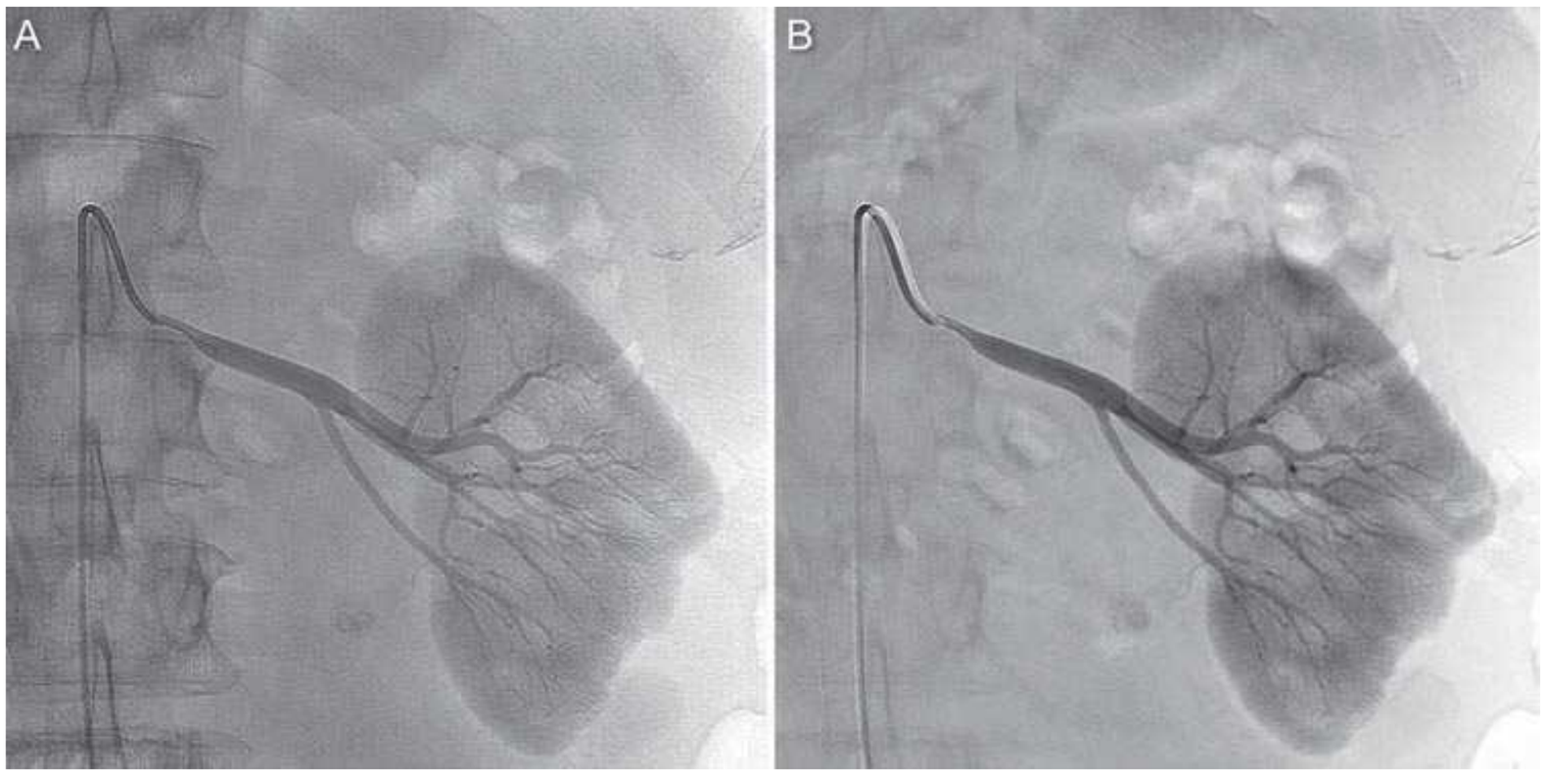


FIGURE 12.9 Illustration of digital subtraction angiography. **A:** Unsubtracted image showing both vascular structure (i.e., left renal artery) and background structures including bone and soft tissue. **B:** Subtracted image showing only vascular structure with removal of all background structures.

invasive angiography for diagnostic purposes due to thresholds of serum creatinine (Cr) and eGFR (i.e., Cr > 1.5 mg per dl, eGRF <60 mL per min) utilized by most radiology departments for exposure of patients to iodinated contrast and gadolinium-based contrast agents that are used for CT and MR angiography, respectively.

A necessary preamble to renal artery angiography is the establishment of arterial access. In most circumstances, this is obtained in the right or left common femoral artery (CFA). Less commonly, the brachial or radial artery may be used. The advantages and disadvantages to these various access sites are outlined in Table 12.1.

Depending on the clinical situation (e.g., preprocedural imaging, baseline renal function, procedural indication), abdominal aortography may be performed prior to selective renal artery angiography. This is achieved by placing a flush catheter with multiple side holes at the level of the T12 vertebra and injecting contrast at 20 mL per sec for a total of 40 mL (Fig. 12.10). The abdominal aortogram provides information about the location of the renal artery ostia and the condition of the abdominal aorta (e.g., presence of atherosclerosis) and its major branches.

Selective angiography of the renal arteries is performed by using catheters with preformed shapes to facilitate selective engagement of the renal artery ostia (Fig. 12.11). A variety of catheters are available for this purpose. The choice of a specific catheter is determined by the anatomy of the abdominal aorta, the location of the renal artery ostium, the course of the proximal segment of the renal artery, the arterial access site used, and the experience of the operator. In the authors' practice, the most commonly used catheters when approaching the renal artery from the CFA access site are the

12.1 Advantages and Disadvantages of Various Arterial Access Sites for Renal Artery Angiography and Intervention

Access Site	Advantages	Disadvantages
Femoral	• Large caliber vessel with low risk of ischemic complication	• Greatest risk of bleeding complication • Difficult engagement of renal artery with prominent inferior course of proximal segment
Brachial	• Medium caliber vessel. Easier to access than radial and accomodates larger caliber sheath	• Moderate risk of both bleeding and ischemic complications
Radial	• Lowest risk of significant bleeding complications • No risk of ischemic complication in presence of negative Allen's test • Well suited for engagement of renal artery with inferior course of proximal segment	• Small caliber typically limits use of sheath sizes of no greater than 6-French which may limit ability to deliver certain interventional devices

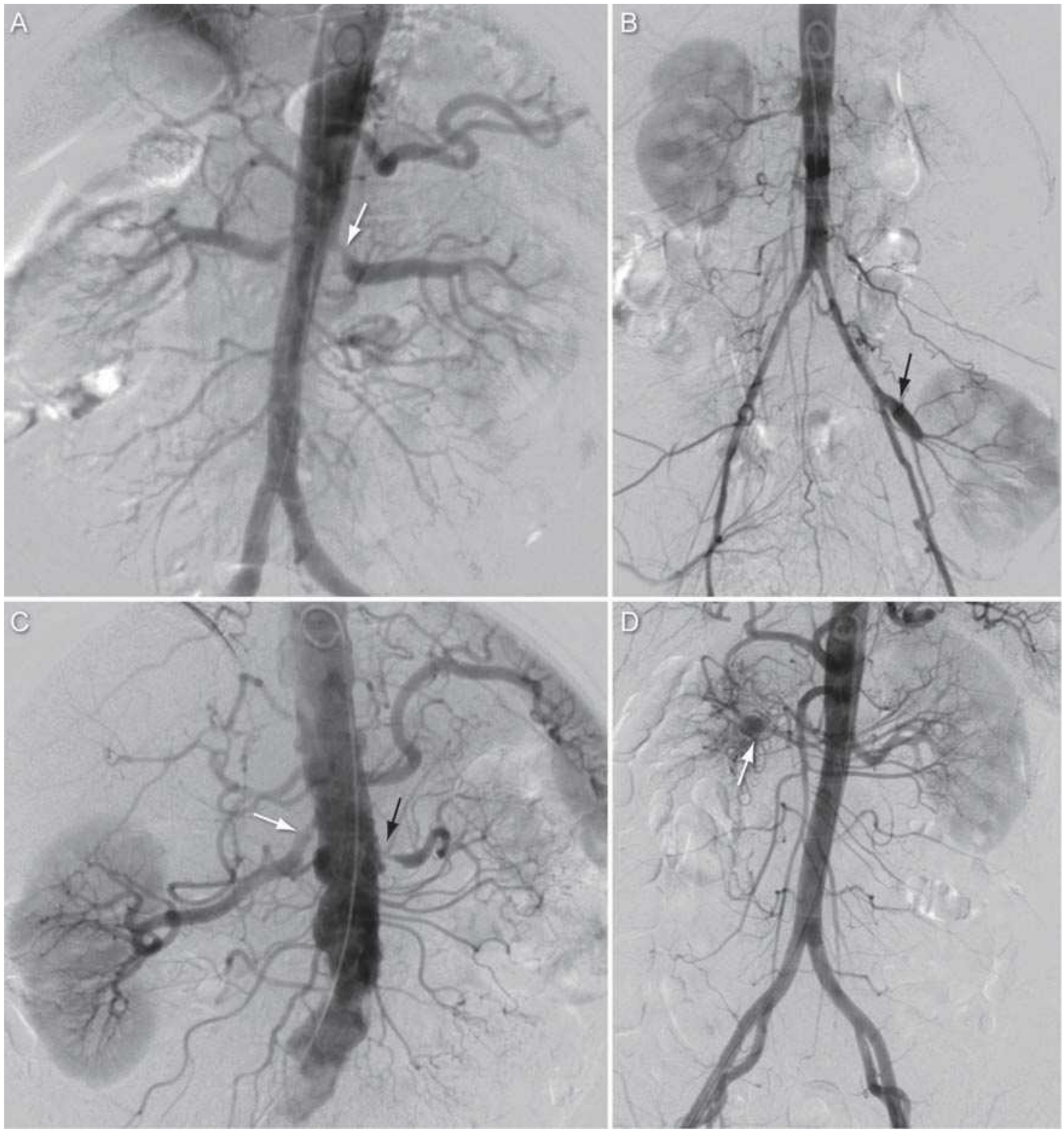


FIGURE 12.10 Examples of abdominal aortograms performed prior to selective renal artery angiography. **A:** Severe stenosis at origin of left main renal artery (*arrow*). **B:** Transplanted kidney with vascular origin from left external iliac artery (*arrow*). Native left renal artery presumed to be occluded based on absence of left renal nephrogam. **C:** Severe atherosclerosis is evident in the abdominal aorta. Critical stenosis noted at origin of left renal artery (*black arrow*). Right renal artery indicated by *white arrow*. **D:** Normal abdominal aorta and left renal artery. Aneurysm of right renal artery noted (*arrow*) with poor opacification of the right renal nephrogram.

Sos, Simmons, Cobra, and JR4 catheters. When approaching from the upper extremity access sites, the most commonly used catheter is the multipurpose catheter (Fig. 12.12).

Images are acquired using DSA. Typically, adequate imaging of the renal arteries is achieved by injecting contrast at 4 mL per sec for total of 8 mL through the catheter. Due to the anterior and posterior location of the right and left renal ostia, respectively, the ostia and proximal segments of both renal arteries are best visualized with the image intensifier in the left anterior oblique projection.[3] Delayed imaging is generally performed to allow visualization of the nephrogram, which provides an estimate of the renal size and the contour of the kidney (Fig. 12.13). In certain clinical situations (e.g., aneurysmal disease), imaging in multiple planes or rotational angiography may be required to elucidate the precise relationship of the pathology to branch points, which can influence treatment decisions.

RENAL ARTERY INTERVENTION

In current practice, the majority of renal artery intervention is performed for the treatment of atherosclerotic disease, and to a lesser extent FMD, and will thus constitute the major

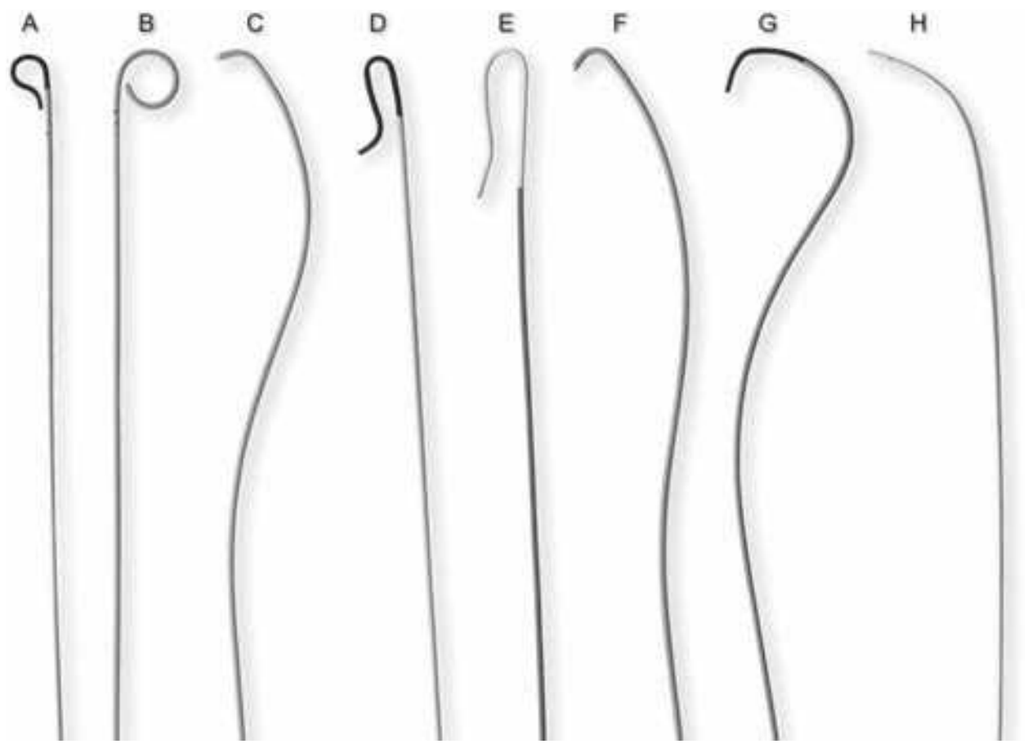


FIGURE 12.11 Catheters used to perform abdominal aortography **(A,B)** and selective renal artery angiography **(C–H)**. **A:** Omni flush, **(B)** pigtail, **(C)** JR4, **(D)** Sos, **(E)** Simmons, **(F)** internal mammary, **(G)** Cobra, and **(H)** multipurpose.

focus of this section. Less commonly treated pathologies are briefly discussed. Finally, the emerging therapy of renal artery denervation is outlined as there is considerable excitement about the potential of this therapy in the treatment of an array of medical renal disorders.

Atherosclerotic Disease

Atherosclerotic disease of the renal arteries is a common entity, particularly in patients with HTN (prevalence of 1% to 5%) and in those with documented atherosclerotic disease in other vascular territories (prevalence of 30% to 40%). Roughly 90% of all cases of renal artery stenosis (RAS) are due to atherosclerotic disease, which is characterized by a typical location at the renal artery ostium or proximal segment of the main renal artery (Fig. 12.14). This is due to the fact that renal artery atherosclerosis typically reflects an extension of atherosclerosis involving the abdominal aorta (Fig. 12.15).

The most challenging component of renal artery intervention for atherosclerotic RAS is case selection, rather than the execution of the intervention itself. Ischemic nephropathy is a complex entity, and considerable controversy exists over the appropriateness of renal revascularization for this disease.[14–16] Unfortunately, randomized trials comparing medical therapy with renal artery intervention have not proved to be particularly helpful in defining the patient populations that benefit from revascularization. In the absence of such guidance, the authors attempt to identify those patients who are most likely to benefit from revascularization for atherosclerotic RAS by asking two fundamental questions:

1. Is the patient's clinical condition being caused by atherosclerotic RAS?
2. If so, is there a reasonable chance that revascularization will have a meaningful clinical benefit?

The answer to the first question is typically answered by the nephrologist taking care of the patient and is provided by matching the patient's clinical presentation (e.g., resistant HTN, acute renal failure with initiation of angiotensin converting enzyme inhibitor, flash pulmonary edema in the setting of uncontrolled HTN, acute or subacute renal failure) with the anatomy of the RAS (unilateral vs. bilateral, severity of stenosis) and anatomic and functional data regarding the parenchymal function and perfusion of each kidney.

The second question is typically arrived at following a discussion between the nephrologist and interventionalist. In general, it is felt that the more severe the RAS, and the less severe the parenchymal disease in the affected kidney, the more likely that revascularization of the main renal artery is likely to have a clinical impact on the patient. The variables that are used to help make this determination and their impact on clinical outcomes following renal revascularization are summarized in Table 12.2. It should be accepted that these variables are most useful when the goal of intervention is to preserve renal function. When the goal of intervention is to improve the control of HTN,

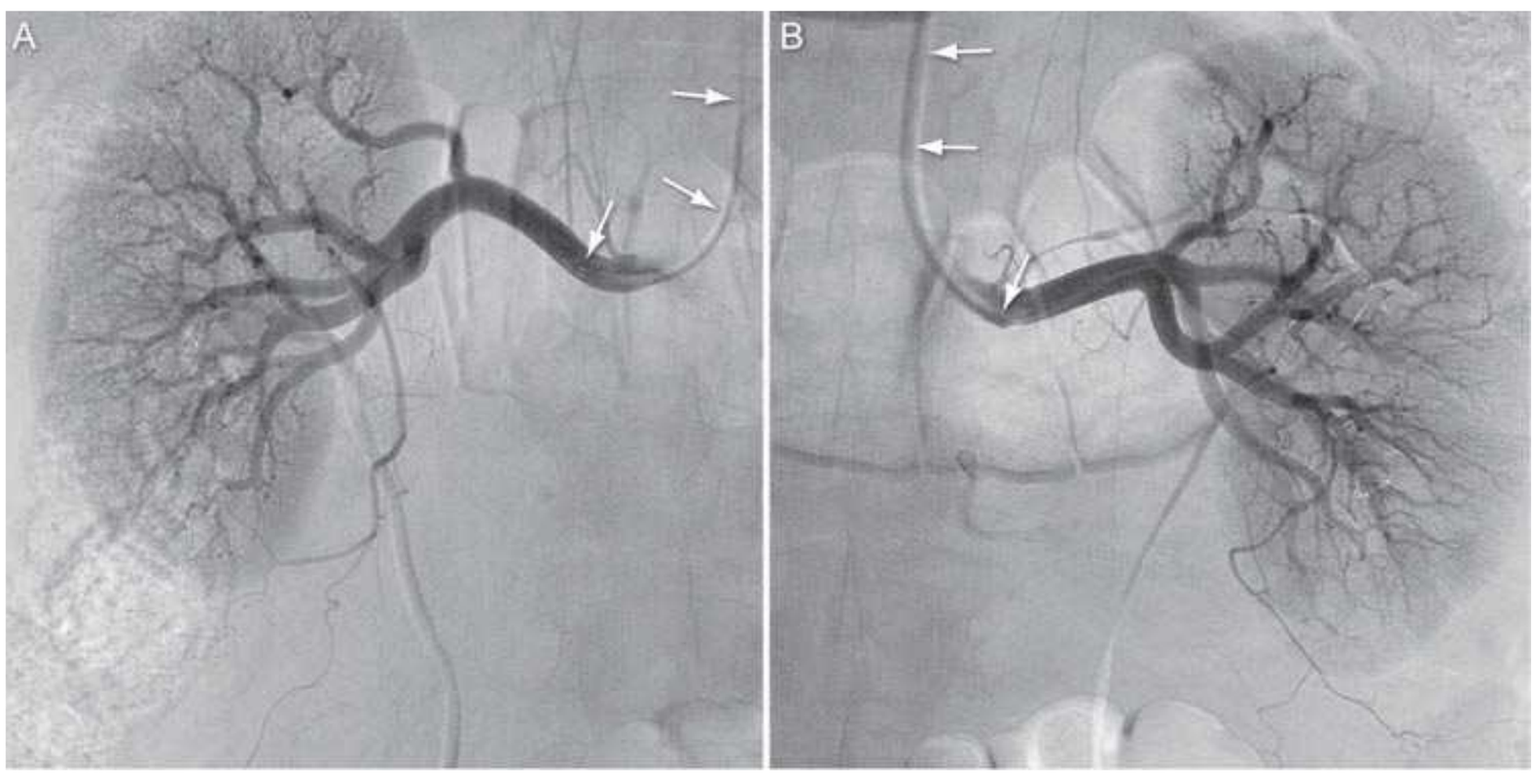


FIGURE 12.12 Selective angiography of the right and left renal arteries using upper extremity access and multipurpose catheter (*white arrows*).

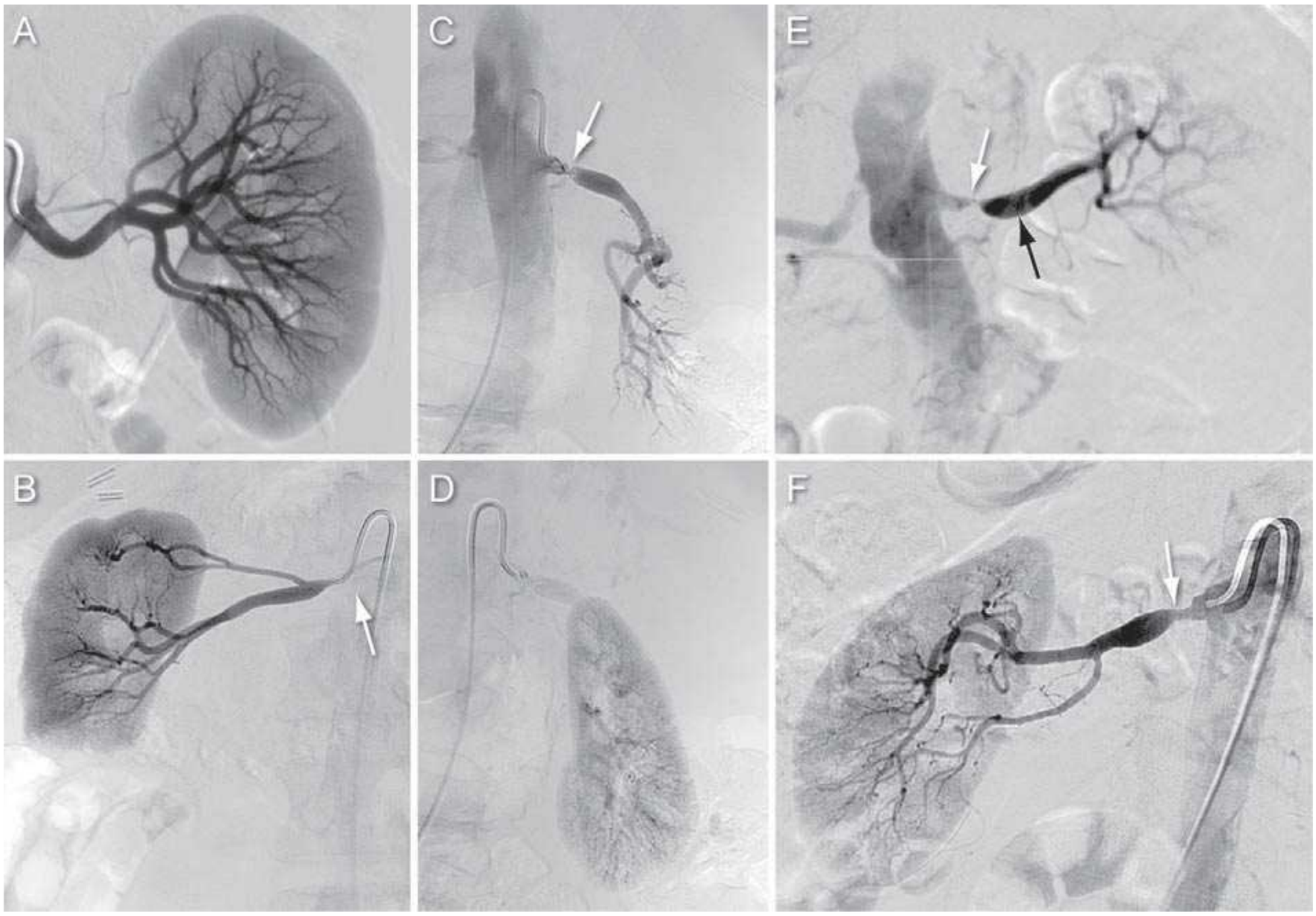


FIGURE 12.13 Spectrum of angiographic findings on renal artery angiography in patients with renal artery atherosclerosis. **A:** Angiogram of left renal artery in a 35-year-old woman without renal artery atherosclerosis to serve as comparison. Note smooth and cylindrical contour of main renal artery, extensive arborization of the renal artery branches, and smooth contour, kidney bean-like shape, and size of the nephrogram. **B:** Selective right renal artery angiogram showing severe ostial stenosis (*arrow*) with lobular contour of small sized nephrogram. Early **(C)** and delayed **(D)** images from selective left renal artery angiogram in patient with long-standing critical stenosis at ostium (*arrow*). Note small sized nephrogram with loss of typical shape. **E:** Selective left renal artery angiogram showing critical right renal artery stenosis (*white arrow*) and significant poststenotic dilatation (*black arrow*). **F:** Selective right renal artery angiogram showing severe renal artery stenosis (*arrow*), severe pruning of the renal artery branching pattern, and diminished size of nephrogram.

decision-making is more complicated, as it can be very difficult to predict the effect of revascularization on renin output from a severely ischemic kidney that may have little parenchymal function.

Execution of Intervention for Atherosclerotic RAS

Arterial access is obtained in the common femoral artery (CFA) or upper extremity artery. A guide catheter is used to engage the ostium of the culprit renal artery (Fig. 12.16). Guide catheters are significantly stiffer than diagnostic catheters and have a greater potential for causing injury to the aorta or renal artery ostium. Because atherosclerotic disease of the renal artery is typically a manifestation of aortic atherosclerosis that encroaches on the renal artery, great care with manipulation of guide catheters in the abdominal aorta is warranted in order to prevent atheroembolism to the lower extremity, mesenteric, and/or renal arteries. A variety of techniques (e.g., no-touch technique) have been described that minimize this manipulation.[17]

Once the guide is engaged at the ostium of the renal artery, an interventional wire is advanced into one of the segmental vessels. In current practice, 0.014-inch wires with atraumatic coil tips are typically used.[18] The stenosis is dilated with an angioplasty balloon with a balloon-artery ratio of ~0.8. Regardless of the initial result with angioplasty, the lesion is then typically stented using a balloon-expandable stent (Fig. 12.17). This is based on two considerations: (1) stenting achieves a highly predictable acute result by overcoming the issue of elastic recoil and treatment of any dissections that can lead to subsequent abrupt vessel occlusion, and (2) stenting reduces the high rate of restenosis that is seen with angioplasty alone.[19] Most of these stents are composed of stainless steel, but a small minority are of composed of cobalt-chromium. Balloon-expandable stents are

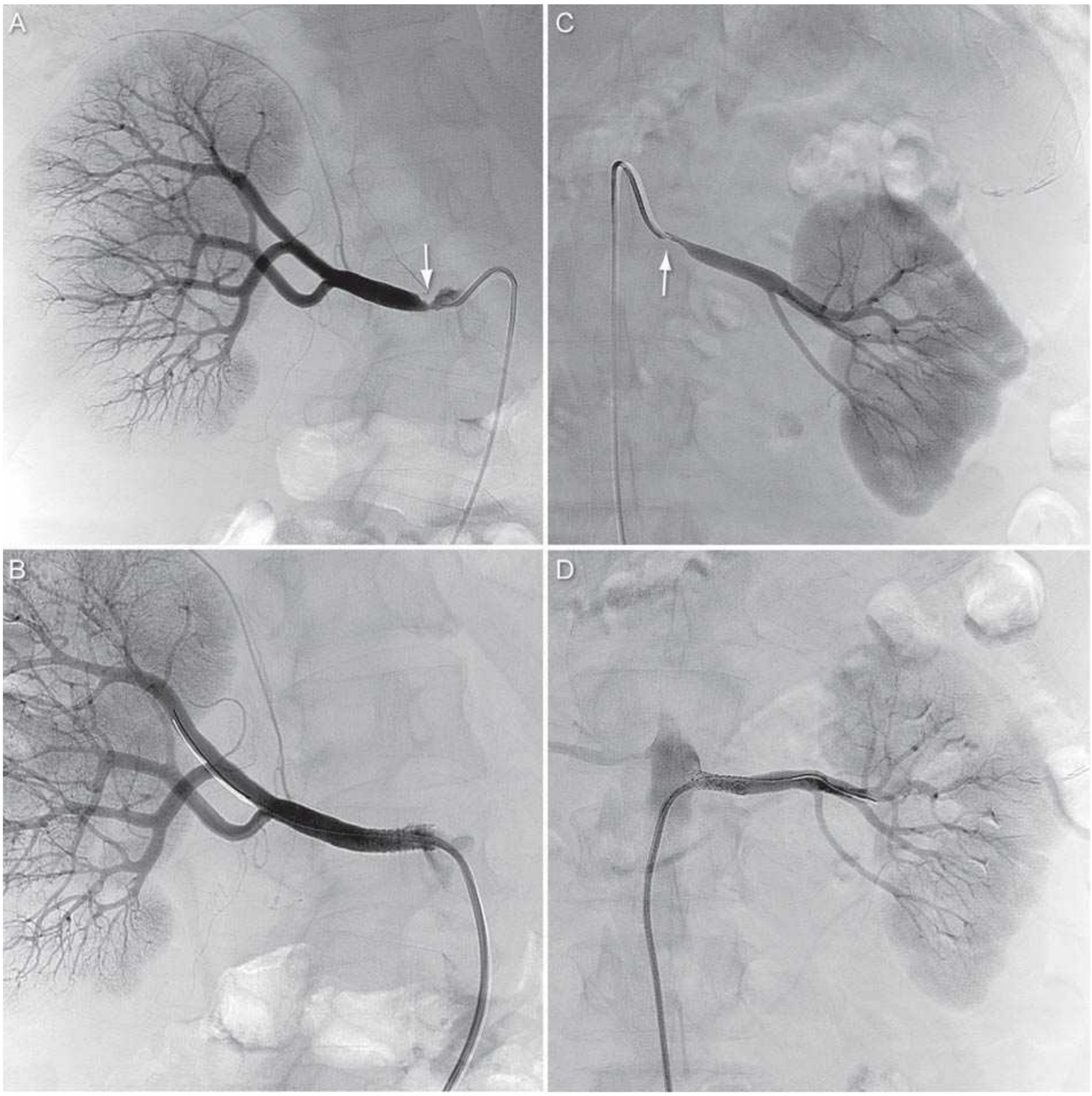


FIGURE 12.14 Examples of renal artery atherosclerotic lesions treated with balloon-expandable stents. **A,B:** Baseline and poststenting angiograms, respectively. **C,D:** Baseline and poststenting angiograms, respectively.

used in this location because of their high radial strength, which is an important consideration at the ostial location. Positioning the stent such that the ostium of the renal artery is covered by stent is a critical component of renal artery stenting. This will generally result in 1 to 2 mm of the stent projecting out into the abdominal aorta. Failure to achieve this objective is associated with high rates of restenosis. The stent is typically postdilated using a noncompliant balloon with a balloon–artery ratio of ~1.0.

The use of embolic protection during renal artery intervention is inconsistent in current practice. In theory, these devices should help prevent atheroembolism to the kidney as a result of angioplasty and stenting, and thus improve the safety of renal artery intervention by minimizing any adverse impact on renal function. Filter-type embolic protection devices have been used with good clinical success.[20–22] A broad range of these devices are available for use, based on U.S. Food and Drug Administration (FDA)-approved indications for use in saphenous vein graft intervention in the coronary circulation,[23–25] and during stenting of the carotid artery (Fig. 12.18).[26,27] Technical challenges associated with their use in the renal artery include the absence of a sufficient landing zone for placement of filter, the presence of early branching of the renal artery preventing embolic protection of both branches, renal artery diameters that exceed the recommended diameter recommendations

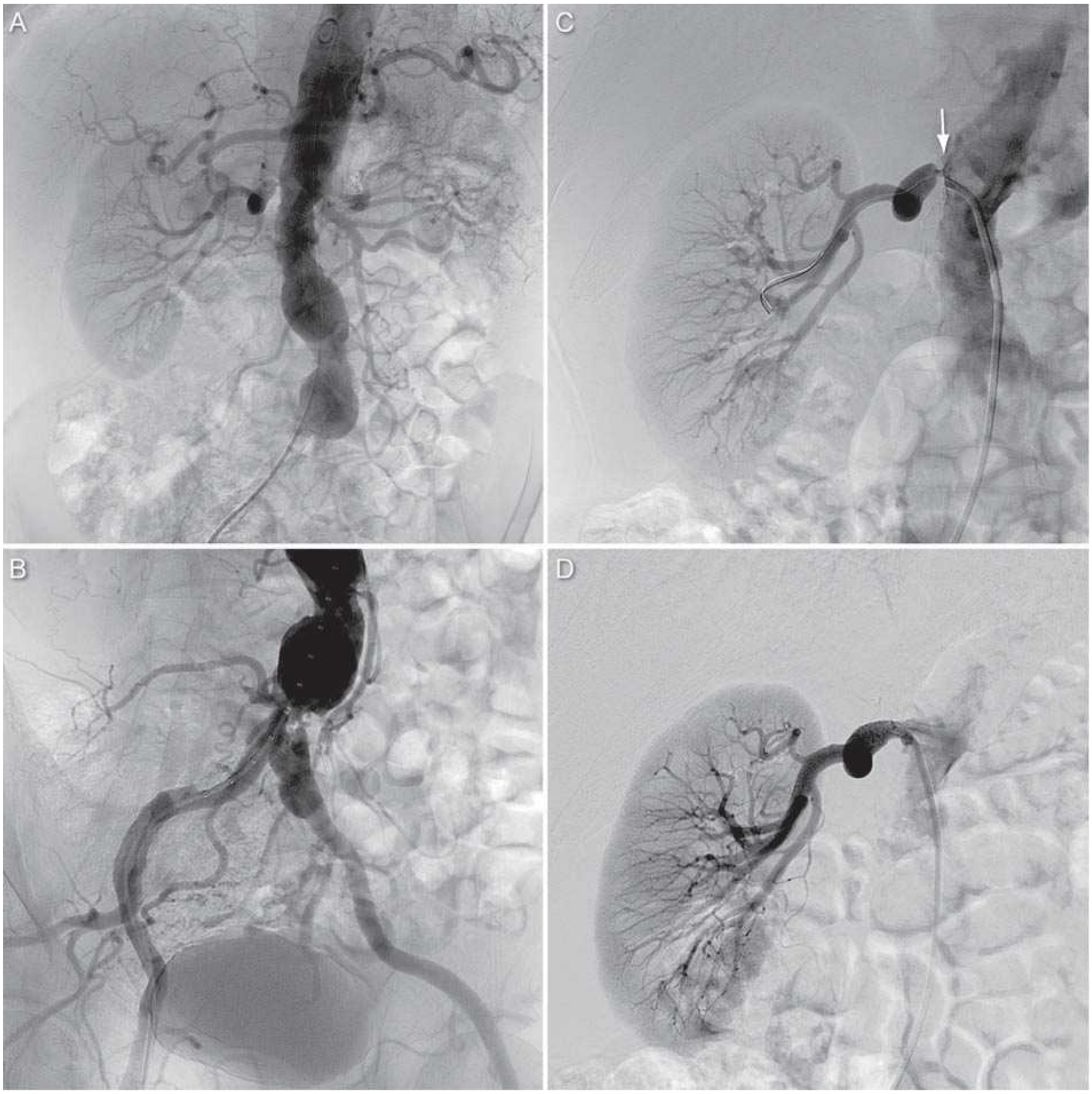


FIGURE 12.15 Clinical case reinforcing concept that renal artery atherosclerosis represents extension of atherosclerosis in the abdominal aorta. **A:** Abdominal aortogram showing extensive atherosclerosis and areas of ectasia. **B:** Pelvic angiogram showing severe stenosis at the aortoiliac bifurcation. **C:** Selective angiogram of right renal artery showing critical stenosis at ostium of right renal artery. **D:** Selective right renal artery angiogram following placement of balloon-expandable stent.

for filter use, and difficulty in delivering the filter through critical stenoses without prior angioplasty. Assuming the absence of these technical issues, it seems reasonable to recommend the use of these devices during renal artery intervention for atherosclerotic RAS, particularly when treating patients with a single functioning kidney, and when baseline renal function is impaired to a degree that even small amounts of atheroembolism would be poorly tolerated.

All renal artery interventions for atherosclerotic RAS are performed using anticoagulation (e.g., unfractionated heparin, bivalirudin) to prevent device-related thrombosis during the procedure. Following the procedure, all patients receive lifelong aspirin (81–162 mg daily) and statin therapy (based on the diagnosis of atherosclerotic disease) and our practice is to recommend clopidogrel 75 mg daily for 1 month. The latter recommendation is based on an extrapolation of data from the coronary circulation where the use of dual antiplatelet therapy with clopidogrel and aspirin was the most effective regimen in preventing acute (<24 hours) and subacute (24 hours to 30 days) stent thrombosis.[28]

12.2 Summary of Variables Used to Predict Favorable or Unfavorable Response to Revascularization for Treatment of Atherosclerotic Renal Artery Stenosis

Variable	Favorable	Unfavorable
Renal function		
Serum Cr	Elevated (but <3 mg/dl)	Normal
Trajectory of serum Cr prior to intervention	Steep increase	Flat or frequent fluctuations
Duration of renal failure	Acute, subacute (<6 months)	Chronic
Urinary protein	<1 gm/24 hours	≥1 gm/24 hours
Kidney size	>10 cm	<8 cm
Renal contour	Smooth, bean-shaped	Lobulated
Resistive index	<0.8	≥0.8
Severity of renal stenosis	Severe (≥70%–90%), critical (≥90%)	Mild (≤50%), moderate (50%–70%)

Cr, creatinine.

Clinical Outcomes following Percutaneous Revascularization of Atherosclerotic RAS

The interpretation of clinical outcomes of percutaneous revascularization for atherosclerotic RAS is a controversial topic with remarkably disparate interpretations of the same data, often based on the inherent biases of the observer. A significant body of data exists. However, given the controversial nature of this topic and the inherent limitations of single center observational data, only data from prospective randomized trials and prospective registries with core-lab adjudication of events will be discussed.

There have been three randomized trials comparing angioplasty with medical therapy that are summarized in Table 12.3.[29–31] In general, these trials enrolled patients with HTN with preserved renal function. The threshold level of ≥50% RAS for inclusion into the trials likely resulted in the

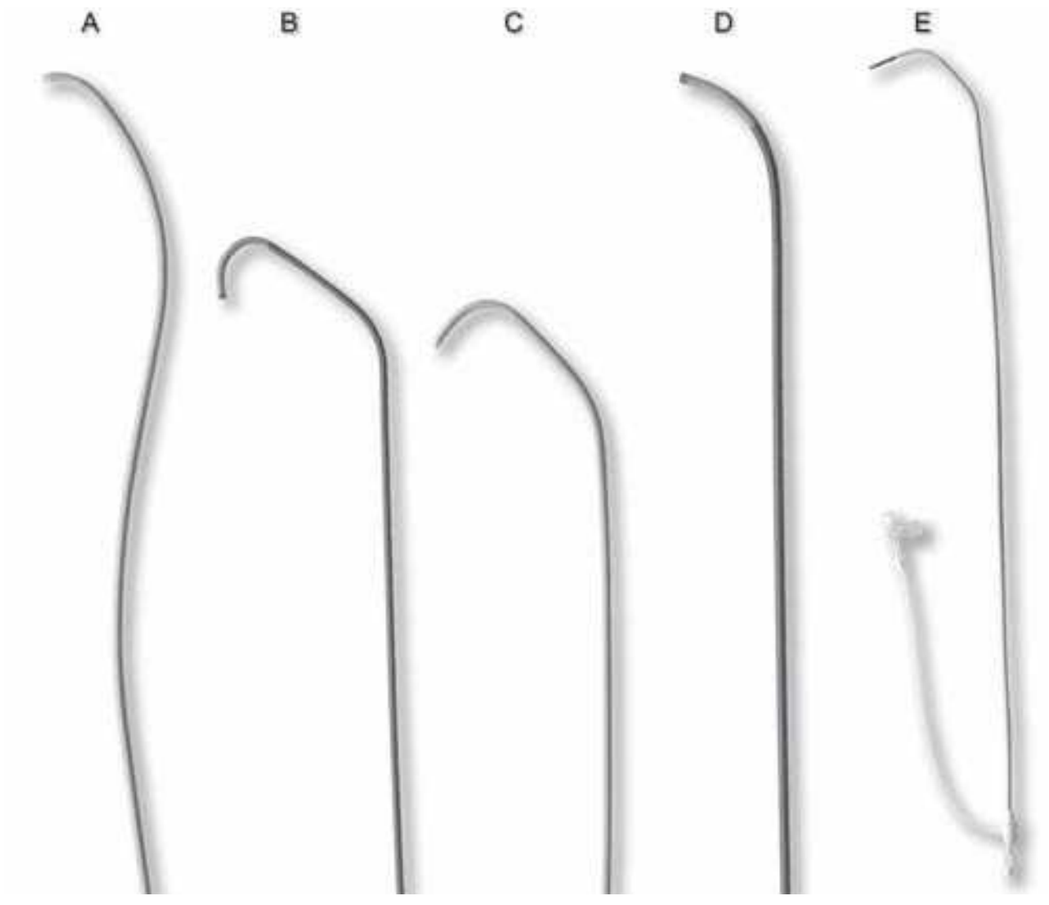


FIGURE 12.16 Guides and guide-sheaths used to perform renal artery interventional procedures. **(A)** JR 4 guide, **(B)** renal standard curve (RES) guide, **(C)** renal double curve (RDC) guide, **(D)** multipurpose guide, and **(E)** RDC guide-sheath.

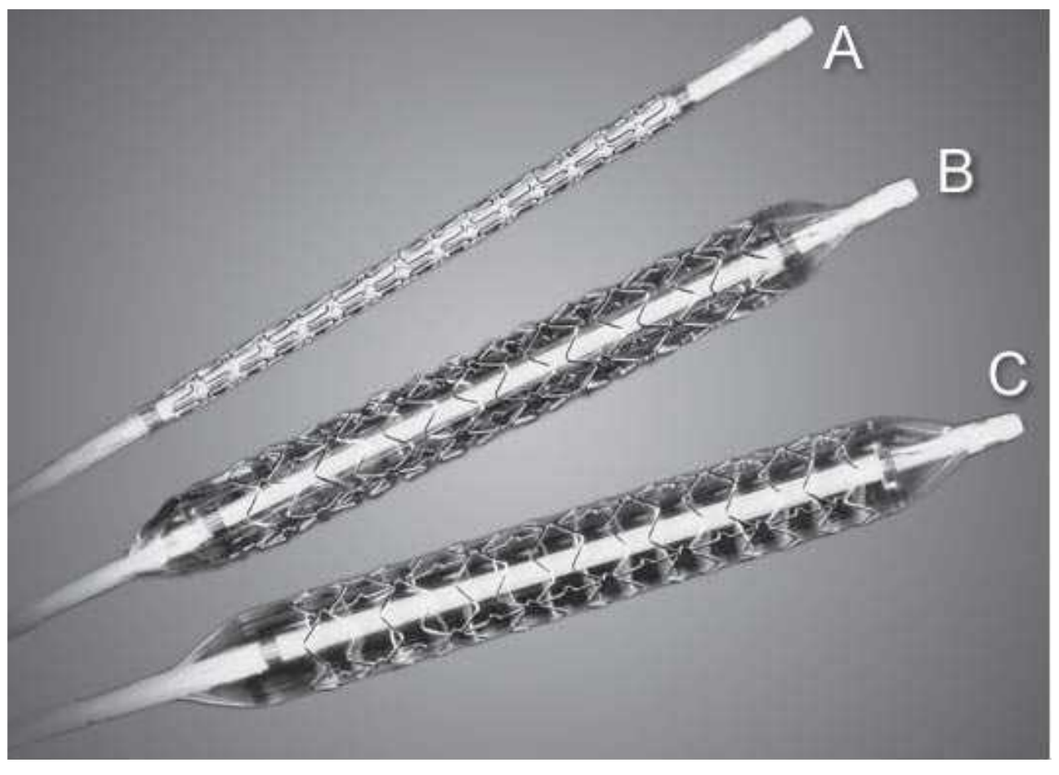


FIGURE 12.17 Balloon-expandable stent. **A:** Stent mounted on balloon as it is introduced into the patient. **B:** Appearance of stent following partial inflation of balloon on which the stent is mounted. **C:** Appearance of stent following complete inflation of balloon on which the stent is mounted.

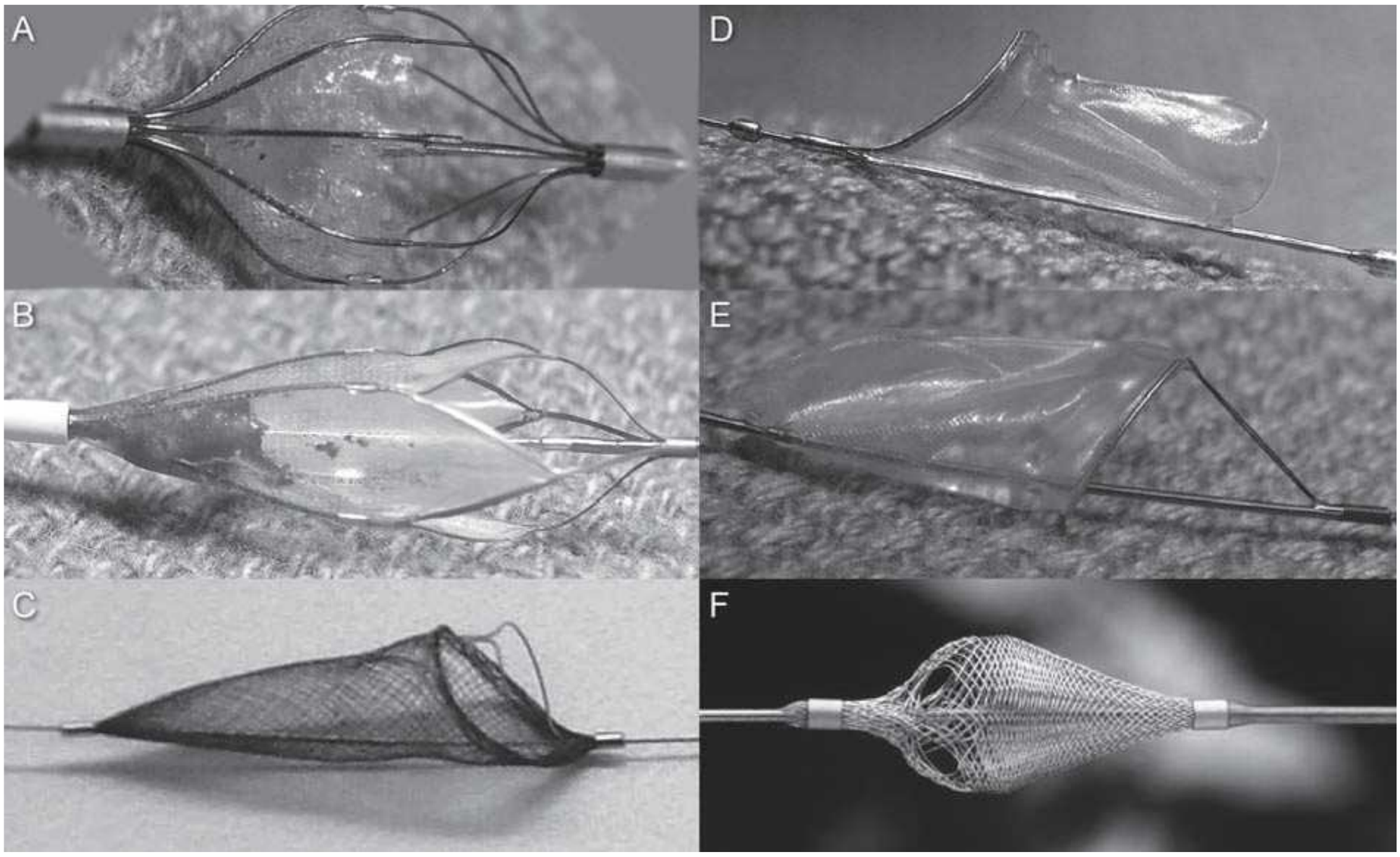
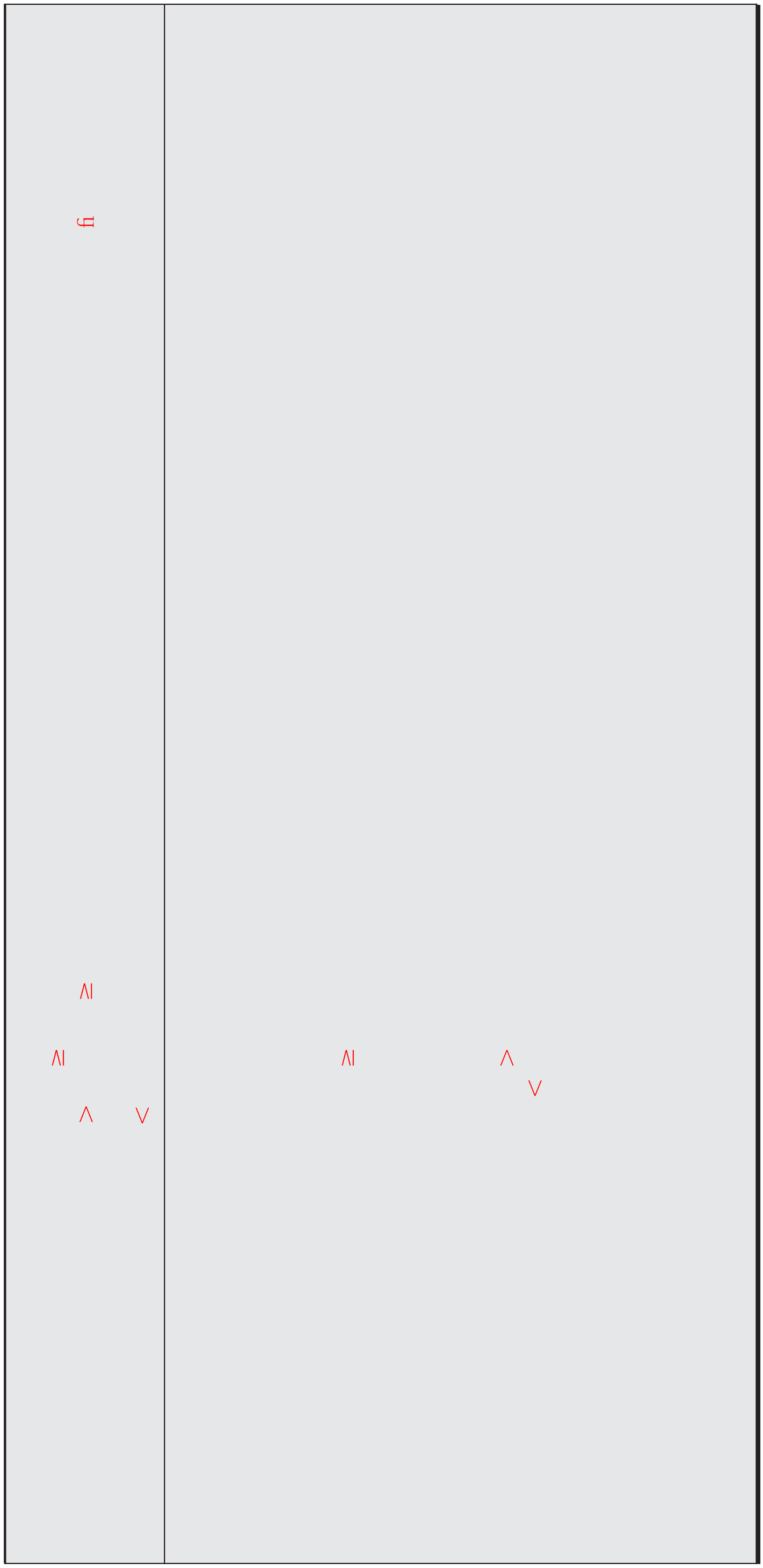


FIGURE 12.18 Sample of filter-type embolic protection devices used during renal, coronary, and carotid artery intervention. **(A)** Angioguard XP (Cordis, Warren, NJ), **(B)** Accunet (Abbott Vascular, Santa Clara, CA), **(C)** Spider (ev 3, Plymouth, MN), **(D)** FilterWire EX (Boston Scientific, Natick, MA), **(E)** FilterWire EZ (Boston Scientific, Native, MA), and **(F)** Interceptor (Medtronic, Minneapolis, MN).

treatment of renal stenoses that were not hemodynamically significant, and may have attenuated any possible treatment effect of renal revascularization. Thus, based on the nature of the patient populations that were included in these studies, it could have been predicted that it would have been difficult to show a benefit of renal revascularization. This was further compounded by the very small size of the patient cohorts in each of these trials (n = 49, 55, and 106) and the high rate of crossover from the medical arm to the angioplasty arm in two of the three studies. With such a high crossover rate, the use of an intention-to-treat method of analysis that was uniformly applied greatly underestimates the potential benefit of any intervention. Accepting these limitations, the conclusion of these trials was that angioplasty was associated with a modest improvement in blood pressure control that manifested itself in the use of less antihypertensive medications when compared to medical treatment alone.

There have been two notable large nonrandomized prospective registries of renal artery stenting that are distinguished from other nonrandomized studies by their prospective nature and the use of independent core laboratory monitoring of angiographic and ultrasound data.[32,33] As a result, there is greater confidence about the reliability of the data reported. These studies enrolled a largely hypertensive population who had an inadequate response to angioplasty for treatment of a severe stenosis (≥70%) of the renal artery. The acute procedural success achieved with stenting was very high (>93%). Significant complications occurred in 5% to 8% of cases, with the majority of these being related to complications at the arterial access site. Restenosis, as determined by duplex ultrasound, occurred in 12% to 17% of patients. Significant improvements in blood pressure control with less use of antihypertensive medication were observed, and renal function was stable over a 24 to 36 month period of follow-up. The major contribution of these studies is in the assessment of the safety profile of the procedure of renal artery stenting.

Two randomized trials comparing renal artery stenting with medical therapy have been completed (summarized in Table 12.3).[34,35] Neither of these studies demonstrated a benefit of renal revascularization. The study by Bax et al. had a number of significant shortcomings, most notably a small sample size (n = 140) that make it difficult to assess the usefulness of the data.[34] By contrast, the ASTRAL trial was the largest randomized trial of renal artery intervention ever performed (n = 806).[35] However, the design and execution of this study were flawed in a number of respects.[36] Only patients in whom the physician was uncertain about the likely benefit of renal artery revascularization were included. This immediately resulted in the selection of a low-risk population who were less likely to benefit from renal revascularization. The use of a threshold of ≥50% stenosis in the renal artery for inclusion in the study resulted in ~40% of patients with stenoses between 50% and 70% being enrolled. Such a degree of stenosis would not be anticipated to be



hemodynamically significant, and therefore would not be associated with clinical benefit from revascularization. In fact, the absence of a core lab to adjudicate the severity of RAS from the renal angiograms in this study makes it likely that a higher percentage of patients with <70% stenosis were included, given the propensity for operators at primary study sites to overestimate the degree of stenosis using visual estimates. The quality of the operators performing the renal stenting procedures is called into question by the alarming rate of major complications reported in this trial (9%). Events such as renal artery occlusion and perforation, and lower extremity gangrene occurred in this trial, but were not observed in either of the large prospective registries performed in the United States, where access site complications were the most common events. The ASTRAL trial was performed in the United Kingdom, raising the possibility that there are possible differences in outcomes with renal artery stenting in different continents, an hypothesis that has gained some traction based on data from carotid artery stent trials based in Europe and the United States.[37–39]

In summary, the ASTRAL trial confirmed that there is no benefit from renal artery revascularization in the setting of an incidental finding of RAS or a RAS of uncertain clinical significance. The trial failed to answer the question of the role of renal artery revascularization in clinical situations where the treating physician is confident that renal artery revascularization is of benefit. Unfortunately, there has been a tendency by several authors, including the primary authors of the ASTRAL trial, to oversimplify and misinterpret the findings of the ASTRAL by concluding that there is no benefit of revascularization in all patients with atherosclerotic RAS. This has contributed to an inappropriate nihilism toward renal revascularization for atherosclerotic RAS and the failure to offer this therapy in patients for whom renal revascularization has a real clinical benefit.

FIBROMUSCULAR DYSPLASIA

Fibromuscular dysplasia (FMD) is a nonatherosclerotic, noninflammatory disorder of small and medium sized arteries that results in the development of stenoses, aneurysms, and dissections in the affected arteries.[40,41] Pathologically, the disorder is classified according to the layer of the arterial wall affected (i.e., intima, media, adventitia) and the pathology observed in that layer (e.g., fibroplasia [accumulation of collagen], hyperplasia [smooth muscle hyperplasia without collagen accumulation]).[42,43] Medial and perimedial fibroplasia account for the vast majority of cases, and have a characteristic "string of beads" appearance on angiography (Fig. 12.19). The remaining pathologic types are difficult to distinguish by angiography, and are often grouped together. Both concentric and long smooth stenoses are felt to be the typical angiographic appearance associated with these nonmedial fibrodysplasias. The renal arteries are involved in 60% to 75% of patients with FMD, with bilateral involvement in ~35% of patients. In contrast to atherosclerotic RAS, the most common location of renal artery involvement by FMD is in the mid- and distal segments of the main renal artery and the branch vessels. It is estimated that ~10% of all cases of RAS can be attributed to FMD.

Interventional Treatment of FMD in Renal Arteries

The dominant indication for the interventional treatment of renovascular FMD is the treatment of HTN in the following clinical circumstances[40,41]:

1. New onset of HTN where the goal of revascularization is to achieve cure of HTN
2. Patients with poorly controlled HTN despite medical therapy
3. Patients who are intolerant of antihypertensive medications
4. Patients who are noncompliant with antihypertensive medications
5. Documentation of loss of renal volume due to ischemia

Fortunately, renal dysfunction due to renovascular FMD is uncommon, and renal failure is rare.

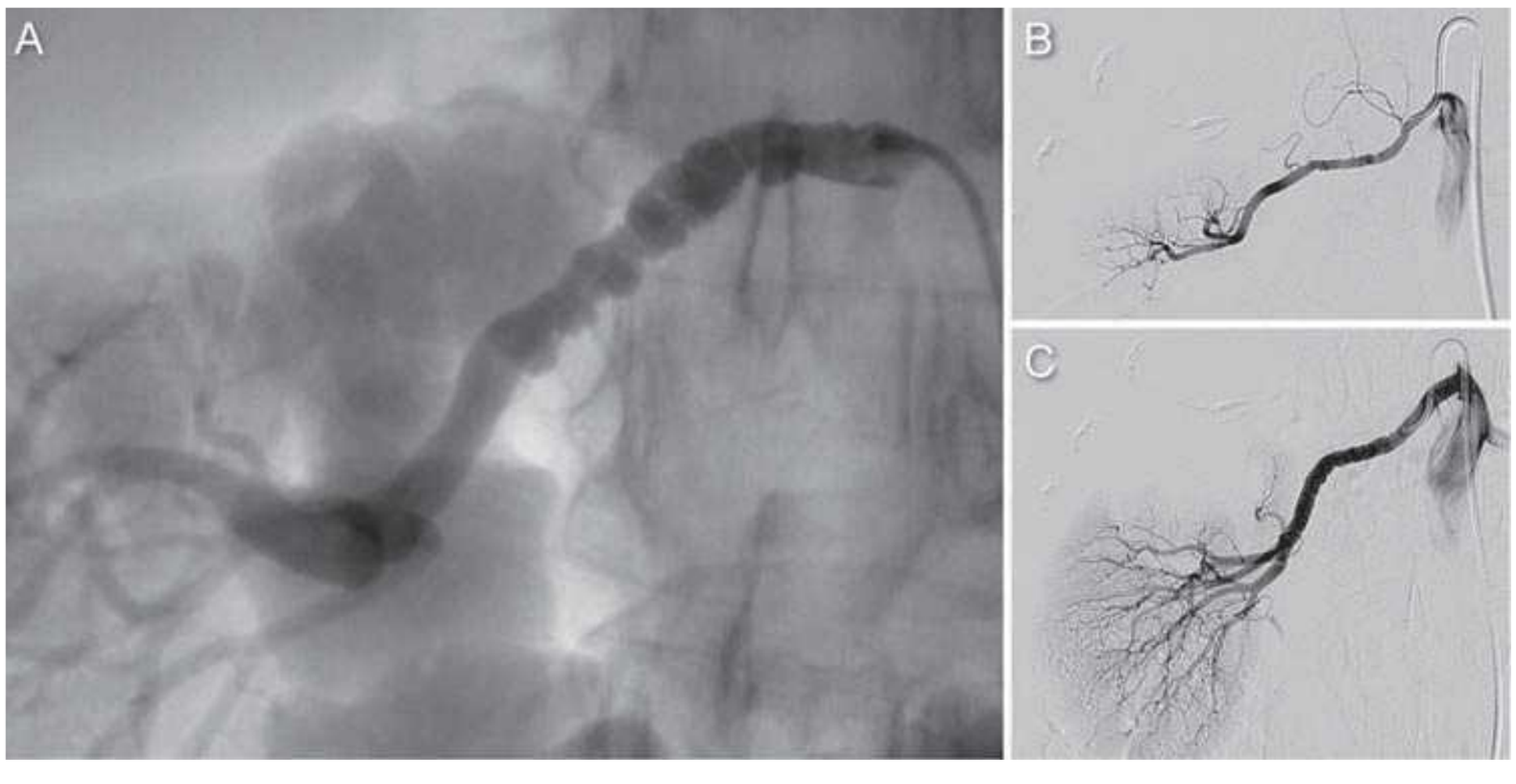


FIGURE 12.19 Typical angiographic apperance of fibromuscular dyplasia involving the renal arteries. **A:** Prominent "string of beads" appearance of right renal artery. **B:** More subtle string of beads appearance in main right renal artery and accessory right renal artery.

Much of the data regarding the interventional treatment of renovascular FMD are derived single center observational series, with no prospective randomized studies (summarized in Table 12.4).[44–52] Accepting the limitations of such studies, the following generalizations regarding the interventional treatment of renovascular FMD are accepted by most experts in the field. In contrast to atherosclerotic RAS, angioplasty is established as the gold standard for treatment of stenotic renovascular FMD. This is based on acute procedural success rates of >90% in most series, and acceptable rates of restenosis that are significantly lower than those observed in the treatment of atherosclerotic RAS (i.e., 7% to 27% at 3-year follow-up). The clinical outcome in terms of blood pressure control varies significantly across studies, likely due to disparities in the spectrum of the disease in these relatively small patient groups. However, a lack of any response is uncommon with a reported frequency of <20% in most series. Variables that have been associated with an increased likelihood of complete cure or improvement in blood pressure control include younger patient age, shorter duration of HTN, less severe baseline HTN, absence of extrarenal arterial involvement, and smaller kidney size.[44,47,50] The true predictive value of these variables derived from small series needs to be interpreted with caution, but do argue in favor of an attempt at angioplasty in the newly diagnosed younger patient with FMD complicated by HTN due to an increased likelihood of complete cure.

The interventional technique of angioplasty for renovascular FMD is similar to that described above for atherosclerotic RAS.[53] One of the significant differences, however, is that it can be difficult and sometimes impossible to assess the precise site of stenosis along a length of a diseased segment of vessel. The location of the stenosis can be most accurately ascertained by using a 0.014-inch pressure wire introduced into the renal artery or branch vessel (e.g., PrimeWire Guidewire, Volcano Therapeutics Inc,

12.4 List of Studies Reporting Procedural and Clinical Outcomes of Angioplasty for Treatment of Fibromuscular Dysplasia of the Renal Arteries

Study	Year	Patients (n)	Success rate (%)	Effects on Blood Pressure[a]			Mean Follow-up (months)	Complications (%)
				Cured (%)	Improved (%)	Unimproved (%)	Mean (range)	
Sos[70]	1983	31	87	59	34	7	16 (4–40)	6
Baert[71]	1990	22	83	58	21	21	26 (6–72)	NR
Tegtmeyer[52]	1991	66	100	39	59	2	39 (1–121)	13
Bonelli[72]	1995	105	89	22	63	15	43 (0–168)	11 (major)
Jensen[73]	1995	30	97	39	47	14	12	3 (major)
Davidson[47]	1996	23	100	52	22	26	NR	12 (minor)
Klow[74]	1998	49	98	26	44	30	9 (1–96)	0
Birrer[46]	2002	27	100	74[c]	26	NR	7.4	33
Surowiec[51]	2003	14	95	79[c]	21	NR	28.5	43
de Fraissinette[48]	2003	70	94	14	74	12	39 (1–204)	11
Alhadad[45]	2005	69	95	24	39	37	84 (28–140)	20
Kim[49]	2008	15	79	13	80	7	24 (1-60)	16

[a]The percentage shown is the total for cured and improved.
NR, not reported.
Adapted from Olin JW, Pierce M. Contemporary management of fibromuscular dysplasia. Curr Opin Cardiol. 2008;23:527–536.

San Diego, CA; and PressureWire Certus, St. Jude Medical, St. Paul, MN). In the presence of a significant stenosis, a pressure gradient between the transducer located near the tip of wire and the guide catheter located at the ostium of the renal artery is observed. By slowly withdrawing the pressure wire, the location of the stenosis can be determined, which guides the location of the angioplasty. It is important to confirm the absence of any pressure gradient at the completion of the procedure. Imaging tools such as intravascular ultrasound may assist in this process, but in the authors' experience do not have the same fidelity for localizing the precise site of the hemodynamically significant stenosis or stenoses.

RENAL ARTERY ANEURYSM

The true incidence of aneurysms of the renal artery is difficult to estimate. Among patients undergoing angiography, an incidence of 0.6% to 1% has been reported.[54] In the current era, the majority of patients have single, small (i.e., <2 cm) aneurysm that is asymptomatic. However, renal artery aneurysms that are bilateral, multiple, and up to 8 cm in size have been reported. In most series, the dominant underlying etiology for renal artery aneurysmal disease includes FMD and atherosclerosis, with a small number being attributed to renal artery dissection, arteritis (giant cell and Takayasu) and trauma, and connective tissue diseases (i.e., Marfan syndrome, Ehlers Danlos syndrome).[55] The majority of renal artery aneurysms are located in the body of the main renal artery (usually near the junction with the branch vessels), with the remainder in the branch vessels within the parenchyma of the kidney. Aneurysmal disease in accessory renal arteries is rare.

Renal artery aneurysms may manifest following rupture or renal infarction due to embolization of thrombus within the aneurysm. Treatment in such circumstances where the aneurysm is symptomatic is clearly indicated. However, the management of asymptomatic patients is more controversial. In such patients, the major risk is felt to be spontaneous rupture. As such, parameters that are felt to be associated with an increased risk of rupture are used to guide the decision to intervene. Although no large epidemiologic studies exist, there appears to be some consensus that larger aneurysms (i.e., >2 cm), enlarging aneurysms, and aneurysms in pregnant women or in those with a potential for pregnancy should be treated. The latter opinion is based on multiple case reports of ruptured renal artery aneurysms in pregnant women.[56,57]

The decision to offer surgical versus endovascular treatment of a renal artery aneurysm is dependent on the morphology of the aneurysm and the anatomic relationship between the aneurysm and the main renal artery and its branches. High quality three-dimensional (3D) reconstructions of the renal artery and aneurysm using either CT angiography or invasive rotational angiography are of paramount importance in this regard.

A variety of surgical techniques have been described (Fig. 12.20), including tailoring (i.e., partial resection of aneurysm followed by reconstruction of a vessel of normal caliber from the remaining arterial wall), use of saphenous vein or polytetrafluoroethylene (PTFE) graft, and aneurysm resection followed by direct reanastomosis of the renal artery.[58] Frequently, a combination of these techniques may be required.

In terms of the endovascular treatment of renal artery aneurysms, a number of techniques have been described (Fig. 12.21).[59–62] These include embolization techniques using coils or liquid embolic agents. Occasionally, a balloon is inflated or a stent is implanted across the neck of the aneurysm to facilitate these embolization procedures in patients with wider aneurysm necks. Stent grafts (i.e., stents with a PTFE covering) have been used but are limited by their inflexibility and high profile, making delivery through smaller and tortuous vessels difficult. In addition, side branches that are covered by the stent graft are obstructed. The complexity of the decision-making in these procedures underscores the need for interventionalists with significant experience with management of aneurysms and renal arterial intervention to lead such cases.

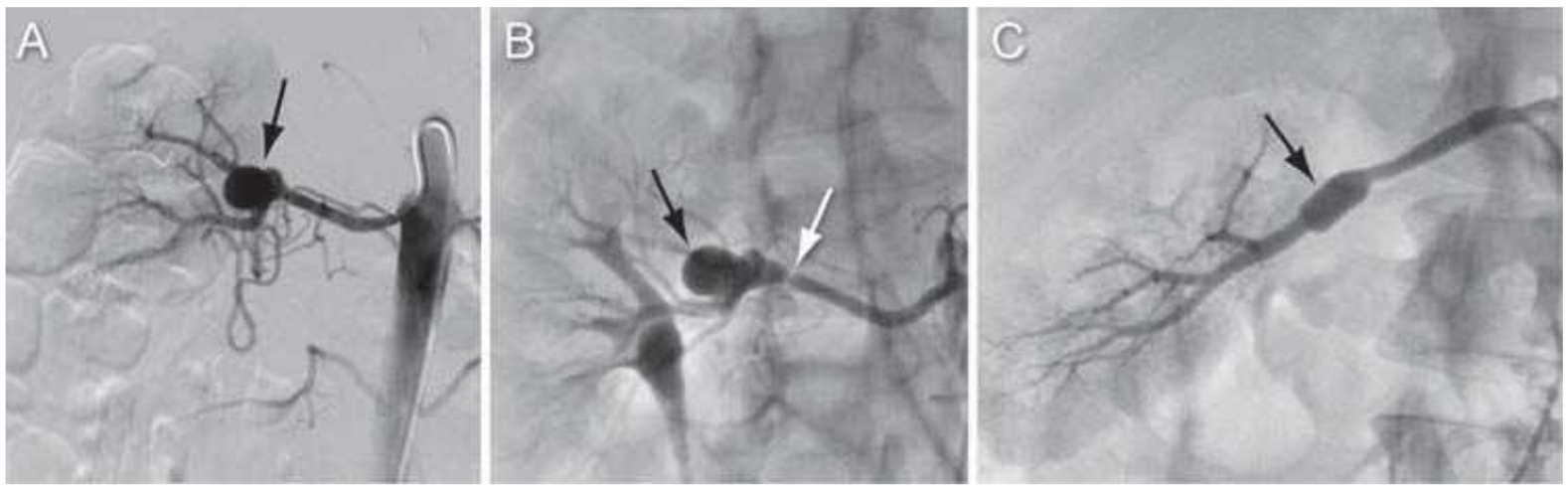


FIGURE 12.20 Surgical repair of renal artery aneurysm. **A:** Selective right renal artery angiogram of right renal artery showing aneurysm at junction of main renal artery and branch vessels. **B:** Selected image from rotational angiogram of right renal artery showing aneurysm (*black arrow*) and critical stenosis (*white arrow*) just proximal to the aneurysm. **C:** Right renal artery angiogram from same patient following surgical repair of renal artery aneurysm. Arrow indicates location of graft.

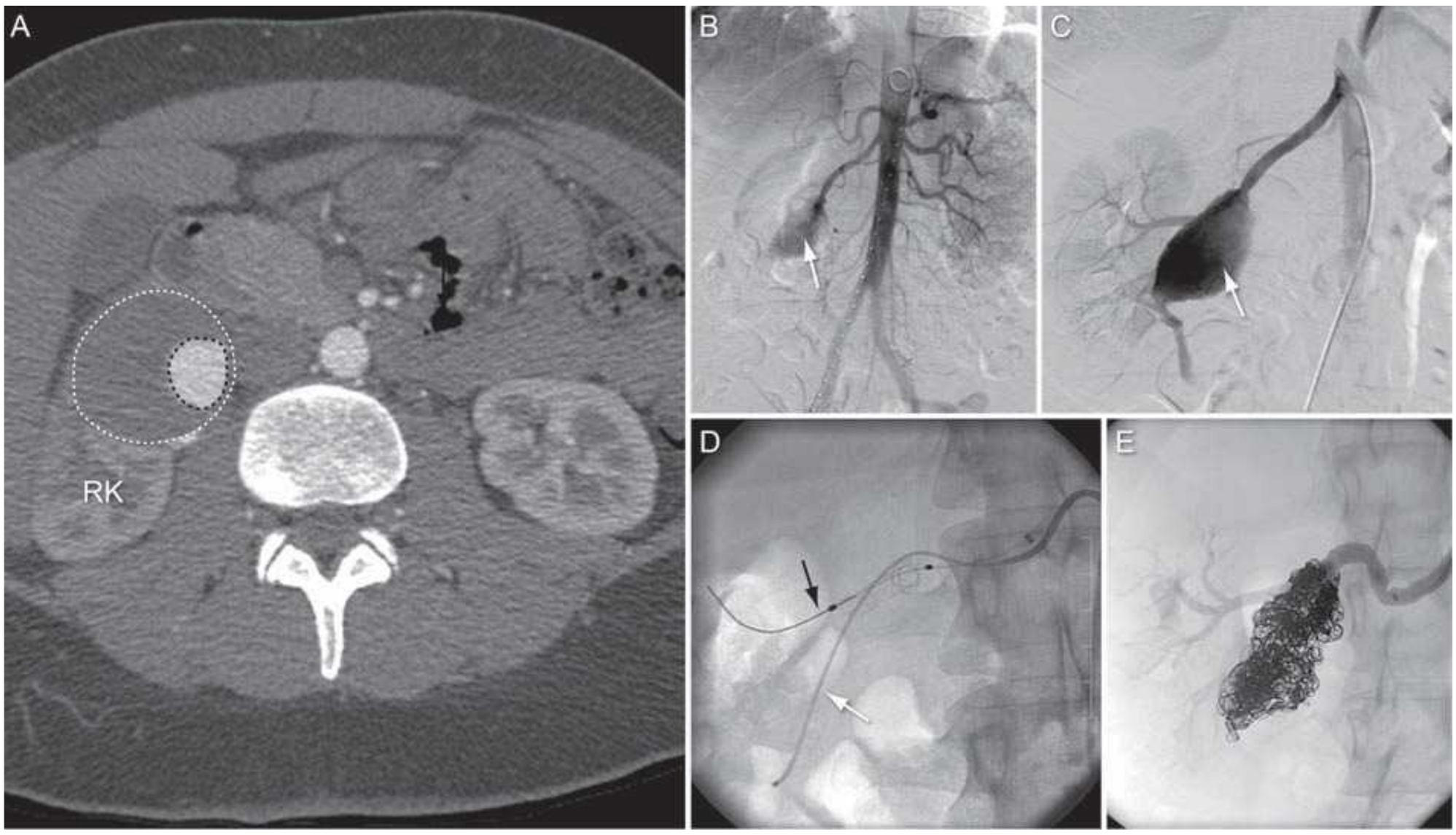


FIGURE 12.21 Endovascular repair of renal artery aneurysm. **A:** Computed tomography angiogram showing large aneurysm of right renal artery (outline of wall of aneurysm shown by *interrupted white line*). Contrast in lumen of aneurysm shown by interrupted black line. **B:** Preprocedural abdominal aortogram. **C:** Preprocedural selective right renal artery angiogram (aneurysm indicated by *arrow*). **D:** Following dual arterial access, stent-assisted coil embolization of the aneurysm was performed. *Black arrow* indicates wire in renal branch to be preserved. *White arrow* indicates wire in aneurysm sac. **E:** Completion angiogram showing successful exclusion of the aneurysm.

RENAL ARTERY EMBOLIZATION AND ABLATION

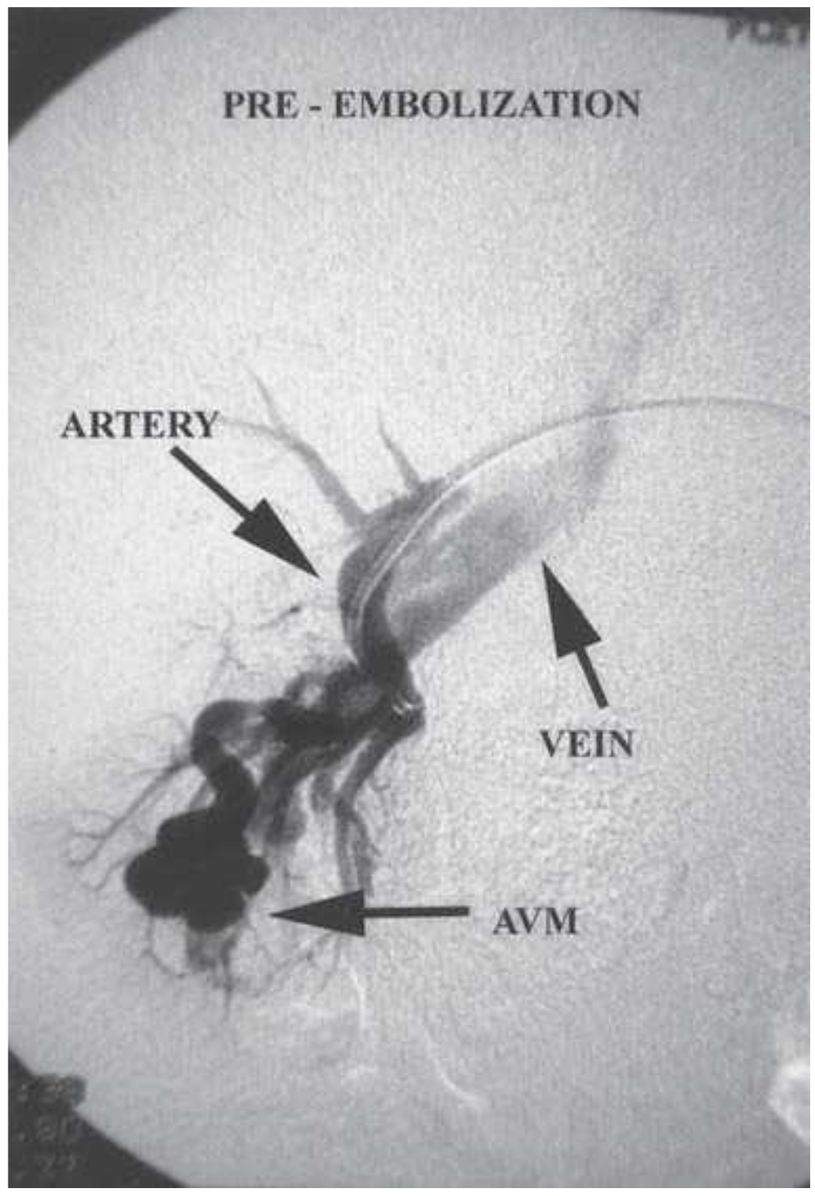
In addition to the treatment of renal artery aneurysms, renal artery embolization may be performed for a variety of other clinical indications including the control of active hemorrhage, obliteration of arteriovenous malformations (Fig. 12.22) or fistulae, treatment of renal artery pseudoaneurysms, devascularization of renal and adrenal tumors, and the treatment of conditions in which ablation of the parenchyma of the kidney is desired (i.e., medical nephrectomy [e.g., hyperreninemia-related HTN, severe nephrotic syndrome, chronic ureteric fistulae in high risk surgical patients, irreversible transplant rejection]).[63,64]

There are three categories of embolic agents that may be used to achieve renal artery embolization: solids, particulates, and liquids (sclerosants). The particular agent(s) used depend largely on the clinical indication and the specific anatomy of the renal artery and its branches (Figs. 12.23 and 12.24). For example, for the clinical indication of medical nephrectomy, embolization of the entire kidney is typically achieved by embolization of the small vessel branches with injection of liquid and particulate agents, followed by deployment of metallic coils in the main renal artery.

Success rates of over 90% are generally reported for renal artery embolization, although highly complex cases where preservation of renal function is paramount are likely to have lower rates. By definition, all patients will suffer a renal infarct following renal artery embolization, which is typically manifest by flank pain and fever that begins 1 to 3 days following the procedure. The impact on renal function will be determined by the size of the renal infarct, the function of the infarcted territory, and the baseline renal function. In addition to loss of renal function, the most feared complication is inadvertent non-target embolization of the arterial supply to the spinal cord, bowel, or lower extremity. Careful attention to technique should minimize these risks.

PERCUTANEOUS RENAL SYMPATHETIC DENERVATION

Since the early 1970s, a considerable body of data has emerged demonstrating a significant role of the afferent and efferent nerves of the renal sympathetic system in the initiation and maintenance of systemic HTN.[65–67] These nerves run in the adventitia of the wall of the main renal arteries and have long been investigated as a potential target for therapeutic intervention. In fact, surgical methods of sympathetic denervation were practiced prior to the availability of modern pharmacologic antihypertensive agents, but were abandoned due to high rates of perioperative mortality and morbidity. With the recognition that nearly half of all patients



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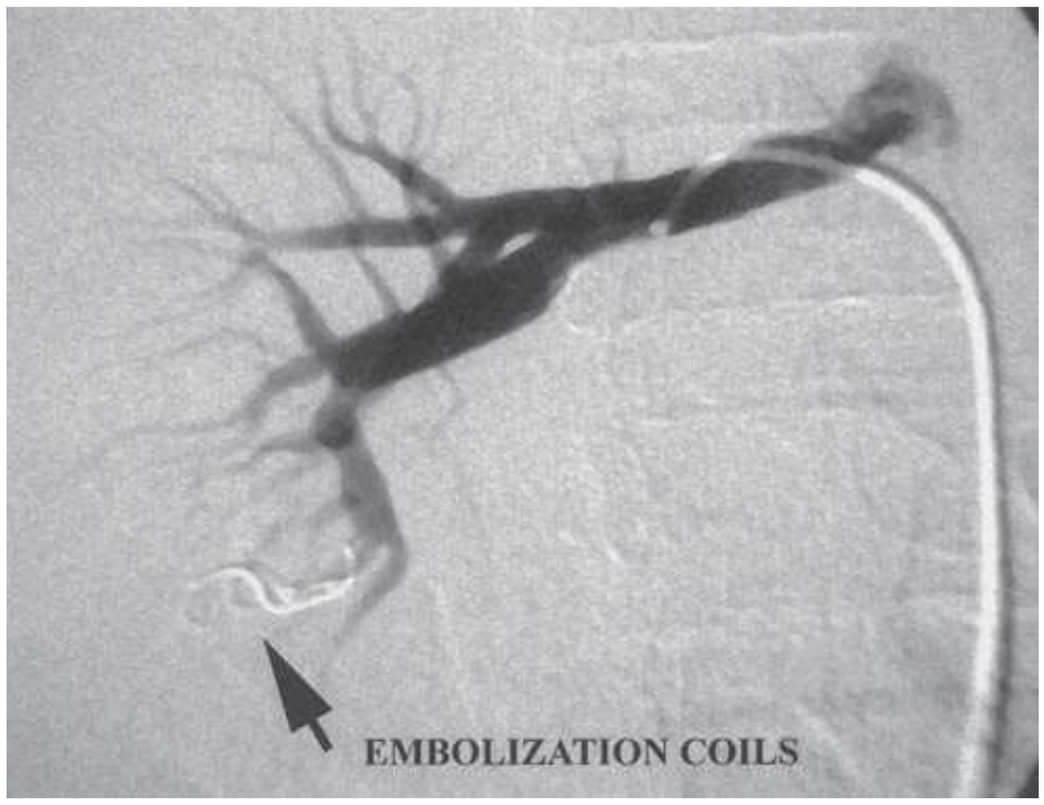
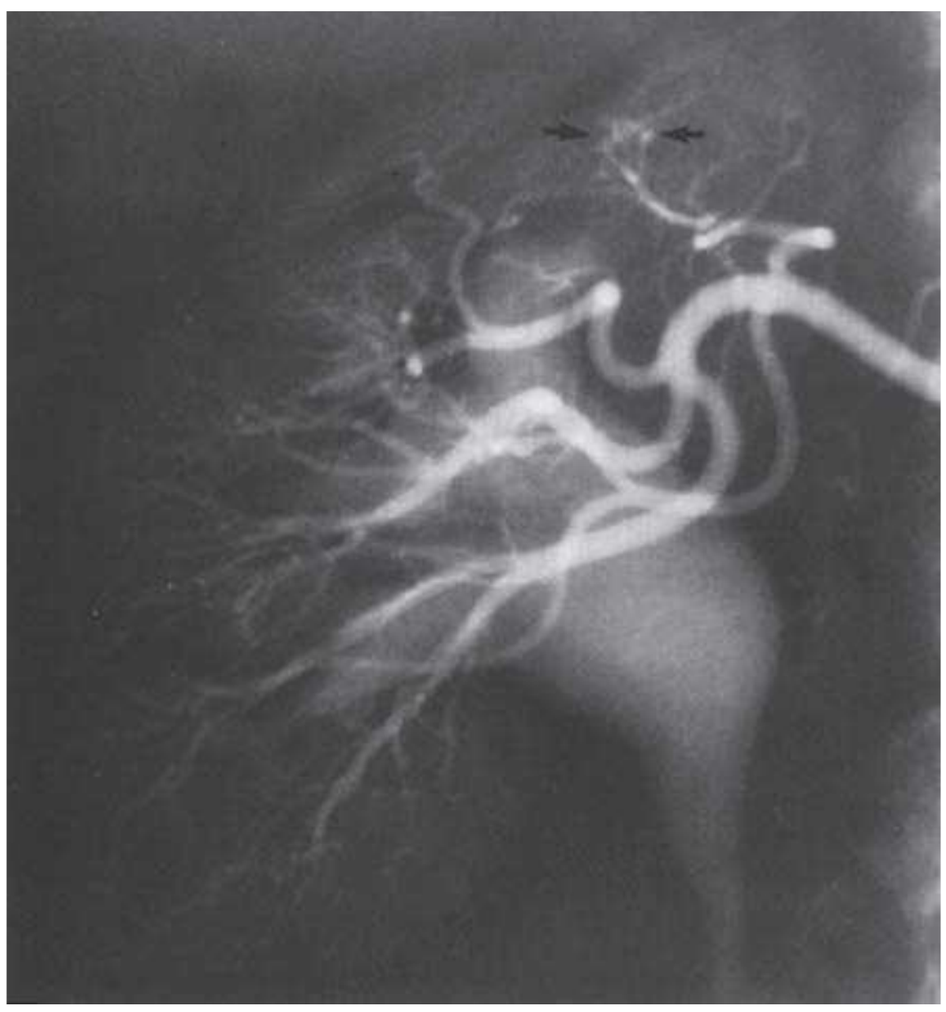
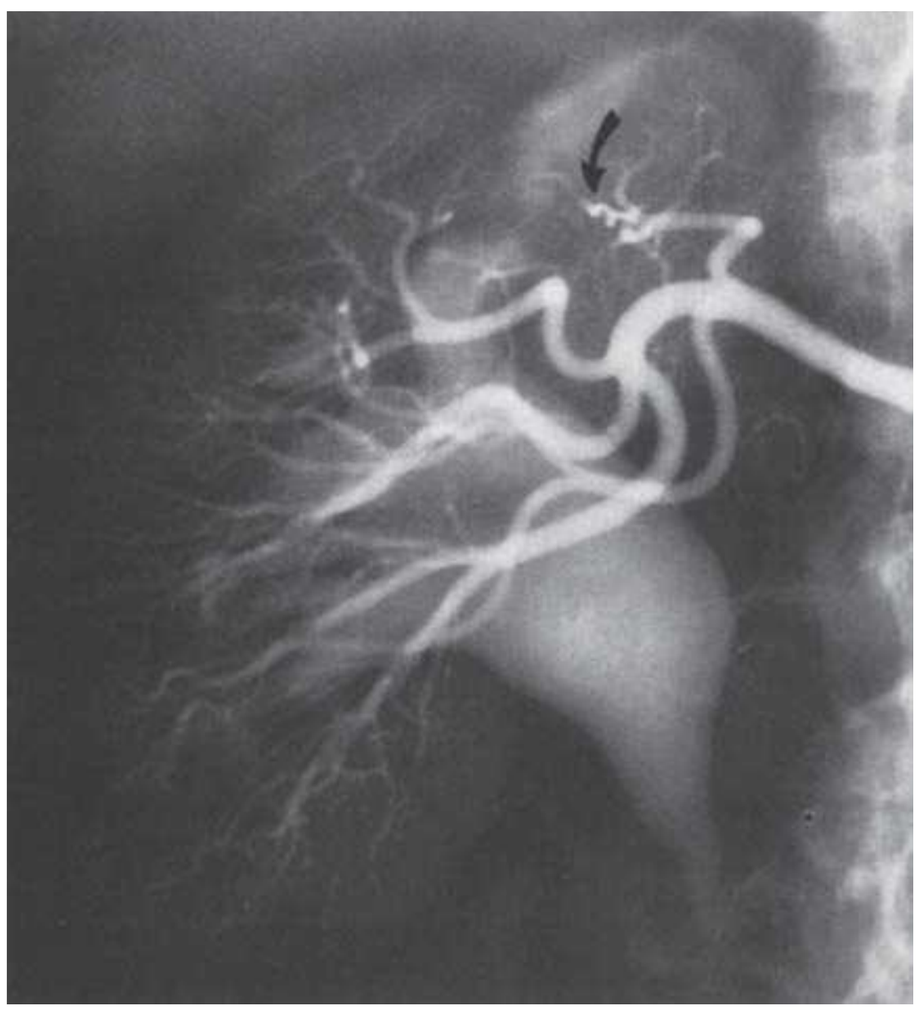


B

FIGURE 12.22 Digital subtraction selective angiography showing an arteriovenous malformation (AVM) of the lower pole of the right kidney, in a patient with hematuria. **A:** Preembolization angiogram depicting the feeding artery and draining vein of the AVM. **B:** Postembolization angiogram demonstrating the stainless steel coils successfully occluding the feeding artery of the AVM. (Reproduced with permission from Morris CS, Rimmer JM. Diagnostic and therapeutic angiography of the renal circulation. In: Schrier RW. *Diseases of the Kidney and Urinary Tract*, 8th ed. Philadelpha: Lippincott Williams and Wilkins; 2007:420–449.)



A



B

FIGURE 12.23 Renal hemangioma in a patient presenting with hematuria. **A:** The typical cluster of abnormal vascular structures (*arrows*) adjacent to a cortical infarct. **B:** Occlusion of the feeding artery after superselective embolization with a platinum microcoil (*curved arrow*). (Reproduced with permission from Morris CS, Rimmer JM. Diagnostic and therapeutic angiography of the renal circulation. In: Schrier RW. *Diseases of the Kidney and Urinary Tract*, 8th ed. Philadelpha: Lippincott Williams and Wilkins; 2007:420–449.)

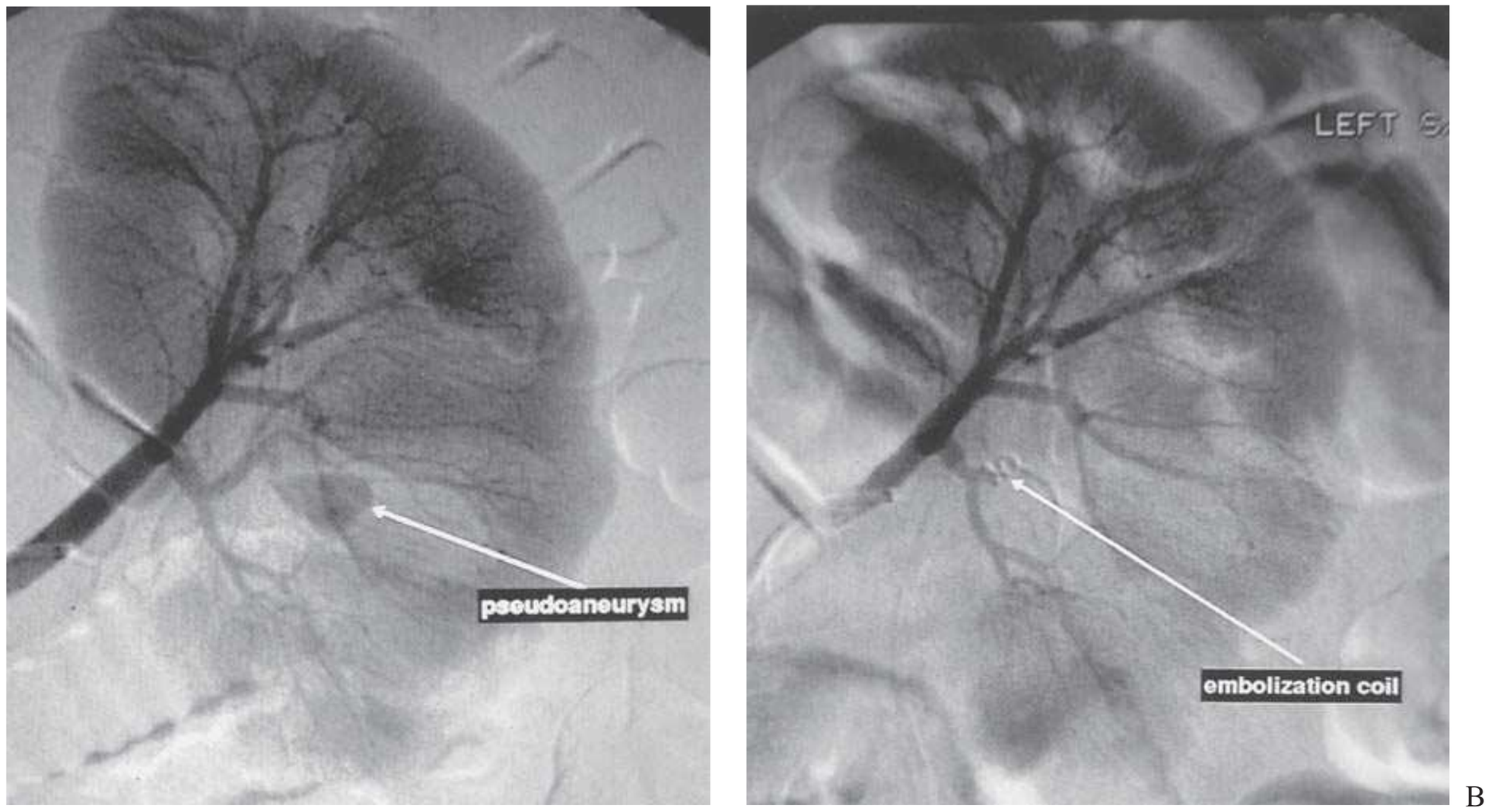


FIGURE 12.24 Digital subtraction angiography of a lacerated left kidney in a 14-year-old boy caused by blunt trauma during a bicycle accident, which resulted in massive hematuria and a large perinephric hematoma. **A:** Preembolization angiogram demonstrates a pseudoaneurysm. **B:** Postembolization angiogram shows the successful placement of a single stainless steel coil, occluding the feeding artery to the pseudoaneurysm. (Reproduced with permission from Morris CS, Rimmer JM. Diagnostic and therapeutic angiography of the renal circulation. In: Schrier RW. *Diseases of the Kidney and Urinary Tract*, 8th ed. Philadelpha: Lippincott Williams and Wilkins; 2007:420–449.)

with HTN do not achieve optimal blood pressure control, a search for a nonsurgical approach to renal sympathetic denervation was explored. At present, one commercial product has been developed and tested in clinical trials (Simplicity Catheter System, Ardian, Palo Alto, CA, recently acquired by Medtronic Inc., Minneapolis, MN) with several other companies and investigators exploring other technologies and techniques to achieve renal sympathetic denervation.

Technique of Renal Sympathetic Denervation

The current commercial product (Simplicity Catheter System) achieves renal sympathetic denervation by applying low power radiofrequency (RF) treatment (i.e., <8 watts) to the length and circumference of the proximal segment of both main renal arteries. This is performed by engaging the main renal artery with a guide catheter through which the radiofrequency catheter is directly inserted into the main renal artery. A total of four to six RF treatments, each lasting 2 minutes, are applied longitudinally and circumferentially in the proximal segment of each renal artery. The RF treatments are typically associated with diffuse visceral abdominal pain that does not persist beyond the duration of the treatment and is managed with usual narcotic and sedative medications (e.g., fentanyl and midazolam). Technically, these procedures are straightforward and much less challenging than stenting of atherosclerotic RAS.

Clinical Outcome Data for Percutaneous Renal Sympathetic Denervation

To date, one proof of principle registry and one randomized trial have been completed using the Simplicity Catheter System.[68,69] The registry study treated 45 patients with resistant HTN (i.e., systolic blood pressure [SBP] >160 mm Hg and on three antihypertensive medications) and a glomerular filtration rate (GFR) of 45 mL/min/1.73 m^2 at five European and Australian sites.[69] In terms of safety, there were two procedure-related complications: one was an arterial access site complication, and the second was a renal artery dissection due to manipulation of the guide catheter that did not have a clinical consequence. Among the treated patients, there was a significant and sustained reduction in the mean systolic and diastolic pressures out to 12 months (27/17 mm Hg at 12 months). In fact, the reductions in blood pressure increased over the 12 month period, suggesting that the effects of denervation are durable.

Using the same enrollment criteria, the Symplicity HTN-2 Trial randomized 106 patients to renal denervation versus medical therapy.[68] At 6-month follow-up, there was a significant reduction in office-based blood pressure measurements in the renal denervation group (32/12 mm Hg) and no significant change in the medical treatment group (1/0 mm Hg). There were no major device- or procedure-related complications, underscoring the straightforward

nature of the technique and the safety of the procedure. Renal function was stable in both groups, indicating no adverse effect on the kidney parenchyma.

Given the positive results from these studies, considerable excitement about this technology has been generated. Further studies in the United States are planned to achieve FDA-approval. In addition, trials of this therapy in other disease entities in which renal sympathetic activation is thought to play a key pathogenic role (e.g., chronic kidney disease, congestive heart failure, hepatorenal syndrome, cirrhosis with ascites) are at various stages of planning.

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CHAPTER

13 Renal Biopsy: Indications and Evaluation

I. David Weiner • C. Craig Tisher • Byron P. Croker

This chapter discusses the indications for performing a renal biopsy, describes the procedure and methods of tissue preparation, and demonstrates the manner in which biopsy specimens are interpreted using a combination of light microscopy, electron microscopy, and immunohistologic microscopy. The technique of percutaneous renal biopsy was introduced clinically in the early 1950s. Iversen and Brun[1] are generally credited with describing its initial use. They believed the technique would be quite useful in obtaining more information about diseases that caused acute kidney injury. At that time, the diseases were referred to as lower nephron nephrosis. The renal biopsy technique was used with increasing frequency during the 1950s, and it has enjoyed wide usage throughout the world since the early 1960s. The technique has provided a wealth of information about the histopathology, pathogenesis, and classification of renal disease that could not have been obtained by any other means.

Proponents of the biopsy procedure employ this technique to diagnose kidney disease, to assess prognosis, to monitor disease progress, and to aid in the selection of a rational approach to therapy. It is used extensively both in younger[2–7] and older patients.[8–14] However, the procedure is not without morbidity and, occasionally, mortality. Therefore, the risk/benefit ratio must be considered carefully in each patient who is being evaluated for a biopsy.

As originally described, the biopsy was performed with the patient in the sitting position, and the procedure involved aspiration of the tissue sample. Brun and Raaschou[15] used the Iversen-Rohlm cannula and syringe, which yielded a cylinder of tissue approximately 1.5 mm in diameter and of variable length. Kark and Muehrcke[16] chose to place the patient in the prone position and initiated the use of the Franklin modification of the Vim-Silverman cutting needle (Popper & Sons, Inc., New Hyde Park, NY) in place of the aspiration technique. Today, most nephrologists position the patient in the prone position and use a spring-loaded, semiautomatic biopsy device. The use of either ultrasonography (US) or computed tomography (CT) to locate the kidneys and to aid in positioning the biopsy needle has greatly simplified the technique and improved its safety.

Adequate tissue samples are obtained in greater than 95% of procedures. In a retrospective study, Bolton and Vaughn[17] reported that renal tissue was obtained in 97% of their patients with the use of image-amplification fluoroscopy, compared with 81% without the use of fluoroscopy. Percutaneous renal biopsies performed with renal imaging using either US or CT were successful in ~98% of patients in several series.[18–21]

The percutaneous renal biopsy is a safe and reliable technique in the hands of the experienced operator. The most common complication is bleeding, which occurs in the majority of patients if they are studied carefully after biopsy using ultrasonography[22] or CT.[23,24] However, the bleeding is self-limited and rarely requires an operative intervention or a blood transfusion. In a survey[25] of the results of over 5,500 percutaneous renal biopsies, the rate of complications, including the need for a blood transfusion or a nephrectomy, the puncture of other organs, or the presence of a clinically evident perinephric hematoma, was 2.1%. The overall mortality is approximately 0.1% to 0.2%,[25–27] which is comparable to that reported for percutaneous liver biopsy or coronary angiography.[25] In a study from a single institution[28] in which 1,000 consecutive percutaneous renal biopsies were analyzed, a total of 94 complications were observed in 81 patients. Gross hematuria, including the passage of blood clots, represented 73% of the complications. Two patients underwent exploration for the evacuation of perirenal hematomas, but no kidneys were lost. One patient died of multiple complications after biopsy.

Multiple factors are associated with an increased risk of complications from the renal biopsy procedure. In one study, the presence of a serum creatinine of at least 5.0 mg per deciliter was associated with a 2.3-fold increase in the risk of a complication.[29] Other studies have identified uncontrolled hypertension, thrombocytopenia, and anemia as predictors of increased risk for complications.[30–32] The simultaneous presence of both hepatitis C and HIV infection is associated with as much as a 5.7-fold increase in complications,[31] but the presence of amyloidosis or monoclonal gammopathy is not.[33,34]

The timing of postprocedure complications has important implications regarding how long patients should be observed prior to discharge. Most studies have shown that the great majority of complications can be identified in the initial 6 to 8 hours after the procedure,[32,35] suggesting that a renal biopsy can be performed safely as an outpatient procedure. However, some studies report that as many as 33% of complications are not identified after 8 hours of observation.[29] Screening tests such as the presence or absence of a postprocedure perirenal hematoma may be helpful in assessing the risk of a clinically significant complication.[36,37]

TECHNIQUES

Prior to an elective renal biopsy, it is important to screen the patient for the presence of bleeding disorders. A careful history should be obtained to determine whether abnormal bleeding occurred during previous surgical procedures. A history of severe menorrhagia, if female, and other evidence of abnormal bleeding, as well as a family history of bleeding disorders should be sought. Screening laboratory studies may include an assessment of the platelet count and bleeding time. In addition, it is advisable to obtain the hematocrit and hemoglobin levels within 24 hours prior to the procedure. Renal imaging, typically by ultrasonography, should be performed prior to a biopsy to assess for the presence of anatomic abnormalities, including solitary kidney, horseshoe kidney, hydronephrosis, small kidneys, or other anatomically abnormal kidneys, which may adversely affect the risk of a renal biopsy. Currently, most percutaneous biopsies are performed with the guidance of US or CT to permit an accurate localization of the kidney. The use of a premedication, such as midazolam (Versed), to help alleviate patient anxiety may make the procedure less unpleasant for the patient. We routinely place an intravenous access in the patient.

Most operators prefer to biopsy the lower pole of the left kidney to reduce the risk of inadvertently passing the biopsy needle through a major renal artery or vein. After the completion of the biopsy, patients are instructed to remain at bed rest for 6 to 8 hours. In our institution, we screen with US or CT in the immediate postprocedure period for the presence or absence of a perirenal hematoma and its size, if present. We assess the blood pressure and pulse every 15 minutes for 1 hour, every 30 minutes for 1 hour, then hourly for the next 4 to 6 hours. The patient is asked to save an aliquot of each voided urine in a separate clear plastic specimen jar labeled with the date and time, which is kept at the patient's bedside for inspection. This provides a visual check for evidence of bleeding into the intrarenal collecting system. The hemoglobin and hematocrit are determined 6 to 8 hours after the biopsy, or earlier if hemodynamic instability or gross hematuria is observed. If the hemoglobin and hematocrit are stable, the patient is relatively pain free and there is no hemodynamic instability or gross hematuria, we discharge the patient home with instructions to call immediately should there be a change in his or her clinical condition. If the patient does not meet these criteria, we admit the patient overnight for further observation.

An outpatient renal biopsy, as described in the previous paragraph, is a component of an ongoing trend to identify approaches to optimize the use of health care resources. An ample amount of literature demonstrates the safety of this approach in both native and transplanted kidney biopsies in both children and adults.[38–40] Ultrasonographic evidence suggests that most episodes of major bleeding occur within the initial 6 hours after a renal biopsy and that the size of perirenal hematomas actually decreases thereafter.[39] These data confirm an earlier report by Carvajal et al.[2] who found only three significant bleeding episodes in 890 consecutive percutaneous biopsies performed in pediatric patients. These data, when linked with the experience in the outpatient setting thus far, suggest that in carefully selected patients in whom the procedure is performed without difficulty, the use of ambulatory percutaneous renal biopsy can be justified. If patients are free of pain at the site of biopsy, have clear urine, and have stable cardiovascular signs for a minimum of 4 to 6 hours after the procedure, they can be safely discharged.[40] Activity should be restricted for at least 24 hours, and patients should be cautioned to seek medical attention immediately if there is macroscopic hematuria or pain over the biopsy site.

Several types of spring-loaded automatic or semiautomatic biopsy guns are employed to perform percutaneous biopsies of both transplanted[21,39–50] and native kidneys.[21,49–63] Based on a sample of almost 2,000 percutaneous biopsy procedures, the rate of complications, including a clinically evident hematoma, nephrectomy, blood transfusion, acute urinary tract obstruction, or biopsy of another organ, was 1%. Adequate samples of tissue were obtained 94% of the time on the initial attempt at biopsy. These data compare very favorably with the published experience with either the Franklin modification of the Vim-Silverman needle or the Travenol Tru-Cut disposable needle (Travenol Laboratories, Deerfield, IL).[17,25] Furthermore, when direct comparisons have been made, the results obtained with the biopsy gun were easily comparable to those achieved with the Travenol disposable needle.[44,48,54,55,60,61] In another study,[21] 1,090 percutaneous kidney biopsies were performed using US guidance and an automated spring-loaded biopsy device. A total of 114 (10.4%) were performed on renal allografts and 976 (89.6%) were performed on orthotopic kidneys. No serious complications, including the loss of kidney, life-threatening hemorrhage, or a persisting hemodynamically relevant arteriovenous (AV) fistula, were encountered. In 98.8% of the patients, sufficient tissue was obtained to make a reliable histopathologic diagnosis.

When combined with real-time US technology, there are several advantages to using the fully automatic biopsy guns. For example, the depth of the biopsy is controlled precisely and can be selected for a particular clinical situation. In the case of one of the most commonly used instruments

(Biopty, Bard Urological Division, C.R. Bard, Covington, GA), the long-throw device has a depth of 2.3 cm, yielding a specimen with a potential length of up to 1.7 cm. The short-throw device has a depth of 1.15 cm and a potential specimen length of 0.9 cm.[64] Fully automatic biopsy guns can be triggered with one hand, thus leaving the operator with a free hand to control the US probe if necessary. Instruction in the use of the biopsy gun is also easier. Many also believe that there is less discomfort with use of the biopsy gun.[21,53,54,60] Some studies have found decreased bleeding with automated biopsy devices,[65] whereas others have not.[60] The use of automated renal biopsy devices has almost completely replaced the use of manual devices.

Currently, there is no universal agreement on the optimum size of the needle that should be used with the various biopsy guns. Many favor the 18-gauge needle, which retrieves almost as many glomeruli per specimen as larger gauge needles.[41–54] This is due, in part, to the fact that the individual specimens have cleaner, sharper edges with less crush artifact. Certainly, in pediatric patients, the 18-gauge needle has been found to be quite adequate.[49,50,52,63] We favor use of a 15- or 16-gauge needle for biopsies in adult patients.

An alternative technique for performing the renal biopsy involves the transjugular approach. In this technique, a guide wire is inserted through the right internal jugular vein, through the vena cava, into the right renal vein, and is then wedged into the lower pole of the right kidney. A transvenous biopsy needle, similar to those used for transjugular hepatic biopsies, is then inserted over the guide wire, advanced into the kidney, and samples are taken. The first description of the procedure is generally attributed to Mal et al.,[66] who reported its use in 50 consecutive patients. All were patients in whom conventional percutaneous renal biopsy was felt to be clinically contraindicated, because of a need for simultaneous hepatic and renal biopsies, severe clotting disorders, respiratory insufficiency, uncontrolled hypertension, morbid obesity, or a solitary kidney. Renal tissue was obtained in 88% of patients, and glomeruli were present in 76% of the samples. Since this initial description, the procedure has become available and is used in a large number of centers. Typically, because of the increased technical difficulty and the cost of the procedure, the transjugular renal biopsy is reserved for patients with contraindications to a percutaneous renal biopsy. Subsequent studies that followed this initial report have confirmed its usefulness in patients in whom a conventional percutaneous approach is contraindicated. In general, adequate tissue is obtained in 85% to 95% of procedures.[67–72] The reported complications include capsular perforation, collecting system puncture, hematuria or loin pain, sufficient bleeding that blood transfusion is necessary, and hypovolemic hemorrhagic shock.[67–71,73,74] Because of the risk of postprocedure complications, most patients should be observed overnight after the procedure. The presence of an underlying clotting disorder is associated with an increased risk of complications,[74] but morbid obesity is not.[70] Thus, the transjugular renal biopsy provides an approach to the renal biopsy in patients in whom a conventional percutaneous approach is contraindicated. The risk of complications, although not inconsequential, is generally considered acceptable if the result of a renal biopsy is important in the patient's management.

Because decreased glomerular filtration rate can lead to platelet dysfunction, which may increase the risk of bleeding, efforts have been made to determine whether specific prebiopsy testing can decrease the risk of clinically significant postrenal biopsy bleeding. Traditional coagulation tests, such as partial thromboplastin time (PTT), prothrombin time (PT), and the International Normalized Ratio (INR), assess coagulation factor-mediated clotting, which is not altered with renal disease. Therefore, such tests are not good predictors of bleeding after a renal biopsy. The bleeding time, sometimes termed the template bleeding time, is more specific for assessing platelet function. Many authors feel that the bleeding time should be a routine component of the pretransplant evaluation,[75,76] whereas others disagree and have instead suggested that failing to measure the bleeding time does not expose the patient to an increased risk of bleeding.[77] Our personal practice is to assess the bleeding time, particularly in individuals with an increased blood urea nitrogen (BUN), in whom the risk of uremic platelet dysfunction is greater.

There are a number of treatment options in patients with uremic platelet dysfunction. Desmopressin (deamino-8-D-arginine vasopressin) rapidly decreases the bleeding time in patients with uremic platelet dysfunction,[78] and can be used to treat patients with a prolonged bleeding time.[75] A recent prospective, randomized clinical trial suggested that the routine use of desmopressin in patients with a serum creatinine less than 1.6 mg per deciliter and normal coagulation parameters, irrespective of bleeding time, decreases both the likelihood of postbiopsy bleeding (treated, 13.7% versus control, 30.5%) and, in those with bleeding, decreases both the size of the hematoma and the duration of hospital stay.[79] Although very intriguing, it is our current belief that confirmatory studies are necessary before adopting routine desmopressin treatment for all renal biopsies. Uremic platelet dysfunction can also be treated either with renal replacement therapy, such as hemodialysis,[80] or with oral estrogen therapy.[81,82] These alternative therapies take longer to improve the bleeding time than is required for desmopressin, and therefore are not routinely used.

INDICATIONS

There is no universal agreement on the precise indications for use of the percutaneous renal biopsy despite almost 60 years of experience with the technique by the nephrology community. The present section describes several clinical situations in which this technique is either routinely or frequently employed to aid in the evaluation and management of a patient with undiagnosed kidney disease.

Acute Kidney Injury

There are many occasions when the etiology of acute kidney injury secondary to intrinsic renal disease is not evident despite a carefully performed history and physical examination and the availability of information gained from various laboratory studies. A biopsy can be very useful in establishing the diagnosis, determining the approach to management, and defining the prognosis in this clinical setting. Retrospective studies from several centers have revealed that the diagnosis of acute tubular necrosis (ATN) cannot be established clinically[76,83–85] in 10% to 25% of patients who present with acute kidney injury. A biopsy in this population can be important because other causes of acute kidney injury are revealed, such as crescentic proliferative glomerulonephritis, interstitial nephritis, Wegener granulomatosis, polyarteritis nodosa, multiple myeloma, amyloidosis, endocapillary proliferative glomerulonephritis, cortical necrosis, hemolytic-uremic syndrome (HUS), systemic lupus erythematosus (SLE), and thrombotic thrombocytopenic purpura, to list just a few. These diseases usually require an approach to management that is different than that normally employed in uncomplicated cases of ATN.

Occasionally, a biopsy can provide helpful clinical information in patients who appear to have ATN on clinical grounds at initial presentation, but who do not regain renal function after 2 to 3 weeks of supportive therapy, including dialysis. The diagnostic possibilities generally are the same as those listed in the preceding paragraph. A careful evaluation of the clinical situation is deemed prudent before a renal biopsy is initiated because this procedure carries a higher risk in the patient with acute uremia.[86]

Nephrotic Syndrome

A renal biopsy in the clinical setting of an acute nephrotic syndrome not associated with systemic disease is influenced greatly by the age of the patient. It is common practice to treat children initially with high-dose corticosteroids, because most younger children have minimal change nephrotic syndrome (MCNS) on a biopsy. The presence of a selective proteinuria and normal renal function and the absence of hypertension strengthen the clinical diagnosis. In children, a biopsy is usually reserved for patients with no response to corticosteroid therapy or in whom the clinical and laboratory features of the illness at the time of initial presentation are distinctly atypical for MCNS. These features would include hypertension, azotemia in the absence of volume depletion, nonselective proteinuria, a highly active urine sediment including red cell casts, and involvement of other organ systems.

Most nephrologists believe that the adult nephrotic patient without signs of systemic disease should undergo a biopsy before therapy is initiated because the majority of these patients, including elderly persons,[87] have a renal disease other than MCNS.[10] The most frequent cause of the nephrotic syndrome in adults is idiopathic membranous glomerulonephritis[10,88]; other frequent causes include focal segmental glomerular sclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), proliferative glomerulonephritis, immunoglobulin A (IgA) nephropathy, and amyloidosis. Because the optimal treatment differs in different conditions, a renal biopsy can provide helpful clinical information. Moreover, fewer than one-third of adult patients have MCNS. Thus, if the physician elects to administer a short course of high-dose corticosteroid therapy equivalent to that employed in pediatric patients, approximately two-thirds of the patients would not be expected to respond favorably. Despite suggestions to the contrary, [25] we believe the risks associated with the use of corticosteroids or other immunosuppressive agents, such as azathioprine, chlorambucil, cyclosporine A, mycophenolate mofetil, and cyclophosphamide, in this population are too great to justify their use in the absence of a specific histologic diagnosis.

Isolated Proteinuria

Isolated nonnephrotic proteinuria of 1 g or less per 24 hours without hematuria or pyuria in an otherwise asymptomatic patient who does not have diabetes mellitus is a relatively common clinical problem. Often, the proteinuria is first detected during a routine physical examination required for participation in school athletics, during a preemployment examination, or at the time of application for life insurance. In young adults, orthostatic proteinuria is commonly identified in this presentation, carries a benign prognosis, and does not require a renal biopsy.[89] Otherwise, unless the patient requests a kidney biopsy for purposes of reassurance, it is currently our policy to merely monitor the clinical course of such patients at periodic intervals of 6 months to 1 year. There is little evidence to suggest that these patients will progress to renal failure or that they are candidates for any type of specific medical therapy in the absence of impaired renal function.[90] If there is any evidence during follow-up of functional deterioration or the development of additional clinical signs or symptoms suggesting the presence of a primary renal disease or kidney involvement secondary to systemic disease, the patient is thoroughly reevaluated and is often advised to undergo a kidney biopsy for diagnosis and possible therapeutic intervention.

In asymptomatic patients who do not have diabetes mellitus and who remain nonnephrotic but persistently excrete more than 1 g of protein per 24 hours, we advise a renal biopsy. It is this group of patients who are more likely to have an underlying renal abnormality. Some of the more common diagnostic possibilities include early idiopathic membranous glomerulonephritis, FSGS, and IgA nephropathy. Patients with urinary abnormalities such as hyaline and granular casts are even more likely to have an underlying glomerular abnormality.[91]

Hematuria with or without Proteinuria

Asymptomatic hematuria, especially in children and young adults, is a frequent cause of referral to nephrologists. It is important that causes of hematuria due to neoplasms in either

the upper or lower collecting system or due to either cystitis or pyelonephritis be excluded before one considers a renal biopsy. In general, the diagnostic value of a renal biopsy in the setting of idiopathic microscopic hematuria relates directly to the extent of associated clinical and laboratory findings. For example, in a series of 76 pediatric patients with isolated hematuria, Trachtman et al.[92] found that almost three-quarters of all biopsy specimens obtained in patients who had either a first-degree relative with hematuria or a history of at least one episode of gross hematuria were abnormal histologically. IgA nephropathy and Alport syndrome were the two most common findings. Schröder et al.[93] performed renal biopsies in 65 children with isolated hematuria of at least a 1-year duration. Of the group, 95% had histologic abnormalities that included IgA nephropathy (16 patients), Alport syndrome (8 patients), thin glomerular basement membrane (33 patients), and nonspecific mesangial abnormalities (5 patients). In a later report, Topham et al.[94] evaluated 165 children and adults with isolated hematuria using cystourethroscopy and renal biopsy. All had a normal intravenous pyelogram, were normotensive with a normal serum creatinine, and were free of both proteinuria and a urinary tract infection. In this group, 47% had significant histologic findings, including IgA nephropathy in 49 patients, whereas only 5 abnormalities were identified on a cystourethroscopy. Renal biopsy abnormalities were most common among patients under 20 years of age (69%), prompting these investigators to conclude that a renal biopsy should replace a cystoscopy in younger patients as the next step in evaluation if renal imaging yielded normal results. Furthermore, because renal histologic abnormalities are quite frequent in the clinical setting of isolated hematuria, these investigators recommended a kidney biopsy in patients over 45 years of age in whom findings at renal imaging and cystoscopy are normal.

The likelihood of identifying significant glomerular pathology is considerably higher when hematuria is accompanied by proteinuria, with or without an abnormal urine sediment that includes red blood cell, granular, hyaline, or white blood cell casts. We believe it is important to establish the histologic diagnosis of the renal lesion in this clinical setting; although admittedly, a biopsy is not required to identify the source of hematuria. Primary renal diseases that can be seen include IgA nephropathy, acute or resolving postinfectious glomerulonephritis, MPGN, and an occasional example of interstitial nephritis. Heredofamilial and multisystem diseases that may be seen include Fabry disease, sickle cell trait and disease, polyarteritis nodosa, Wegener granulomatosis, diabetes mellitus, SLE, and Henoch-Schönlein disease. Many of these systemic diseases may be evident on clinical grounds if a careful prebiopsy evaluation is undertaken, as discussed in the next section.

Systemic Disease

There are many systemic diseases that involve the kidney, although the extent and frequency of involvement varies considerably in different conditions. Patients often undergo a renal biopsy for diagnosis and management on the basis of either the frequency or severity of the renal lesion. These diseases include SLE, Henoch-Schönlein purpura, polyarteritis nodosa, Goodpasture syndrome, Wegener granulomatosis, and various gammopathies.

In approximately 40% to 50% of all patients with type I insulin-requiring diabetes mellitus and comparable percentages with type II adult-onset diabetes mellitus, renal failure develops during the course of the disease.[95,96] The natural history of renal disease in both types of diabetes mellitus has been well studied and is reasonably predictable[96]; thus, in most patients, a renal biopsy is seldom indicated for a diagnosis or management. However, a biopsy can be helpful in patients whose course may be complicated by the sudden development of renal failure, proteinuria, or nephrotic syndrome, or who have serologic evidence of other causes of renal disease.

Although nephrotic syndrome is observed in approximately 10% of all patients with diabetes, its sudden appearance, especially in the young diabetic without previous evidence of functional renal impairment, should not be ascribed automatically to diabetic nephropathy. This point is well illustrated by the experience of Urizar et al.[97] who described five young diabetic patients with nephrotic syndrome in whom the renal disease was not distinguishable histologically from MCNS. Nephrotic syndrome appeared either simultaneously or shortly after the recognized onset of diabetes in three of the children. Treatment with corticosteroids in four patients resulted in a prompt response, with loss of edema, cessation of proteinuria, and normalization of all serum abnormalities. No patient had abnormalities suggestive of diabetic nephropathy. Other investigators have reported similar experiences.[98,99]

Other types of renal disease also can be seen in association with diabetes mellitus, often in the clinical setting of the nephrotic syndrome. Couser et al.[100] reported the coexistence of dense deposits within the glomerular and tubular basement membranes, resembling those seen in type 2 MPGN and lesions typical of diabetic nephropathy in a 24-year-old nephrotic man with type I diabetes mellitus. Other examples of well recognized renal diseases that have been reported to occur in patients with diabetes mellitus in either the presence or absence of diabetic nephropathy include acute postinfectious proliferative glomerulonephritis,[101,102] crescentic proliferative glomerulonephritis,[101] and membranous glomerulonephritis.[103–105]

The renal biopsy is central to the management of SLE with renal involvement (i.e., lupus nephritis). At present, it is our practice to biopsy all patients who present with clinical evidence of active lupus nephritis unless a medical contraindication exists. Border[106] has suggested that patients with more than six red blood cells (RBCs)/high-power field, a urine protein excretion greater than 200 mg per 24 hours, or an abnormal serum creatinine value are candidates for a biopsy. There is no other way to establish the type of renal lesion that is present, and the management of lupus nephritis varies considerably depending on the specific histologic lesion.

The value of renal biopsy in predicting a prognosis has been debated. The results of earlier studies suggested that the biopsy classification of lupus nephritis was useful in predicting the clinical course[107,108] and this issue was challenged[109–111] from a prognostic standpoint but reaffirmed subsequently.[112–116] Correspondingly, we believe it is important to establish as precise a histologic diagnosis as possible because, in general, patients with diffuse proliferative lupus nephritis with signs of disease activity, such as increased cellularity, segmental necrosis, fibrinoid deposits, and crescents in the glomeruli, have a poorer prognosis than individuals with mesangiopathic, focal proliferative, or membranous lupus nephritis.

Controversy also exists concerning the value of renal biopsy in patients with clinically silent lupus nephritis. In 1977, Mahajan et al.[117] described 12 patients with diffuse lupus nephritis but without clinical or laboratory evidence of renal involvement at the time of renal biopsy. A later report, in which 10 of the original 12 patients were followed from 5 to 11 years, revealed deterioration of renal function in 3 years, with one death as the result of renal failure.[118] All patients received prednisone alone or in combination with azathioprine. These investigators concluded that the prognosis for the preservation of renal function appeared better in patients with clinically silent diffuse proliferative nephropathy as opposed to those with clinically active disease, and recommended a biopsy in patients with SLE even in the absence of overt clinical renal involvement.[118] Woolf et al.[119] described eight patients ranging in age from 6 to 26 years, with clinically silent lupus nephritis, who on biopsy had a variety of histologic lesions indicative of active renal involvement. Although no consensus exists regarding the use of a renal biopsy in patients with SLE who are without clinical evidence of renal involvement, it is currently our policy to withhold a biopsy in this group of patients.

Renal biopsy can often aid the clinician in selecting an appropriate therapy for the treatment of vasculitis when renal involvement is present. Polyarteritis nodosa and Wegener granulomatosis require aggressive combination therapy with cyclophosphamide and prednisone. The prognostic value of crescents in antiglomerular basement membrane (GBM) disease and other conditions is discussed in later paragraphs. Other systemic diseases that often exhibit renal involvement and, therefore, can be diagnosed with the aid of a renal biopsy when other diagnostic tests have failed or have not been employed include multiple myelomas, kappa light-chain disease,[120] amyloidosis,[121] fibrillary glomerulonephritis, and mixed cryoglobulinemia with renal failure.[85,122]

Transplant Kidney

Renal biopsies are a valuable diagnostic tool in the management of the transplant recipient. A biopsy of an allograft represents the major clinical exception to avoidance of a percutaneous biopsy of a single functioning kidney. Numerous studies confirm the value and relative safety of a renal biopsy in this setting.[41,42–48,123,124] A biopsy is the most accurate means of determining the presence of lesions, such as cellular or humoral rejection, ATN, drug-induced or viral (especially BK virus) interstitial nephritis, hemorrhagic infarction, calcineurin inhibitor toxicity, and de novo or recurrent glomerulonephritis in the allograft. There are several clinical settings in which a biopsy of the allograft is often indicated. These include failure of the graft to function within the initial 7 to 10 days after surgery, a rapid deterioration in function of unknown etiology after the initial good function, an absence of a response to an adequate antirejection therapy within a reasonable period of time, and an unexplained nephrotic syndrome or nephrotic-range proteinuria.

A large number of cadaveric kidneys are engrafted, and ischemia-reperfusion injury is a frequent complication. Failure to achieve improved renal function within 7 to 10 days after surgery raises the possibility of a more severe form of renal injury, such as an infarction or a superimposed episode of acute rejection. A biopsy is often invaluable in determining the etiology of the renal failure, in guiding subsequent therapy, and in establishing a prognosis. For example, Kiaer et al.[125] reported a 100% graft loss when infarction, capillary thrombosis, and arterial or arteriolar thrombosis were found either singly or in combination on a biopsy. Thus, the presence of these lesions in the clinical setting of an acute kidney injury would obviate the necessity for the continued use of antirejection therapy.

The incidence of acute rejection, characterized by a sudden decrease in renal function, is greatest during the first 6 months after transplantation. In most instances, the suspicion of acute rejection can be made on clinical grounds. However, acute rejection often occurs in the absence of clinical features, such as graft tenderness or fever, and a patient believed to have acute rejection may not respond to a reasonable course of antirejection therapy. It may be desired to tailor the antirejection therapy to vascular versus tubulointerstitial rejection or antibody versus cellular rejection. A biopsy can be extremely helpful at this juncture in the patient's therapy. In particular, the presence of peritubular C4d deposition suggests the presence of acute humoral rejection, whereas its absence is typical in cell-mediated rejection.[126,127] A confirmation of acute humoral rejection involves the demonstration of morphologic evidence of acute tissue injury in combination with circulating antibodies to either donor human leukocyte antigen (HLA) or to other antidonor endothelial antigens.[128] Other complications, such as ATN, drug-induced nephrotoxicity, or overt renal infarction may be diagnosed.

As noted previously, C4d staining of biopsies has been a valuable tool. C4d is produced by the activation of the classic and lectin complement pathways. Thus, ischemia reperfusion (I/R), necrosis, lupus nephritis, and other conditions may exhibit C4d staining and must be considered in a biopsy interpretation.[129–133] Current guidelines recommend the exclusion of loci of I/R, necrosis, and fibrosis when using C4d staining to evaluate for possible humoral rejection.[128,134,135]

The occurrence of the nephrotic syndrome or nephrotic-range proteinuria in a transplant recipient suggests the possibility of either recurrent or de novo glomerulonephritis.[136,137] Those forms of disease that are most likely to recur in the transplant kidney include MPGN, FSGS, diabetic nephropathy, and IgA nephropathy.[136,137] To date, the most common de novo disease reported is membranous glomerulonephritis.[136] Although some would take exception, we believe it is worthwhile to establish the lesion that is responsible for proteinuria, especially if the proteinuria is associated with a decrease in renal function.

Renal Mass or Neoplasm

In addition to a percutaneous kidney biopsy for traditional medical indications, as introduced previously, the past decade has seen renewed interest in percutaneous (core) biopsies for renal masses and other neoplasms. The technique fell into disfavor in previous decades because of bleeding, false-negative results, and other less common complications.[138] Regardless, there is a driving force for tissue diagnosis because 50% of renal neoplasms are now identified as incidental to abnormal imaging for other reasons. A percutaneous renal mass biopsy is often performed to evaluate for a possible lymphoma, a renal abscess, or metastatic disease due to a known extrarenal malignancy. It may also be performed to confirm the diagnosis of a primary renal neoplasm in a patient with known disseminated disease or an unresectable retroperitoneal tumor in whom surgical treatment is contraindicated.[138–144] Although there was initial concern regarding the potential for seeding the biopsy tract with malignant cells, only a total of six cases have been reported, and multiple case series published since 1999 have reported no such events.[145]

CONTRAINDICATIONS

Both the relative and the absolute contraindications for a renal biopsy vary among nephrologists. However, most agree that the risk of complications increases in the presence of severe uncontrolled hypertension, sepsis, known or suspected renal parenchymal infection, a hemorrhagic diathesis, a solitary ectopic or horseshoe kidney (except in the case of a transplanted kidney), or when the patient is unable to cooperate during the procedure.

In 1958, Kark et al.[146] published the results from their initial 500 percutaneous renal biopsies and listed 11 contraindications. These included an uncooperative patient, large cysts, a renal neoplasm, a renal artery aneurysm, marked calcific arteriosclerosis, a hemorrhagic diathesis, a single kidney, a perinephric abscess, hydronephrosis or pyonephrosis, a terminal state of illness, and a rising blood nonprotein nitrogen level greater than 100 mg per deciliter. Hypertension was viewed as a relative contraindication, depending on the importance of the biopsy and the skill of the operator.

Certainly, the presence of a single kidney (except a renal allograft), including a horseshoe kidney, sepsis, or a hemorrhagic diathesis, remain important contraindications to percutaneous renal biopsy.[147] However, in some patients, an open or laparoscopic biopsy may well be justified if the clinical situation warrants the risk. This also holds true for the patient with a renal artery aneurysm or calcific arteriosclerosis and an undiagnosed parenchymal renal disease. Many times, a coagulation disorder can be corrected, thus allowing the biopsy to be performed. In most clinical situations, there is little or no reason to perform a biopsy if the patient has large multiple cysts or a terminal illness. The same is true in the presence of a perinephric abscess, acute pyelonephritis, hydronephrosis, or pyonephrosis. Today, a rising BUN or a BUN greater than 100 mg per deciliter is not considered a contraindication if the rise is sudden or unexplained and quite likely due to an acute and potentially reversible process, as discussed earlier in this chapter. The presence of normal-sized or large kidneys increases the likelihood that an acute rather than a chronic form of renal failure is present.

When a patient is hypertensive, we delay the biopsy until the blood pressure is brought under adequate control. Thus, the presence of hypertension should be considered, at most, a relative contraindication. It is important that blood pressure control is obtained because it is well documented that hypertensive patients are more prone to bleeding after a percutaneous renal biopsy.[17,28] Diaz-Buxo and Donadio[28] not only found a significantly greater incidence of complications in hypertensive patients (11.6%) as compared with normotensive subjects (7.1%) undergoing percutaneous renal biopsy, but the higher incidence also correlated positively with both the severity and the duration of the hypertension.

GROSS INSPECTION AND TISSUE PROCESSING

An evaluation of a kidney biopsy includes both a gross and a histologic examination of the specimen. A standard histologic examination includes light microscopy, immunohistology, and transmission electron microscopy. Other less frequently used techniques include scanning electron microscopy, microbiologic cultures, tissue and cell cultures, quantitative or qualitative chemical analyses, enzyme assays, and molecular pathology. Most of the remainder of this chapter is concerned with the preparation, histologic examination, and actual evaluation of the biopsy specimen for clinicopathologic interpretation.

Gross Examination

The general purpose of a gross examination is to determine adequacy and to divide the specimen into the appropriate portions for subsequent processing. The overall dimensions, color, and consistency should be noted. In particular, the area of viable cortex should be identified and delineated from the medulla, which is generally paler. Areas of infarction, other necrosis, or pyogenic inflammation that are often pale and highlighted by a hyperemic border may be evident by a gross examination. Ischemia with reflow also may be

hyperemic throughout. In general, if the specimen contains both the cortex and the medulla, the medulla is the deeper tissue as it is removed from the needle, although there are several exceptions. If the needle is thrust deeply into the kidney before the core is taken, the cortex may be missed altogether, or if the direction of the needle is obtuse to the pelvis, the needle may pick up the medulla first and then the cortex as it passes completely through the medulla. We have observed specimens that contain the cortex, the medulla, and then more of the cortex. Chronic disease will make it more difficult to delineate the cortex.

Allografts have additional notable characteristics. First, in older grafts, a thick rind of fibrous tissue surrounds the graft. This area may be quite pale and should not be confused with the cortex. In newer grafts, the surface may be deeply colored from a hemorrhage or the presence of granulation tissue. Second, the outermost rim of the cortex may be pale from ischemic atrophy or necrosis. The deeper cortex is of interest for the diagnosis of an additional disease. Many laboratories employ a dissecting microscope or hand lens to identify glomeruli in the cortex and to guide division of the specimen at the time of the biopsy.

Processing for Histologic Examination

Despite considerable effort, it has not been possible to develop a single method of tissue fixation and processing that is optimal for light, immunofluorescence, and electron microscopy. Therefore, it is customary to divide the cortical portion of the biopsy into three parts (Fig. 13.1). We prefer to divide the tissue core along its short axis, as illustrated, to minimize tissue damage. For longer cores, the largest part is taken for light microscopy, a smaller portion is taken for immunofluorescence microscopy, and the smallest portion is taken for electron microscopy. The exact proportions of this division are variable and depend on the total amount of cortex and the clinical setting (e.g., native versus allograft biopsy). The basic consideration in the division of the specimen depends on the definition of an adequate sample for each type of histologic examination. Six glomeruli generally are considered an adequate number in a native kidney for light microscopic evaluation. However, in exceptional circumstances, the limits are broad. For example, in the evaluation of a patient with nephrotic syndrome, a disease that is diffuse and generalized such as uncomplicated idiopathic membranous glomerulonephritis may be diagnosed with a single glomerulus. On the other hand, in the early stages of a focal proliferative disease or with the variable pattern of involvement often observed in lupus nephritis, the diagnosis may not be appreciated with six or more glomeruli. We prefer a core of tissue sufficient in length to provide 12 glomeruli for light microscopic examination. The extent or degree of severity of chronic atrophy also is determined best on larger specimens and is especially important for the assessment of permanent nephron loss.

The factors that determine the adequacy of a sample for immunohistology are somewhat different. The principal role of immunohistochemistry is to evaluate the type, location, and distribution of serum proteins, particularly those commonly identified in immune complexes or directed against a specific antigen, such as seen in anti-GBM disease. The biology of these immune diseases is such that the distribution of immune mediators is more diffuse and generalized, even though the light microscopic pattern may be focal and segmental; therefore, sections containing four to six glomeruli usually are adequate.

Electron microscopy is most useful in diffuse and generalized diseases. It is preferable to examine two or three glomeruli as well as tubules and small vessels whenever possible. After the viable cortex has been divided, any remaining tissue such as the medulla is also processed for light microscopy.

When only a few millimeters of cortex are obtained, we process the entire specimen for electron microscopy. During the examination for electron microscopy, the tissue also is evaluated by light microscopy so that the maximum amount of information can be obtained. In addition, electron microscopic processing is technically most suited for handling small pieces of tissue, and the overall preservation of the tissue is much better. If the core of the cortex is 4 to 8 mm in length, we process equal parts for the electron microscopy and the immunofluorescence microscopy (Fig. 13.1).

Two tissue cores are recommended for allograft biopsies because of the variable nature of rejection.[148] We also modify the division of tissue from transplant biopsies. This is done to expedite the diagnosis when rejection is suspected. Approximately half the cortex is submitted for frozen sections. Most of the remainder is processed for routine light microscopy. Small (1-mm) portions are saved for electron microscopy. Frozen sections are taken immediately for light microscopy stains, hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS), and immunohistology microscopy. These procedures can be completed within 1 to 2 hours. Rejection can be determined from the frozen sections in many patients. The final evaluation has to wait

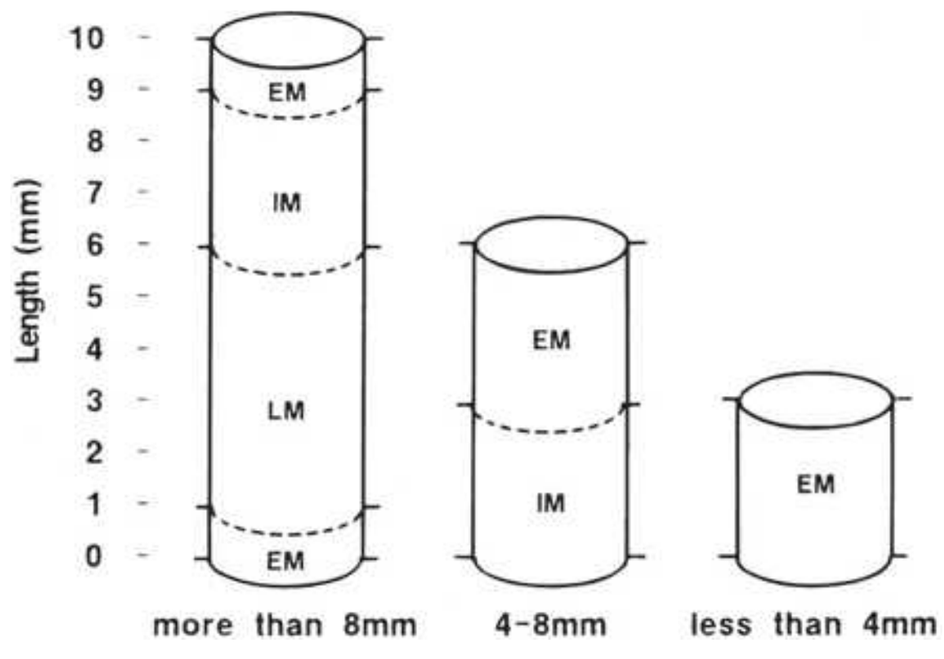


FIGURE 13.1 The methods of dividing cores of native cortical renal tissue obtained by a percutaneous biopsy based on the sample size. *EM*, electron microscopy; *IM*, immunofluorescence microscopy; *LM*, light microscopy.

for routine processing in subtle or complex situations, but rapid processing produces permanent sections in 4 hours.

There is a diversity of opinion regarding the optimal tissue fixation for light microscopy. In part, this is because several fixatives are available that yield generally acceptable results. The fixative for use in general pathology has a formaldehyde base; a 4% solution of formaldehyde in neutral phosphate buffer is acceptable in most situations. Other common fixatives include Zenker, Van de Grift, Helly, and Bouin solutions.[149] Variations in fixation and other processing steps make less and less difference with time and experience.

There are circumstances when a standard fixation and tissue processing should be supplemented with special handling of the tissue. Urate, uric acid, other water-soluble crystals, and glycogen may be dissolved from the tissue during processing in aqueous solutions. Ethanol is the fixative of choice for preservation when the presence of urates and uric acid are suspected. Lipids are extracted from the tissue during the later stages of processing for routine paraffin sections; therefore, frozen sections are preferable for the demonstration of lipids. Some fixatives degrade antibody binding and nucleic acid hybridization to tissue or tissue extracts; therefore, processing must be appropriate for these tests. Because special handling is required in only a few cases, it is important to have a high index of suspicion when these situations arise and to alert the pathologist and the laboratory before the biopsy is actually performed.

After fixation, the tissue is dehydrated and embedded using one of several techniques. We prefer wax embedding because it is automated and permits the use of the greatest variety of special stains. We normally prepare seven slides with 2-μm thick sections. The first, fourth, and seventh slides are stained with H&E, the second and fifth are stained with PAS, and the third and sixth are stained with periodic acid-methenamine silver (PAMS).[149] Additional stains, such as Congo red, are used as necessary. Some laboratories use plastic materials for embedding, which produce very clean, thin, crisp sections that also can be stained with the H&E, PAS, and PAMS procedures. Specimens processed in this manner require separate handling and, in our experience, additional special stains often demonstrate poor contrast.

Many types of fixatives are available for electron microscopy, and several are acceptable for the evaluation of a kidney biopsy within certain limits. We recommend either of two initial fixatives for electron microscopy. The first is buffered formaldehyde. In its early use, formalin solution was maligned as a fixative for electron microscopy because of poor tissue preservation. This was not because formaldehyde is actually a poor fixative, but because formaldehyde produces a highly acidic solution in water and the increased acidity produces many artifacts. Buffered formaldehyde (pH 7) is a good fixative, is inexpensive, and is readily available. It has a long shelf life, and tissue can remain in the fixative for months if necessary before additional processing.

Glutaraldehyde is commonly employed to preserve kidney biopsy specimens for an electron microscopy and is our choice as a primary fixative for ultrastructural preservation, although it may be less readily available in routine histology laboratories. Regardless of the fixative, it is important that the tissue to be processed for electron microscopy is divided into pieces less than 1 mm in any dimension to ensure good penetration of the fixative and all other solutions used in subsequent processing. This is not a problem with needle biopsies, but wedge biopsies must be divided accordingly. After the primary fixation, the tissue is ready for additional processing, which should be performed in a dedicated electron microscopy laboratory.

Tissue for immunohistology can be handled in several ways. For frozen sections, the tissue is placed between gauze sponges, moistened with saline, and taken directly to the laboratory. It is important that the tissue not be allowed to float in saline because tissue specimens left in aqueous solutions absorb water, which distorts the architecture. Ideally, the transit time to the laboratory should be less than 30 minutes. If transport is delayed, the tissue should be kept on ice, but should not be frozen. Alternatively, a second method can be employed. Michel et al.[150] developed a holding solution composed of buffered ammonium sulfate and N-ethylmaleimide, which is used at room temperature for the preservation of biopsy specimens for immunofluorescence microscopy. The original solution has been modified slightly and is even more broadly applicable than originally described.[151] Michel's medium remains valuable for holding the tissue at room temperature or for shipping kidney specimens without refrigeration, provided certain guidelines are followed. First, the tissue pieces should be 2 mm or less in thickness. Second, the tissue should not be kept in the solution for longer than 1 week before it is rinsed and frozen as described for fresh tissue. We block the tissue in gelatin, as described by Burkholder et al.,[152] or routine cryomicrotomy solution, after which it is snap frozen in isopentane or Freon cooled with liquid nitrogen or an electrical refrigeration unit, or in a slurry of dry ice and acetone. Rapid freezing is important to reduce the formation of large ice crystals because they result in tissue distortion and sectioning artifacts. Tissue that is stored frozen before and after sectioning should be protected to prevent desiccation and denaturation artifacts.

Frozen sections are cut and stained according to any of several immunohistologic procedures. Direct immunofluorescence staining for serum proteins with fluoresceinated heteroantisera or monoclonal antibodies remains the standard procedure. Antibodies to IgG, IgM, IgA, C1q, C3, and albumin are used most frequently. With the appropriate interpretation, this panel of antisera allows for the successful identification of most clinical diseases. Staining for selected amyloid proteins and κ and λ light chains is helpful or necessary in many adult cases. A variety of other antigens have been used in special or experimental situations; however, most are not necessary in everyday clinical practice. After staining, the slides are cover-slipped with buffered glycerol at pH 8.2 in preparation for viewing.

A fluorescence microscope equipped with epifluorescence is convenient to use and should be outfitted with adequate illumination, a primary interference filter, and an appropriate secondary filter.

A number of methods employing other fixatives or embedding procedures for the immunohistochemical demonstration of serum proteins in kidney biopsies have been described, including the use of wax sections. Some of these alternative procedures are unreliable, but others[153] are suitable for the demonstration of antibodies and some complement proteins in paraffin sections.

Several other tests are occasionally required that can be performed only on frozen sections. These include neutral fat stains and most enzyme histochemistry. The use of immunohistochemistry, combined with a host of specific monoclonal antibodies, has produced a highly specific and sensitive system for the identification of cell and tissue antigens. The impact of this methodology has been most noticeable in our ability to identify lymphohistiocytic cell infiltrates and to classify cellular immune responses in the kidney such as cellular rejection, interstitial nephritis, and posttransplant lymphoproliferative disease (PTLD). We have found that the avidin biotin complex (ABC) procedure yields the best combination of sensitivity, specificity, quality control, and time for the completion of the test.[154] There are excellent monoclonal antibodies commercially available for the identification of B cells, T-cell subsets, and monocytes. Many of these may be used in tissues following the fixation in buffered formalin.

Immunoperoxidase staining of frozen sections can also be used in addition to or instead of immunofluorescence staining. The advantage of immunoperoxidase techniques over immunofluorescence microscopy includes a greater sensitivity and permanence of the staining when diaminobenzidine is used as the substrate for color development. The disadvantages of immunoenzyme staining for immunoglobulin localization include the increase in preparation time and the added expense. Endogenous peroxidase and endogenous biotin may produce a bothersome high background and should be blocked.[155]

Molecular Biology

In situ hybridization has been a powerful tool for a host of investigational studies.[156,157] A variety of molecular probes have been used to study gene expression at the level of messenger RNA (mRNA)[158–160] and for the detection of viral sequences.[161,162] For example, Epstein-Barr virus (EBV) probes may be useful in the diagnosis of PTLD. Renal biopsy specimens also can be used as a source of DNA or RNA for extraction and nucleic acid blotting or for polymerase chain reaction (PCR)-based techniques.[161,164–167] Although the potential for molecular diagnosis of renal disease by microarray analysis and other techniques is near at hand, there are few standard applications for clinical renal biopsy diagnosis at the present time.[168] Proteomic studies (e.g., mass spectroscopy) have been used in the subclassification of amyloids in tissue blocks[169,170] and have additional potential.

THE HISTOLOGIC EVALUATION

Light Microscopy

The purpose of this section is to present a systematic approach to the histologic interpretation of a kidney biopsy. The discussion also includes the role of several special stains in a biopsy diagnosis. The evaluation should begin with a review of all tissue that is present on the light microscopic sections at a relatively low magnification to assess the adequacy of the specimen and to identify any major abnormalities. This is followed by a systematic evaluation of the glomeruli, tubules, the interstitium, and the vasculature. It is preferable to establish the histopathologic findings before the clinical history is known to avoid a bias in the final biopsy interpretation. Nevertheless, the biopsy findings must ultimately be reconciled with the clinical presentation, course, and prognosis.

It is important that certain terms be carefully defined before continuing. There are four principal definitions that have evolved largely from light microscopic evaluation of kidney biopsies to describe glomerular disease. Focal denotes a process in which only some of the glomeruli are altered histologically. The majority of glomeruli are spared. Generalized indicates the majority of glomeruli on the biopsy are altered by some process; for instance, proliferation or sclerosis. A local, or segmental, lesion is one in which only a portion of a glomerulus exhibits an alteration. A segmental sclerotic process involves only a portion of a glomerulus. The opposite of a segmental process is one that is global in nature and generally affects the entire glomerulus. The term diffuse has been used ambiguously in the literature. Sometimes it has meant global and other times it has meant generalized; therefore, care must be taken when interpreting this term. We will avoid the term diffuse for these reasons. Glomerular lesions are generally focal and segmental or are generalized and global, but important exceptions exist (e.g., generalized and segmental in lupus nephritis).

The initial evaluation of the specimen is intended to determine the specific regions of the renal parenchyma that are present on the section, which might include the cortex and the outer and inner medulla. If only the cortex is seen, the presence of the renal capsule can aid in the orientation of the specimen. The glomeruli should be counted to provide a rough estimate of the sample size. This is generally accomplished best with the PAS-stained sections, in which the glomeruli (including those that are globally sclerotic) can be identified readily. All the tissue on each slide should be evaluated because certain features may not be present in all sections. Next, the overall condition of the renal architecture is evaluated to differentiate between

chronic or irreversible nephron loss and acute or reversible nephron damage. The type, distribution, and intensity of cellular infiltration are accurately established on the H&E and PAS sections. The PAS and PAMS stains are well suited to evaluate the degree of interstitial fibrosis, tubular atrophy, and glomerulosclerosis. In general, the degree of tubular and interstitial injury relates to the reduction in creatinine clearance when the sample size is adequate and the process producing the injury is uniform. The latter feature is important because, with approximately 1 million nephrons in each kidney, a needle biopsy specimen that contains 10 glomeruli only provides a sample of 1 in 100,000. As a first approximation, we employ a simple procedure to estimate the extent of chronic nephron loss in a biopsy. Using a PAS-stained section, the total number of glomeruli is counted and the percentage that is sclerotic is determined. Sclerotic glomeruli are generally shrunken in appearance because of the complete collapse of the capillary bed and the paucity of cells. In a study of chronic glomerulonephritis, we have shown that global glomerular sclerosis of up to 50% is associated with the maintenance of the serum creatinine near the normal range.[171] However, an increase in glomerular sclerosis of the global type above 50% to 60% is associated with increases in the serum creatinine. This estimate of chronic nephron loss is based on an interpretation of the intact nephron hypothesis.[172] When one portion of the nephron is lost to disease or injury, the remainder of the nephron undergoes atrophy. Thus, the accompanying tubule undergoes atrophy in the presence of global sclerosis. The converse also is true. Tubular atrophy is characterized by a decrease in the outer diameter of the tubule, thickening and wrinkling of the tubular basement membrane, simplification and a decrease in thickness of the tubular epithelium, and an increase in the interstitial connective tissue that surrounds the tubule. However, it is also true that one part of the nephron may take longer than another to atrophy. It is also important to estimate the percentage of atrophic tubules in the specimen because there may be significant tubular atrophy in the absence of glomerular sclerosis. These changes all lead to disruption of the normal architecture of the renal parenchyma. Because some nephrons are destroyed or atrophy, the remaining nephrons may hypertrophy, showing tubules with larger diameters and cells with increased cytoplasm. This picture of atrophy and hypertrophy is visualized in Figure 13.2. In this setting, hyperfiltration is associated with glomerular changes of increased size and mesangial matrix and segmental sclerosis.[173–177] These features are the harbingers of the point of no return or of continued deterioration in renal function.[178] The normal architecture also can be disrupted by more acute changes, such as edema and inflammation. In this setting, there is separation without evidence of atrophy of the renal tubules. Depending on the etiology of the injury, these latter changes may be reversible, as seen in ATN, or occasionally they may progress to the loss of the entire nephron.

Glomeruli

The next step in the interpretation of the biopsy is a detailed evaluation of the glomeruli, which includes a careful assessment of the various structures that comprise the glomerulus or renal corpuscle. The glomerulus is composed of the visceral epithelial cells, the endothelial cells, the mesangial cells with the mesangial matrix and the basement membranes of the capillary loops, the Bowman capsule and the

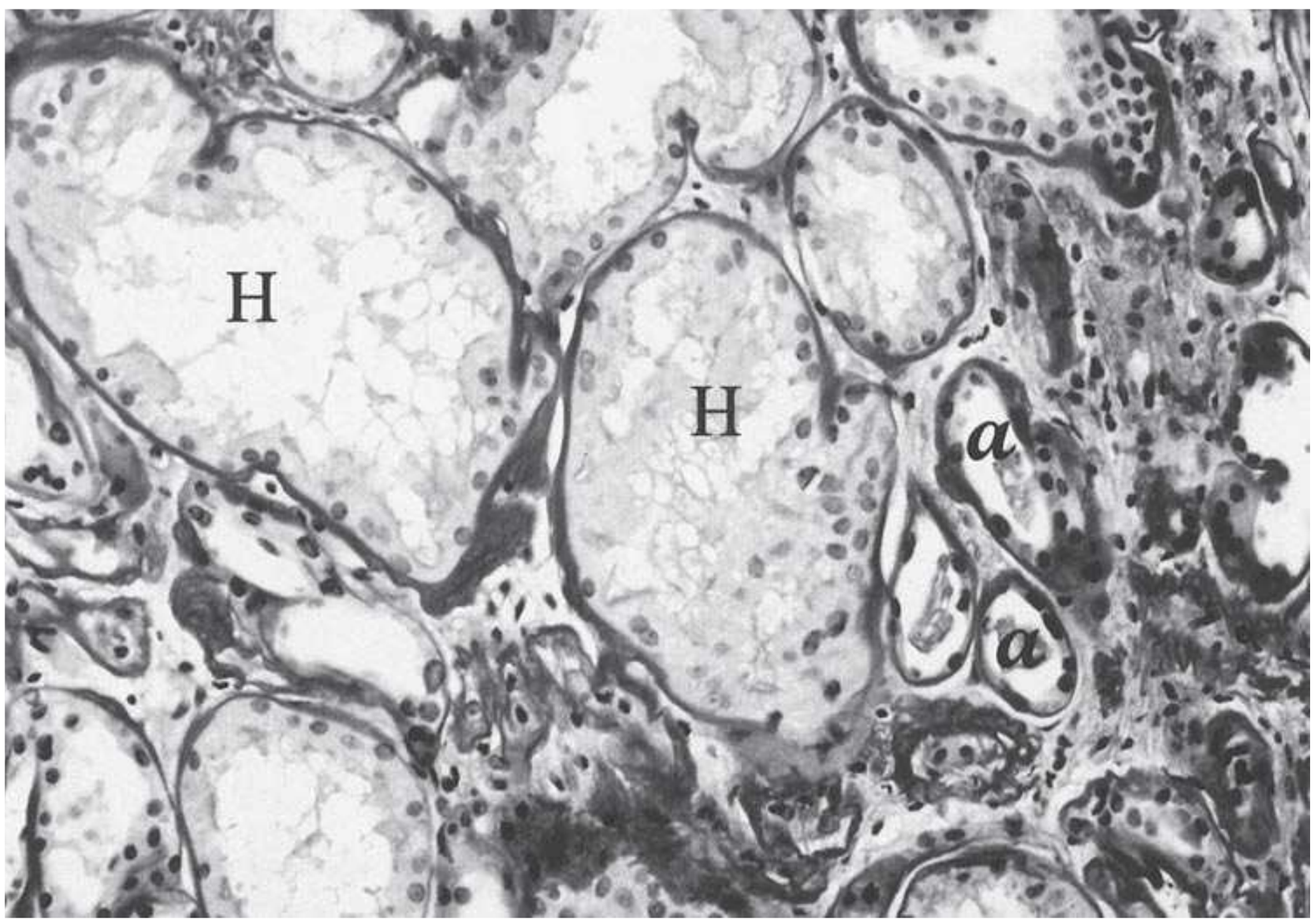


FIGURE 13.2 This figure shows shrunken atrophic tubules *(a)* and hypertrophic tubules *(H)* (PAS, magnification ×210.)

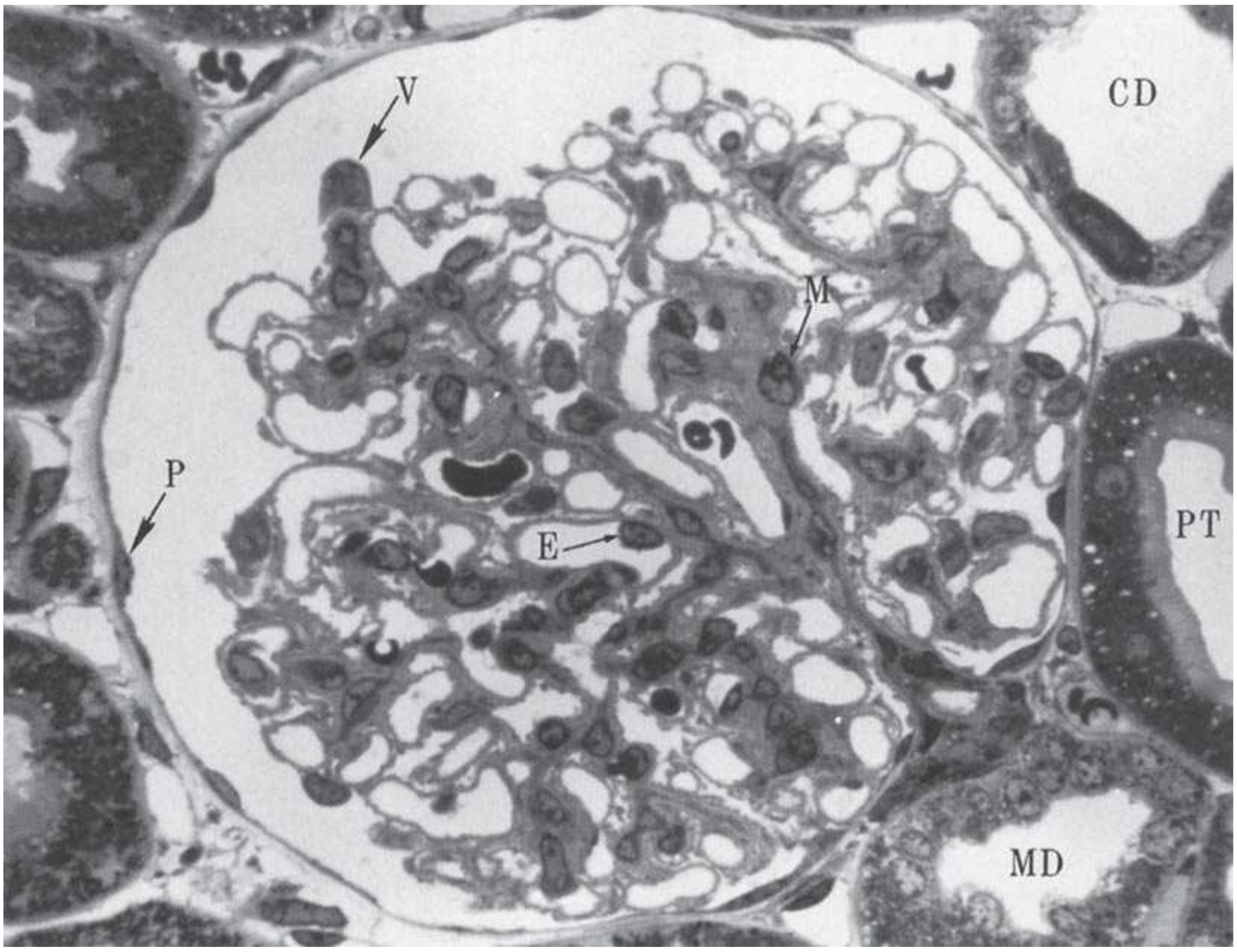
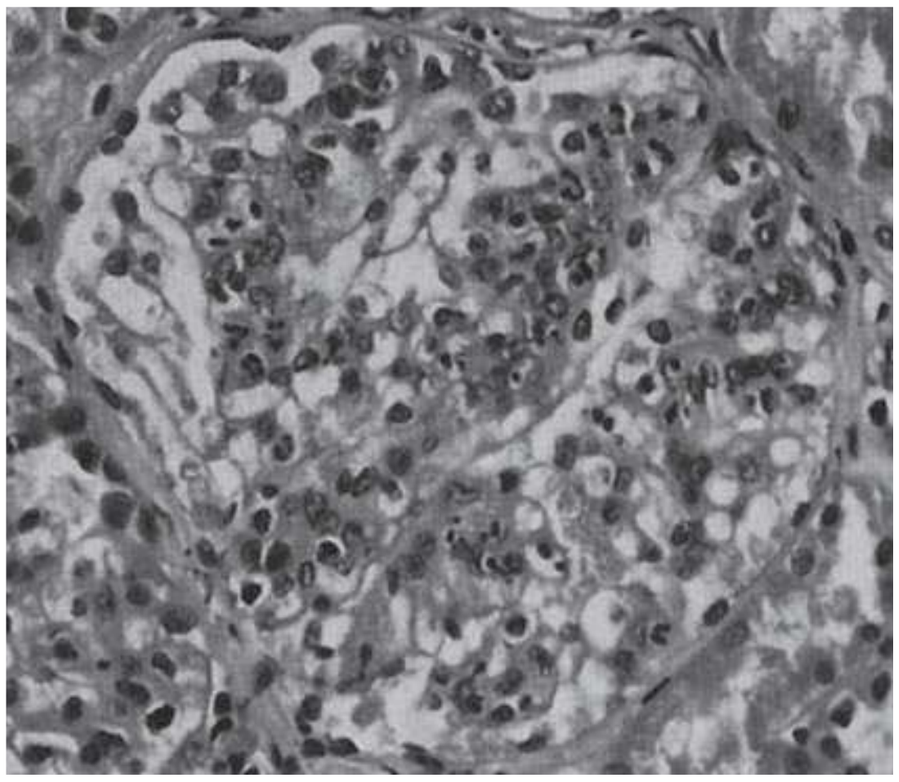


FIGURE 13.3 A photomicrograph of the glomerulus (renal corpuscle) depicting the major cell types and anatomic regions. *V,* visceral epithelial cell; *M,* mesangial cell; *P,* parietal epithelial cell; *E,* endothelial cell; *MD,* macula densa; *CD,* collecting duct; *PT,* proximal tubule (magnification ×750). (From Brenner BM, Rector FC Jr, eds. *The Kidney,* 3rd ed. Philadelphia: Saunders; 1986, with permission.)

overlying parietal epithelial cells, the extraglomerular mesangium, and the afferent and efferent arterioles (Fig. 13.3). The first assessment is a determination of the overall glomerular cellularity, which is evaluated using H&E-stained sections (Fig. 13.4A). The cell type should be established if the glomeruli are hypercellular. This includes an estimate of the number of cells normally present in the glomerulus, including the mesangial, endothelial, visceral epithelial, and parietal epithelial cells; and the inflammatory cells that migrate into the glomerulus, including neutrophils, lymphocytes, and monocytes (Fig. 13.5). Most pathologists principally rely on a qualitative assessment of the cellular composition in sections 2- to 3-μm thick and stained with H&E. There are several guidelines for this assessment. Under normal conditions, a typical cross-section through the mesangium contains one to three mesangial cell nuclei. An entire glomerulus may contain one to two neutrophils, but more than two is abnormal. There are also a small number (1%) of mononuclear cells (monocytes) in the normal glomerular mesangium,[179] but these cells cannot be identified with certainty on routine H&E sections. Similarly, it may not be possible to distinguish monocytes from large lymphocytes or even epithelial cells without immunohistochemistry or electron microscopy. Some cells within the glomerulus may contain inclusions, inspissated material, or numerous vacuoles, such as those that are present in foam cells.

Next, the glomerular capillaries are examined to determine whether they are patent, collapsed, or obstructed with fibrin, platelets, or cells. The PAS and PAMS stains are important in the evaluation of the capillary walls (Fig. 13.4B,C). Both stains will label normal as well as have an abnormally thickened basement membrane. If the basement membrane is thickened, it is important to establish the nature of the changes. Both the epithelial and endothelial surfaces are smooth under normal conditions. If one surface is shaggy or irregular or exhibits projections, often referred to as spikes when present on the epithelial surface, it is distinctly abnormal (Fig. 13.4C). Occasionally, both surfaces of the basement membrane are irregular in configuration. In necrotizing glomerulonephritis owing to virtually any cause, the capillary wall, including the basement membrane, may be ruptured, discontinuous, or completely lost (Fig. 13.6). This histologic picture usually is associated with fibrin deposition. Fibrin deposition associated with necrosis should be distinguished from fibrin deposits that distend the capillary in the presence of an intact basement membrane. Larger immune complexes can be seen on Masson or PAS stains. These deposits can also be identified on 1-μm thick plastic sections stained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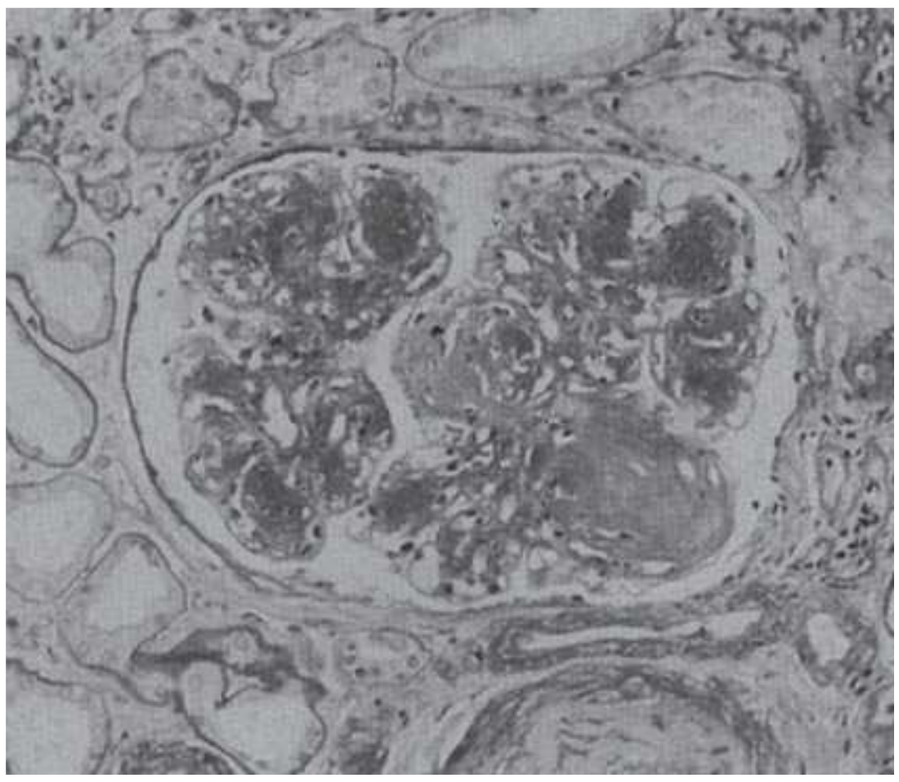
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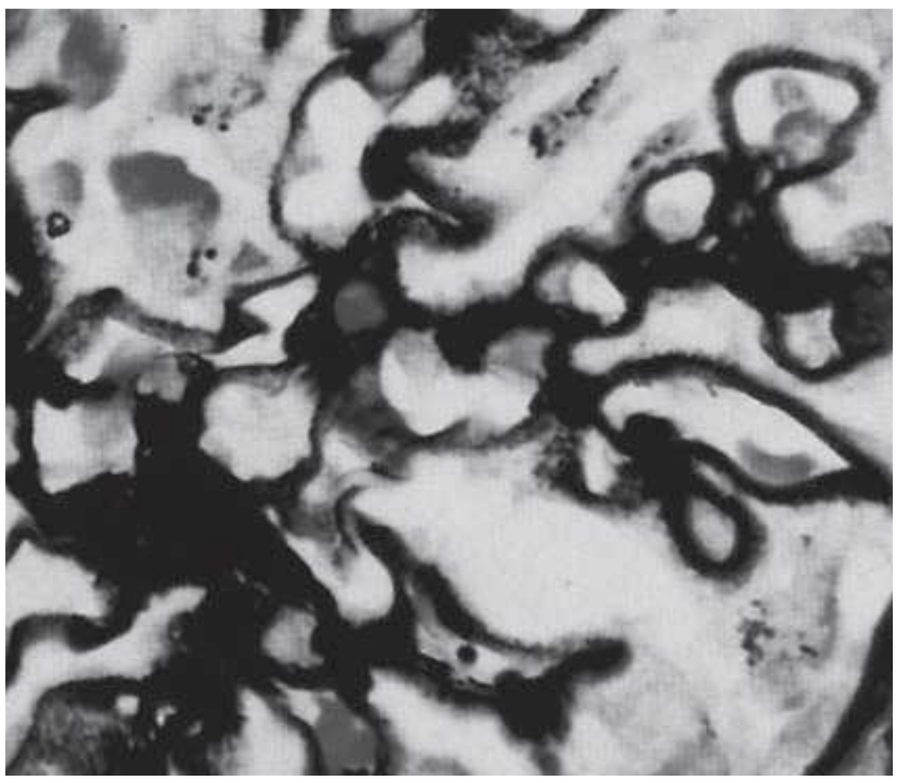
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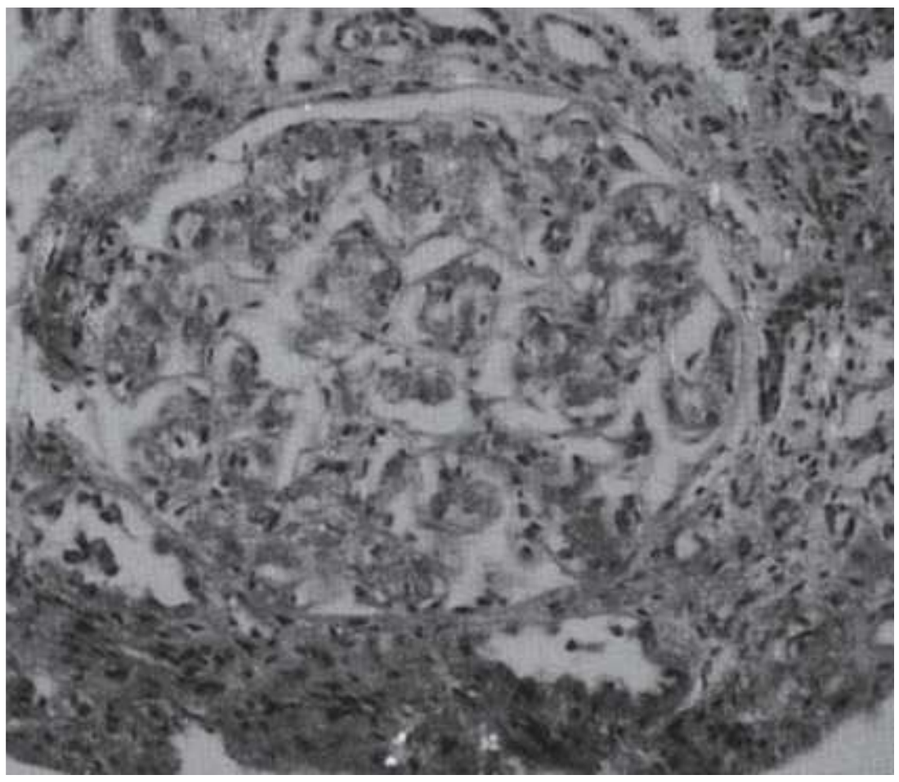
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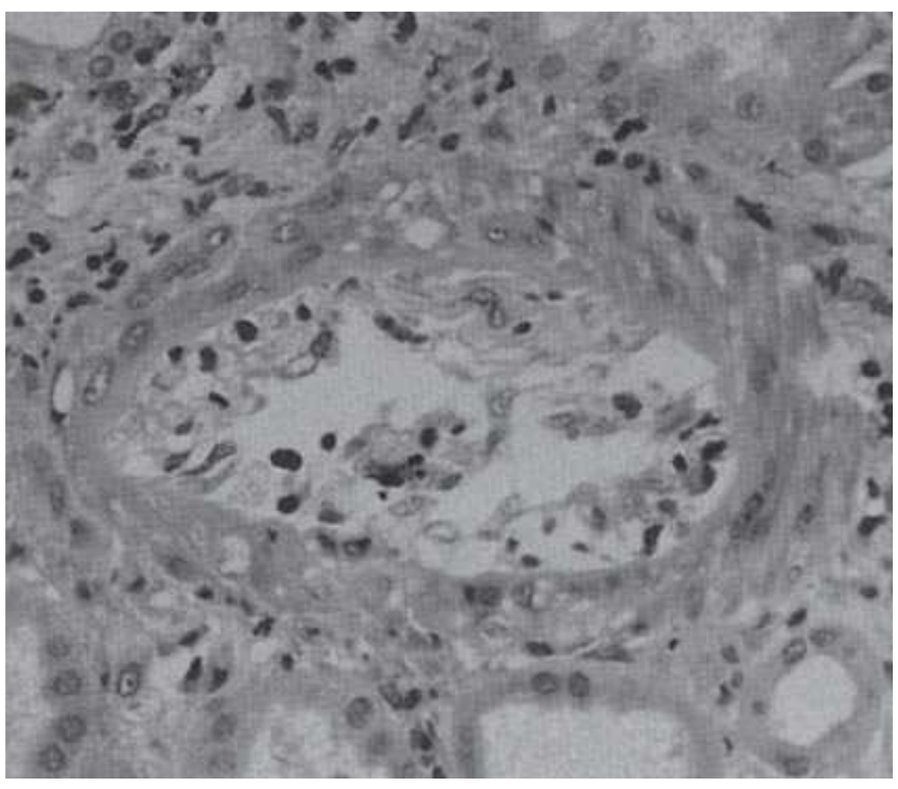
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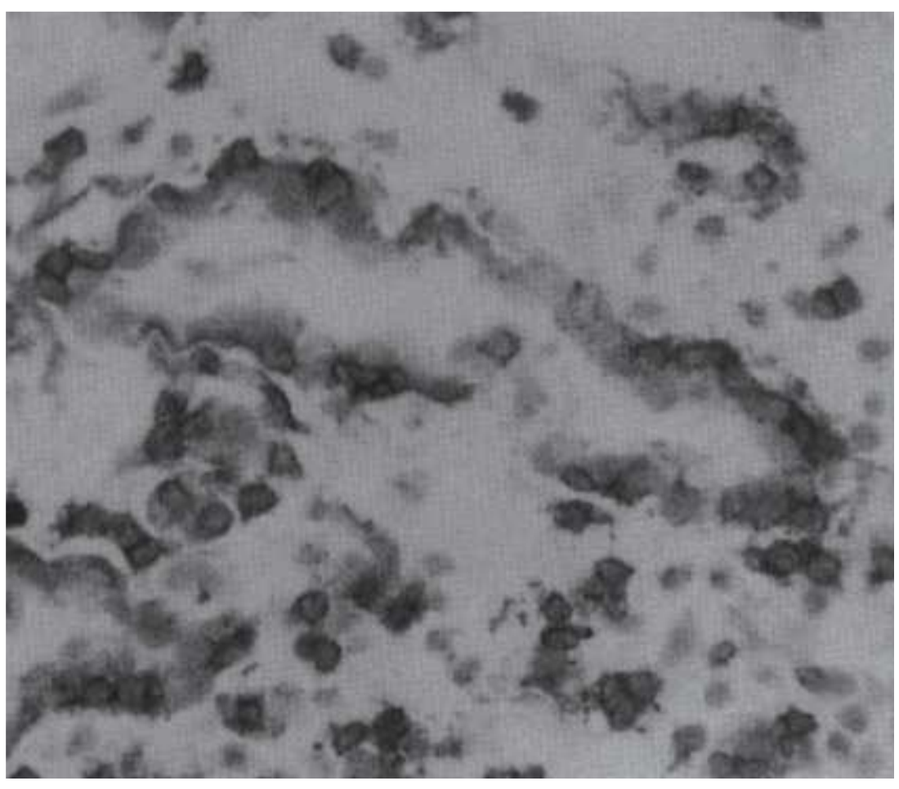
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FIGURE 13.4 **A:** An H&E stain of a glomerulus with moderate neutrophilic infiltrate in acute poststreptococcal glomerulonephritis (magnification ×200). **B:** A PAS stain of a glomerulus, illustrating an early and late nodular mesangial expansion typical of nodular intercapillary glomerulosclerosis of diabetic nephropathy (magnification ×200). **C:** A PAMS stain depicting a portion of a capillary tuft with spikelike projections extending outward from the capillary basement membrane. This picture is characteristic of stage II idiopathic membranous glomerulonephritis (magnification ×1,000). **D:** A depiction of the yellow-green birefringence of the amyloid when stained with Congo red. Other tissue structures (particularly fibrous tissue) may appear white when viewed with the polarizing microscope and should be distinguished from the amyloid (Polarization optics; magnification ×200). **E:** This interlobular artery exhibits inflammation primarily in the intima, which is seen commonly in acute vascular rejection. Compare with the immunofluorescence pattern in Figure 13.22F, which demonstrates the transmural nature of the process (H&E; magnification ×400). **F:** A photomicrograph of an immunoperoxidase preparation using LEU 2A antibody, an antibody to cytotoxic T cells, and diaminobenzidine. Several cross-sections of tubules and interstitium are shown from a typical example of acute cellular rejection. The T cells infiltrate both the interstitium and the tubular epithelial cells (magnification ×200). (See Color Plate.)

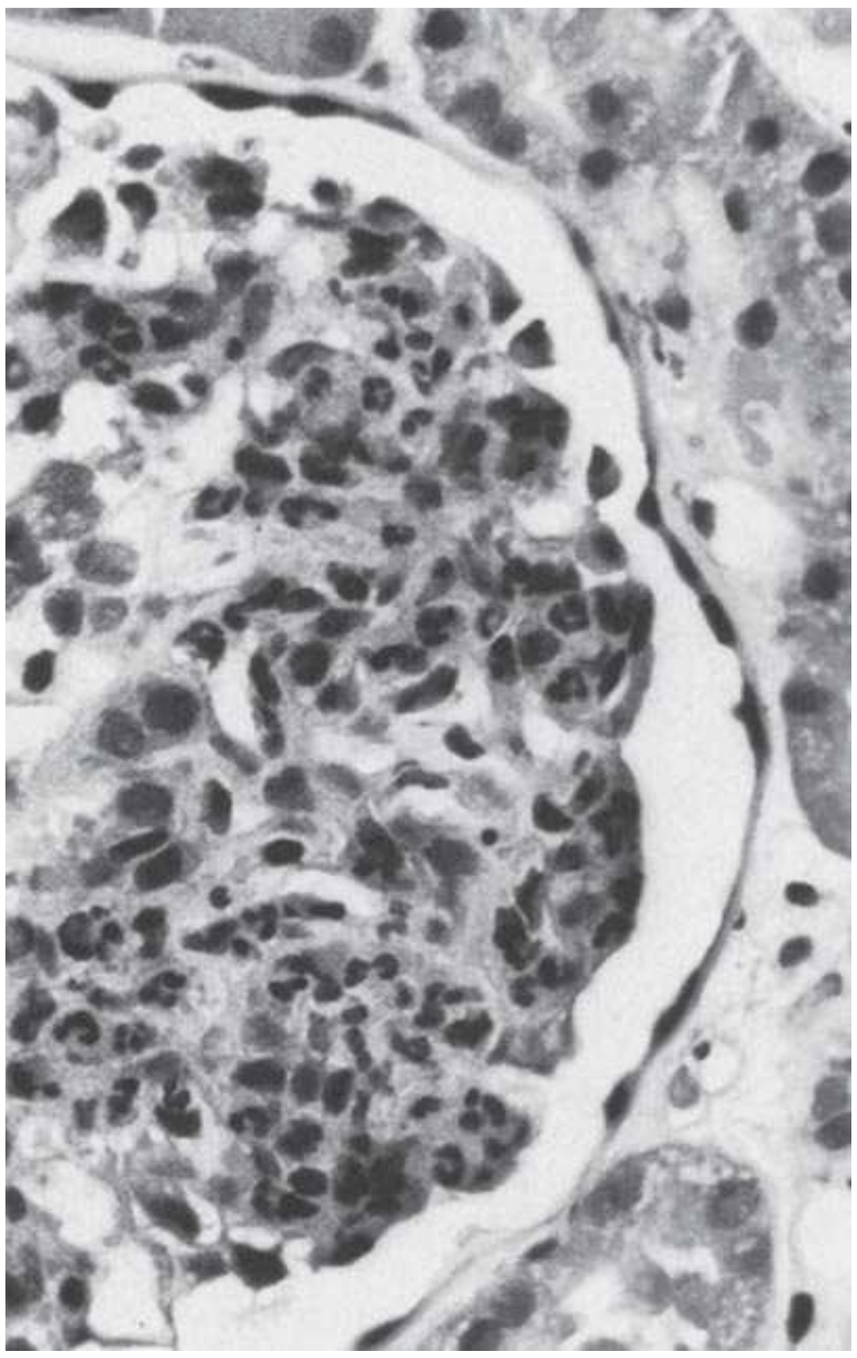


FIGURE 13.5 A photomicrograph of a glomerulus illustrating large numbers of neutrophils and mononuclear cells within peripheral capillary loops in a biopsy from a patient with post-streptococcal glomerulonephritis (H&E; magnification ×560).

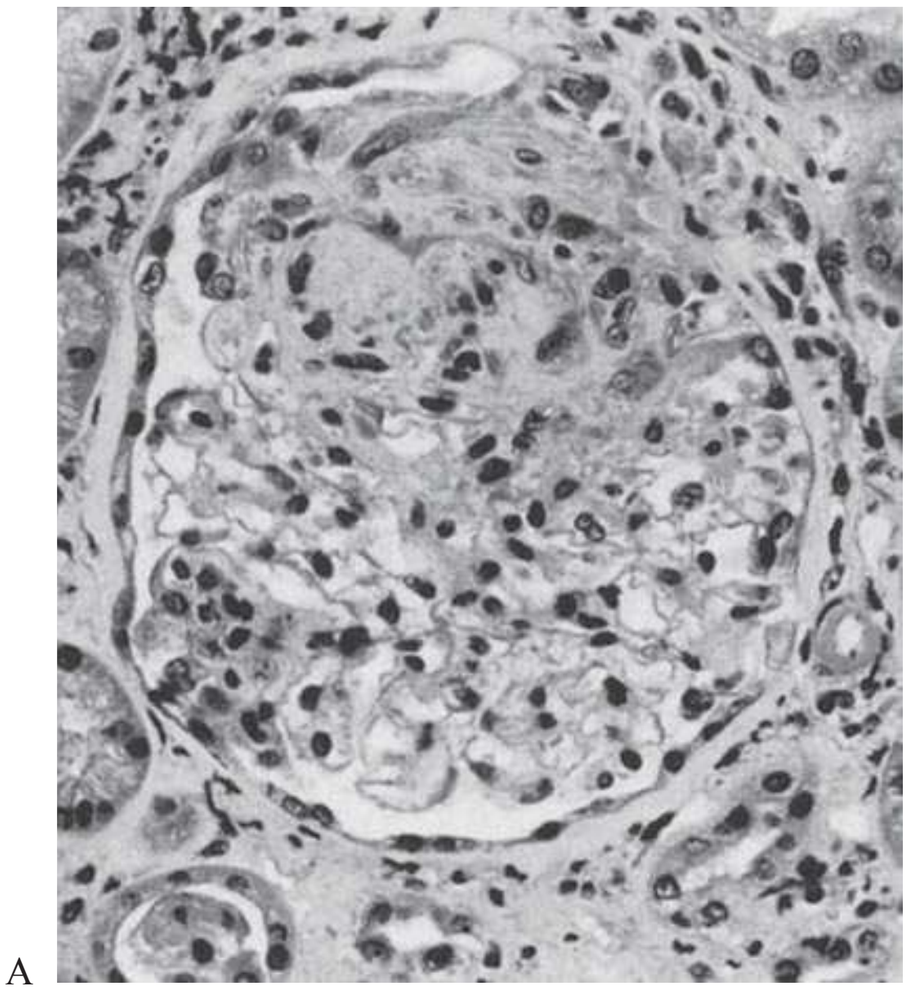
with toluidine blue[180] that are cut from tissue that has been prepared for electron microscopy (Fig. 13.7). The capillary (visceral) epithelial cells may exhibit hypertrophy, atrophy, or hyperplasia.

In addition to the mesangial cellularity, there may be an increase in mesangial matrix material with or without an increase in cells. This is evaluated best with the PAS and PAMS stains (Fig. 13.4B). Mesangial expansion may be global and may involve the entire glomerular tuft; it may be segmental (Fig. 13.8); or it may involve only the stalk region, which is designated the extraglomerular mesangium and forms part of the juxtaglomerular apparatus. Mesangial matrix expansion may extend into the capillary or may be observed in association with subendothelial capillary basement membrane thickening, so-called mesangial interposition.

Mesangiolysis is an alteration of the mesangium that is characteristic of thrombotic microangiopathies, but can be observed in patients with diabetes and other conditions.[181] The lesion appears as a relaxation or a disruption of the attachment of the capillary basement membrane to the mesangium. The basement membrane balloons outward in early lesions. The potential space is filled with disrupted mesangial matrix material, fibrin or platelet thrombi, fragmented erythrocytes, and other material (Fig. 13.9). Eventually, the mesangiolytic lesions sclerose and sclerosis is associated with worsening renal function.

The basement membrane of Bowman's capsule is thicker than the capillary basement membrane in the normal state and, like the capillary basement membrane, may increase in thickness with injury. The parietal epithelium lining the Bowman capsule can respond to injury with hypertrophy or hyperplasia or can exhibit infiltration with monocytes, resulting in the formation of crescents.[182]

The arterioles of the juxtaglomerular apparatus may be markedly abnormal because of the presence of glassy eosinophilic hyaline material (Fig. 13.10), edema, fibrin, other inflammatory changes; hypertrophy; or sclerosis. They may



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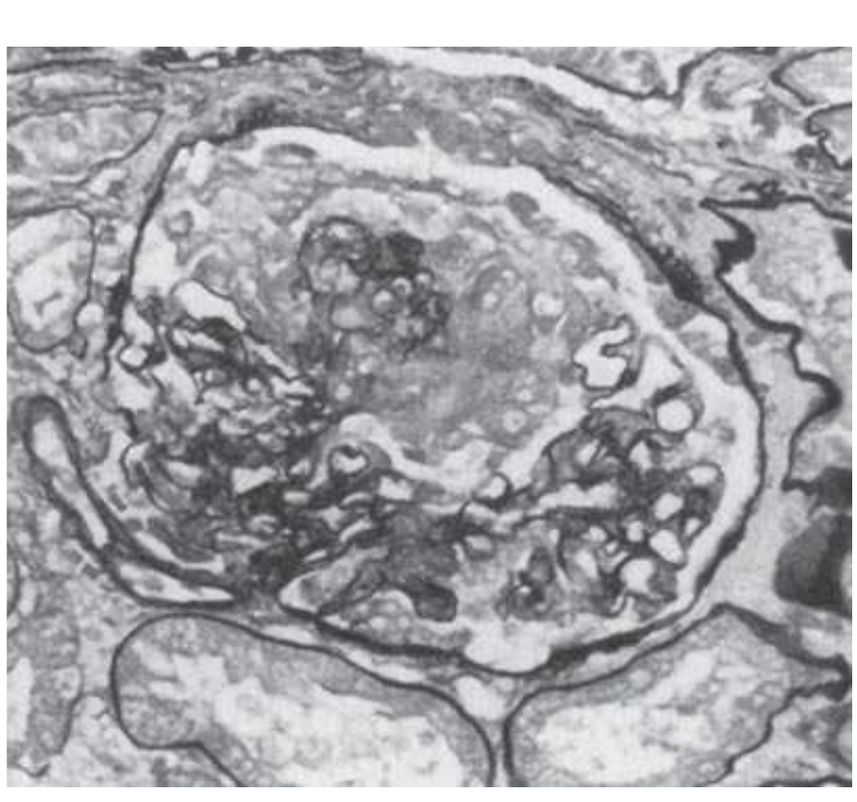


B

FIGURE 13.6 Photomicrographs demonstrating a segmental necrosis of a glomerular tuft in a biopsy from a 60-year-old man with a clinical diagnosis of Wegener granulomatosis (**A:** H&E stain; ×340; **B:** PAMS stain; ×300).

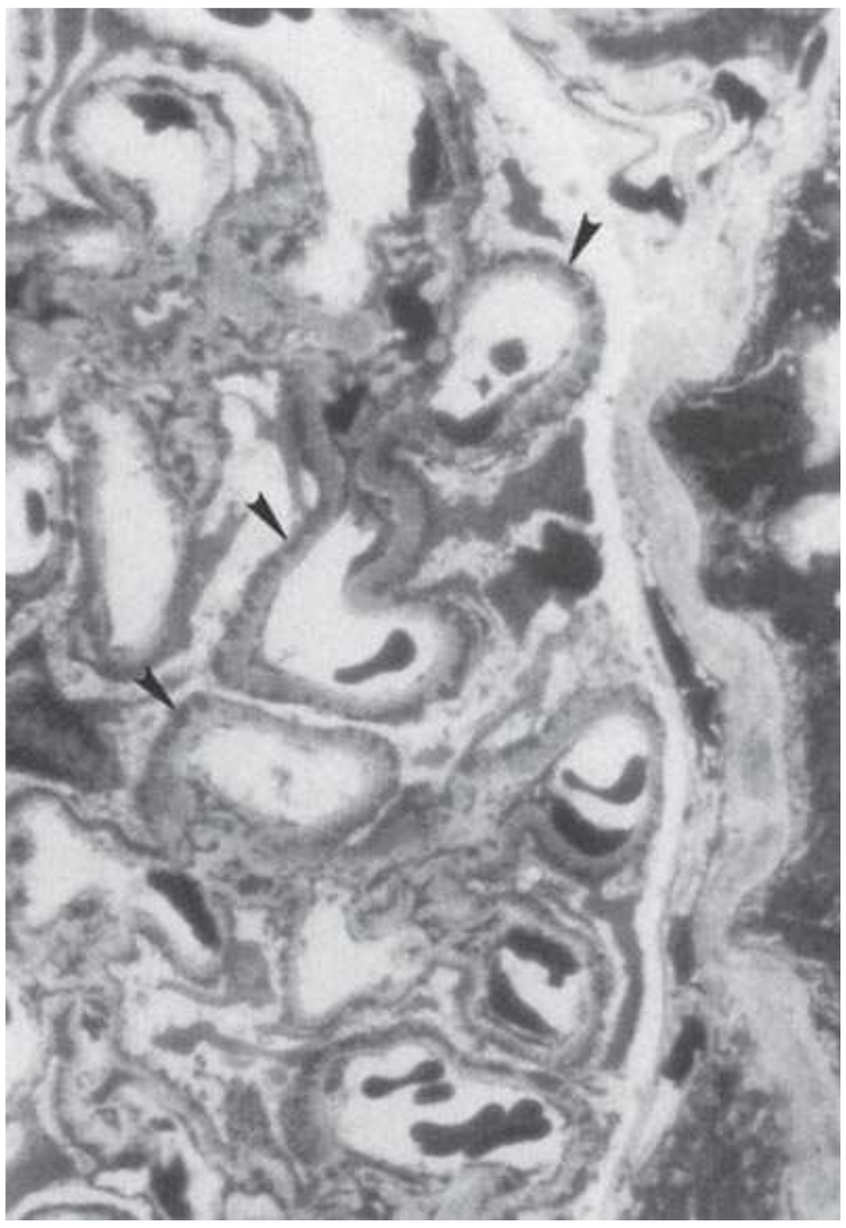


FIGURE 13.7 A micrograph of an Epon section stained with toluidine blue, illustrating subepithelial immune complexes (*arrowheads*) along capillary basement membranes in a patient with idiopathic membranous glomerulonephritis (magnification ×1,050).

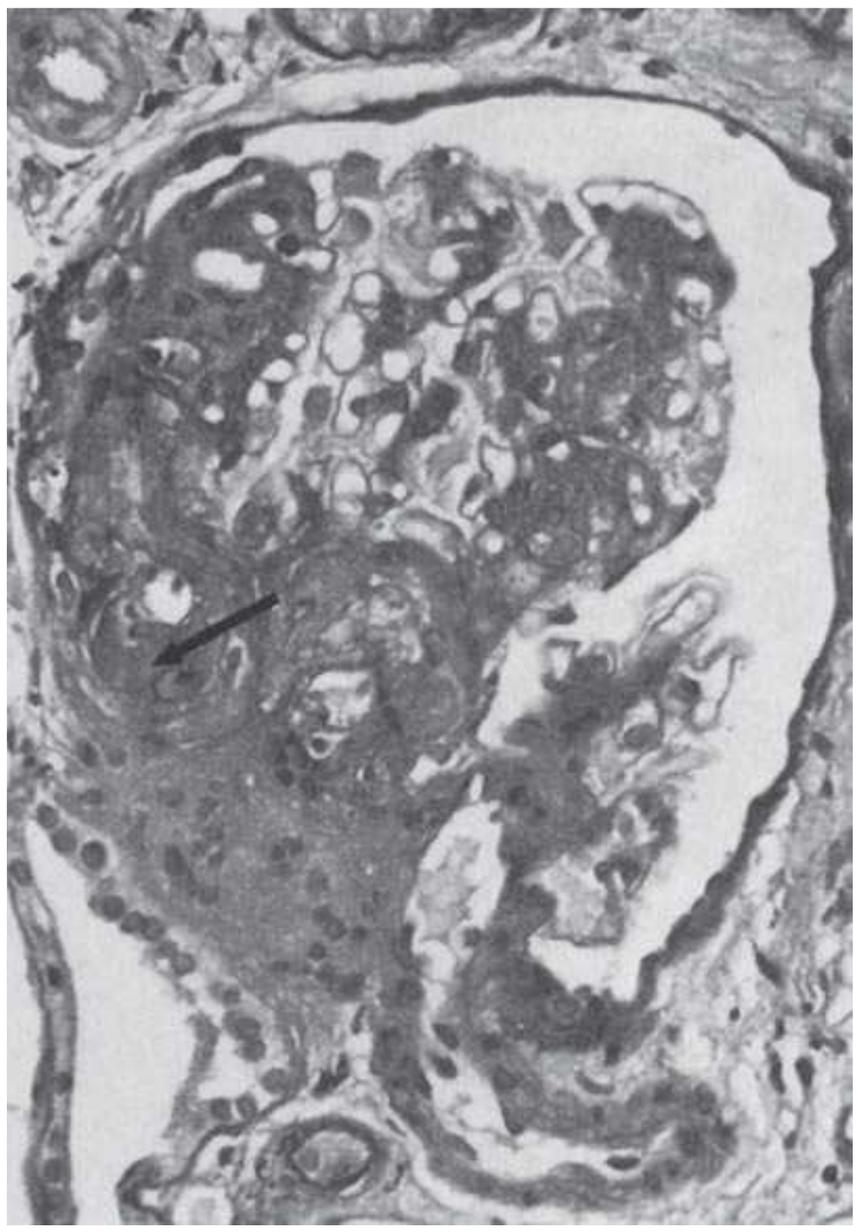


FIGURE 13.8 A glomerulus from a patient with focal segmental glomerular sclerosis, illustrating segmental sclerosis and hyalinosis (*arrow*) (PAS; magnification ×360). (From Newman WJ, Tisher CC, McCoy RC, et al. Focal glomerular sclerosis: contrasting clinical patterns in children and adults. *Medicine* 1976;55:67, with permission.)

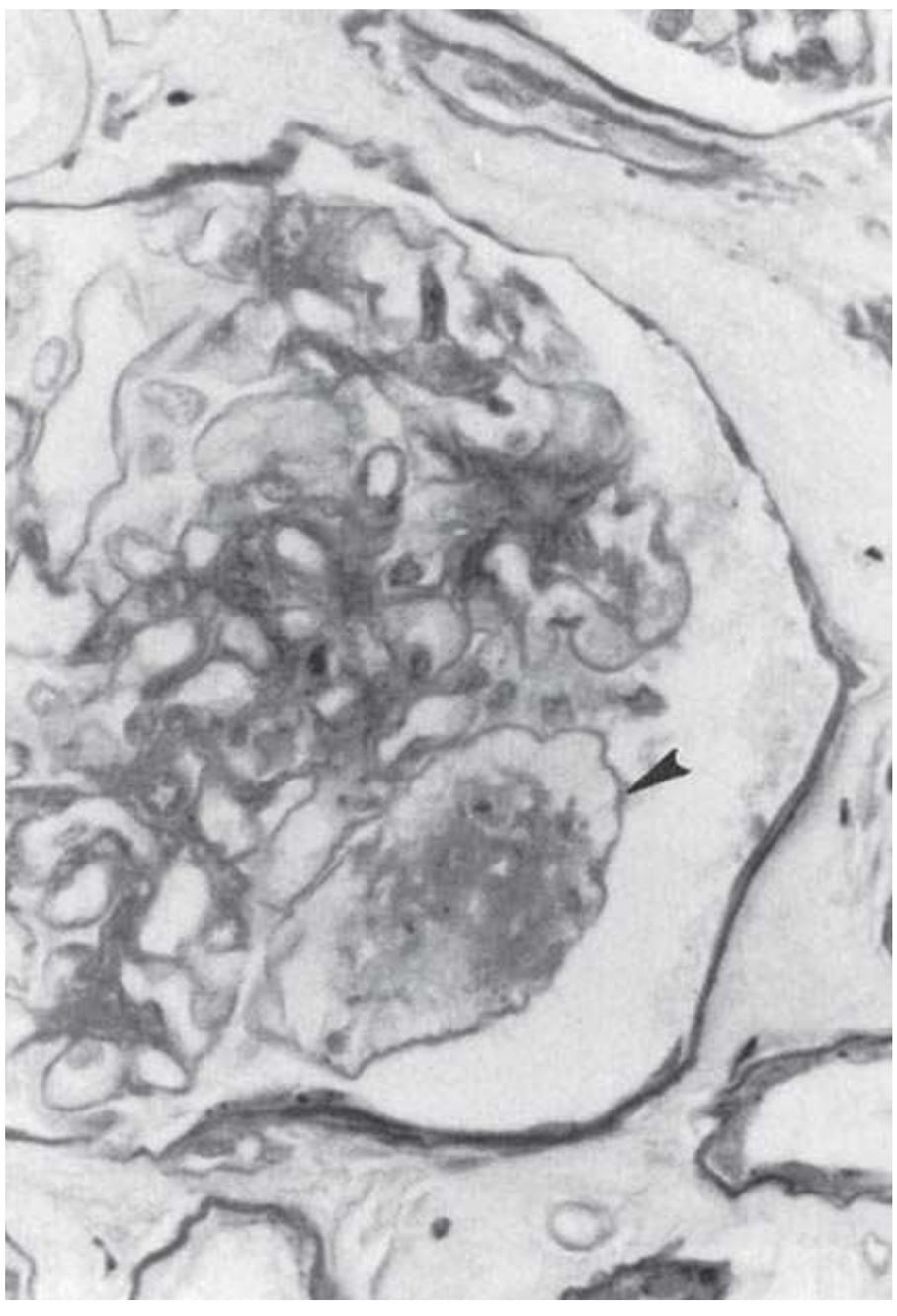


FIGURE 13.9 A light micrograph depicting mesangial lysis (*arrowhead*) in a glomerular tuft (PAS; magnification ×500).

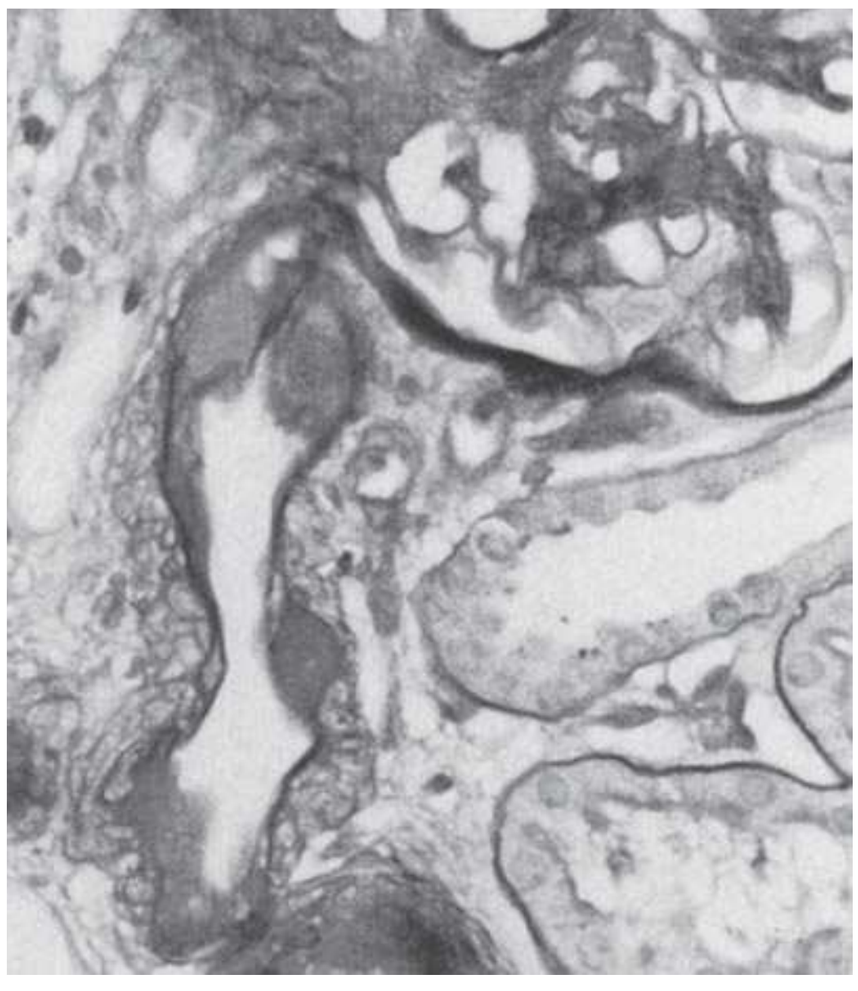


FIGURE 13.10 An arteriole with marked subintimal hyaline arteriolosclerosis from a patient with diabetic nephropathy (PAS; magnification ×460). (From Suki WN, Eknoyan G, eds. *The Kidney in Systemic Disease,* 2nd ed. New York: Wiley-Liss; 1981, with permission.)

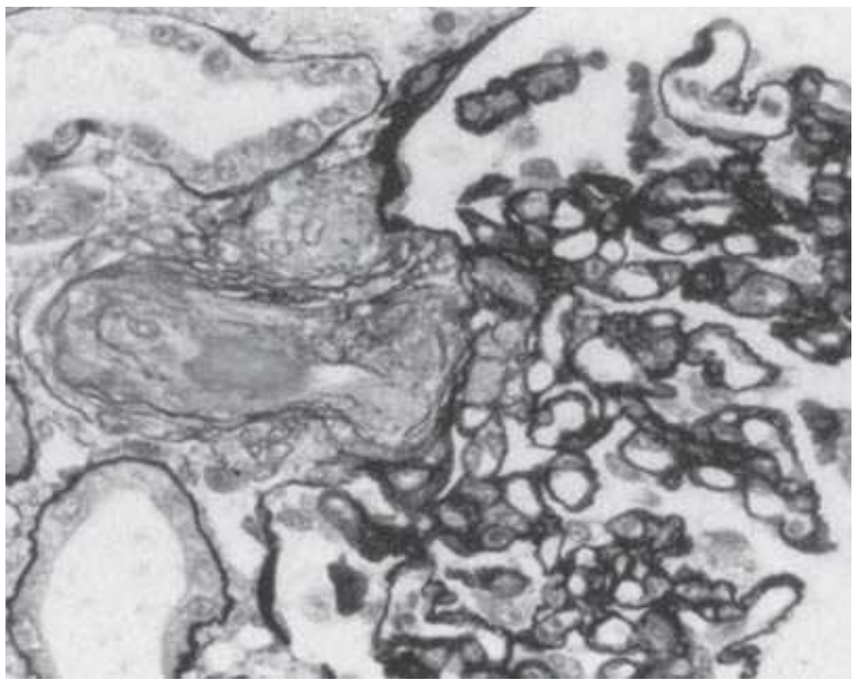


FIGURE 13.11 A fibrin thrombus in an afferent arteriole of a kidney biopsy from a 62-year-old woman with acute kidney injury and a stigmata of a hemolytic-uremic syndrome (PAMS; magnification ×415).

contain fibrin thrombi (Fig. 13.11) or may exhibit segmental fibrinoid necrosis. The pericytes surrounding the arterioles should be examined carefully for evidence of hypercellularity and increased granularity. The PAMS stain is used to screen for the latter feature.

Tubules

Next, the tubules are examined in detail. The proximal tubule epithelium should be tall, columnar, and should possess a PAS-positive brush border and display a deeply eosinophilic cytoplasm. Acute signs of ischemic or toxic injury include swelling of the cell cytoplasm and disruption of the brush border. Cytoplasmic vacuolization is followed by cell necrosis. Later stages include the flattening of the remaining cells with irregular staining of nuclei, and evidence of early regeneration (Fig. 13.12). Apoptosis[183,184] follows a variety of stimuli, including cell-mediated cytotoxicity[185–187] with pyknosis of the nucleus, condensation of the cytoplasm, and extrusion of the tubular cells into the lumen. Characteristic PAS-positive droplets that represent lysosomes are present in increased numbers with proteinuria. Additional findings include lipid droplets and cytoplasmic vacuoles. Large irregular vacuoles are seen with severe hypokalemia (Fig. 13.13); whereas fine, diffuse cytoplasmic vacuoles are observed with exposure to osmotic agents, such as mannitol (Fig. 13.14). Vacuoles are observed in calcineurin inhibitor toxicity (Fig. 13.15). Inflammatory cells may infiltrate the tubule and, if present, the type of inflammatory cell should be characterized (Fig. 13.4F). The lumen may contain extracellular material, such as casts or white or red blood cells. The basement membrane may be thickened by atrophy or the presence of immune deposits, or it may exhibit breaks.

Interstitium

Normally, the tubules are separated by an inconspicuous interstitium, which contains peritubular capillaries and a few interstitial cells. The interstitium may be abnormally thickened or expanded by a variety of extracellular materials, including collagen, other proteins, crystals, or edema. The type of inflammatory cells should be characterized if inflammation is present. Inflammatory cells may display a specific distribution, such as perivascular or periglomerular, marginated in peritubular capillaries, or scattered uniformly throughout the biopsy. Monocytes may aggregate and assume an epithelioid appearance, giving rise to granulomas. Normally, the interstitium is more prominent in the medulla and around the muscular arteries.

Vasculature

Finally, the larger vessels should be examined. The medium-sized renal arteries have a histology typical of arteries elsewhere; however, intrarenal veins have minimal smooth muscle compared to veins of similar caliber in other organs. The most common arterial change is intimal thickening in association with irregular reduplication of the internal elastic lamina, a finding that is seen best with the PAMS stain. The lumen may contain fibrin thrombi, embolized material, or inflammatory cells (Fig. 13.4E). The walls of the arteries

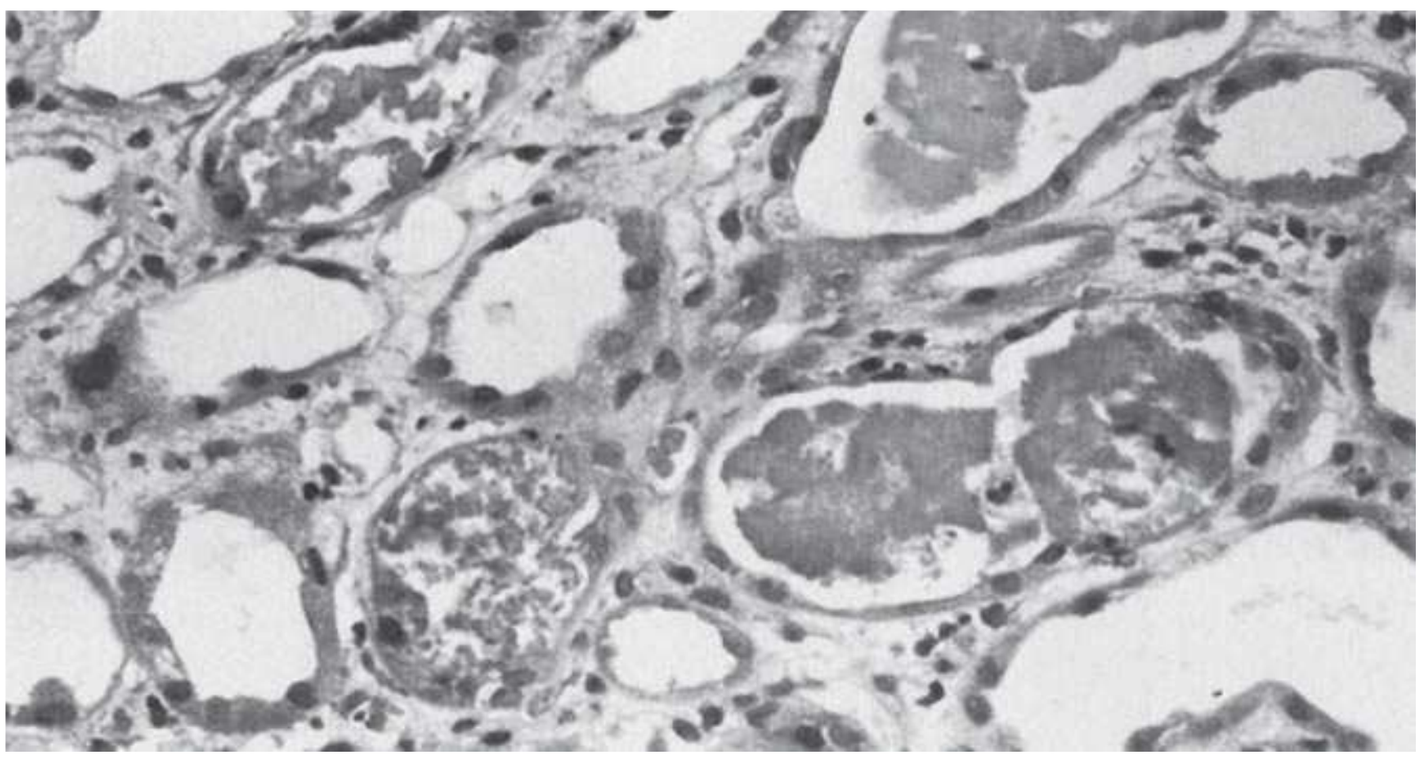


FIGURE 13.12 A photomicrograph depicting acute tubular necrosis (H&E; magnification ×440).

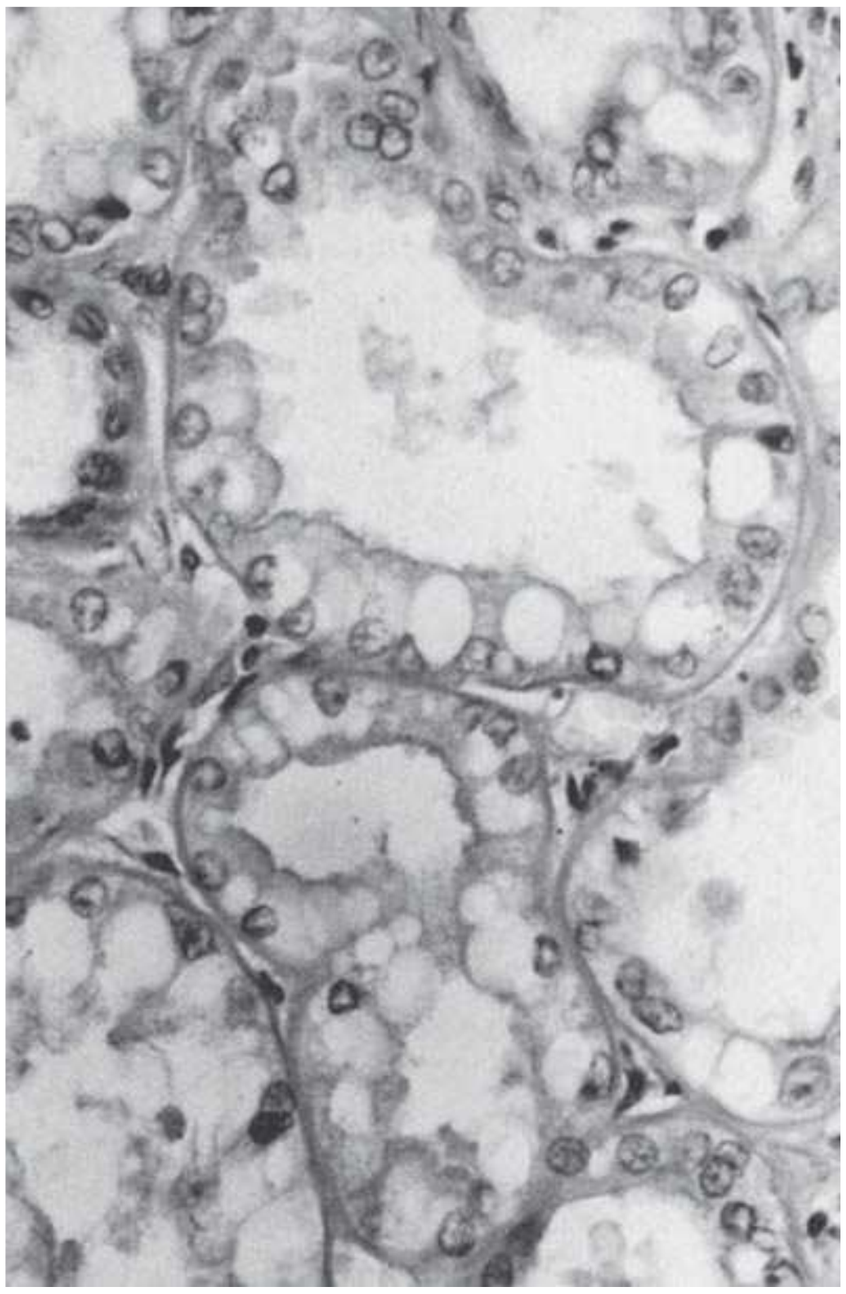


FIGURE 13.13 An example of severe hypokalemic nephropathy. Large irregular vacuoles are evident in the proximal tubule epithelium (H&E; magnification ×460).

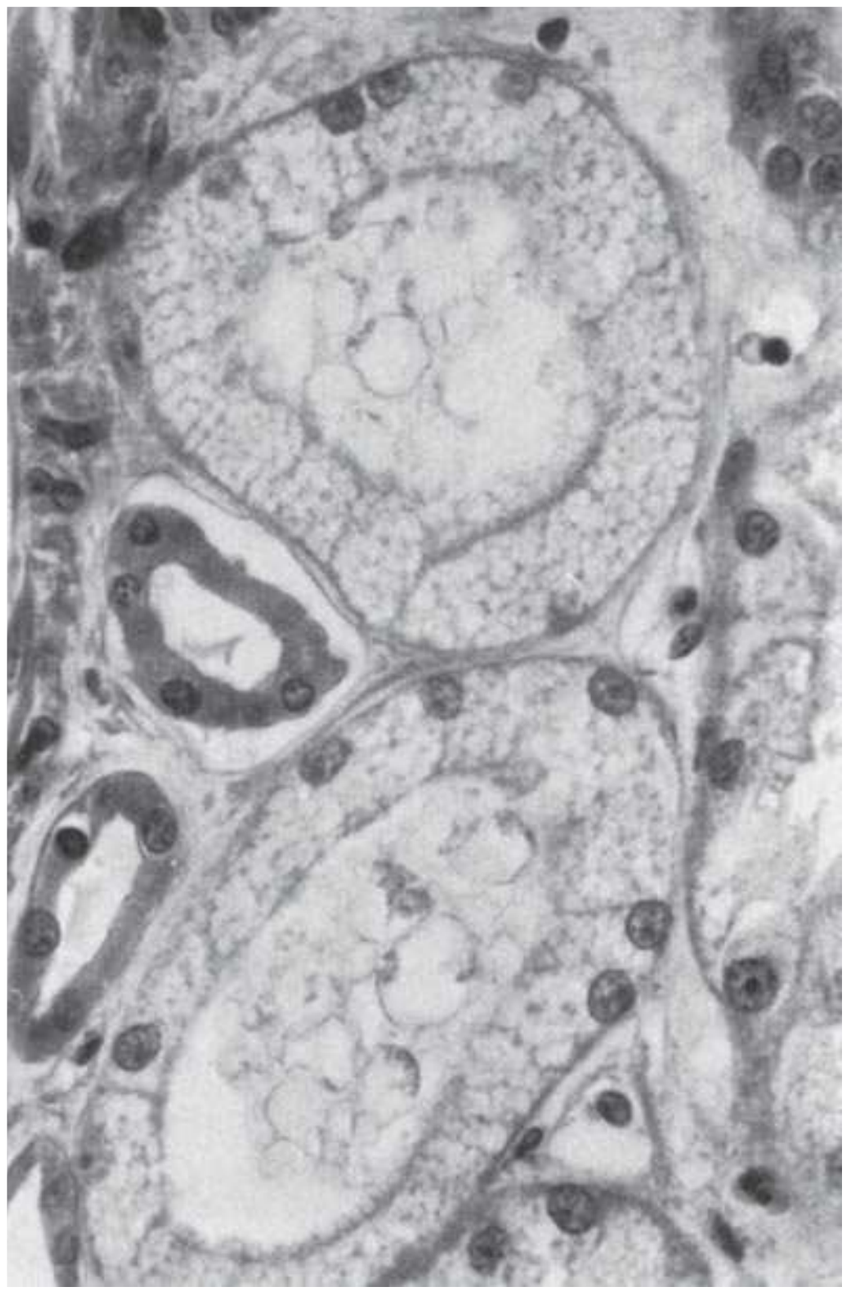


FIGURE 13.14 A photomicrograph illustrating osmotic nephrosis characterized by fine vacuolization of the proximal tubule epithelium (H&E; magnification ×540).

also may be thickened as the result of edema, fibrin, other exogenous material, or inflammation. Similar, although less extensive, changes may be observed in the renal veins.

At this point, it is useful to compare the relative degree and type of involvement of each of the cortical structures. As discussed in the preceding text, when one portion of the nephron is diseased, the remainder of the nephron eventually is affected. The site of the initial insult usually demonstrates the earliest and most severe changes. Thus, in glomerulonephritis, the glomeruli usually exhibit the most

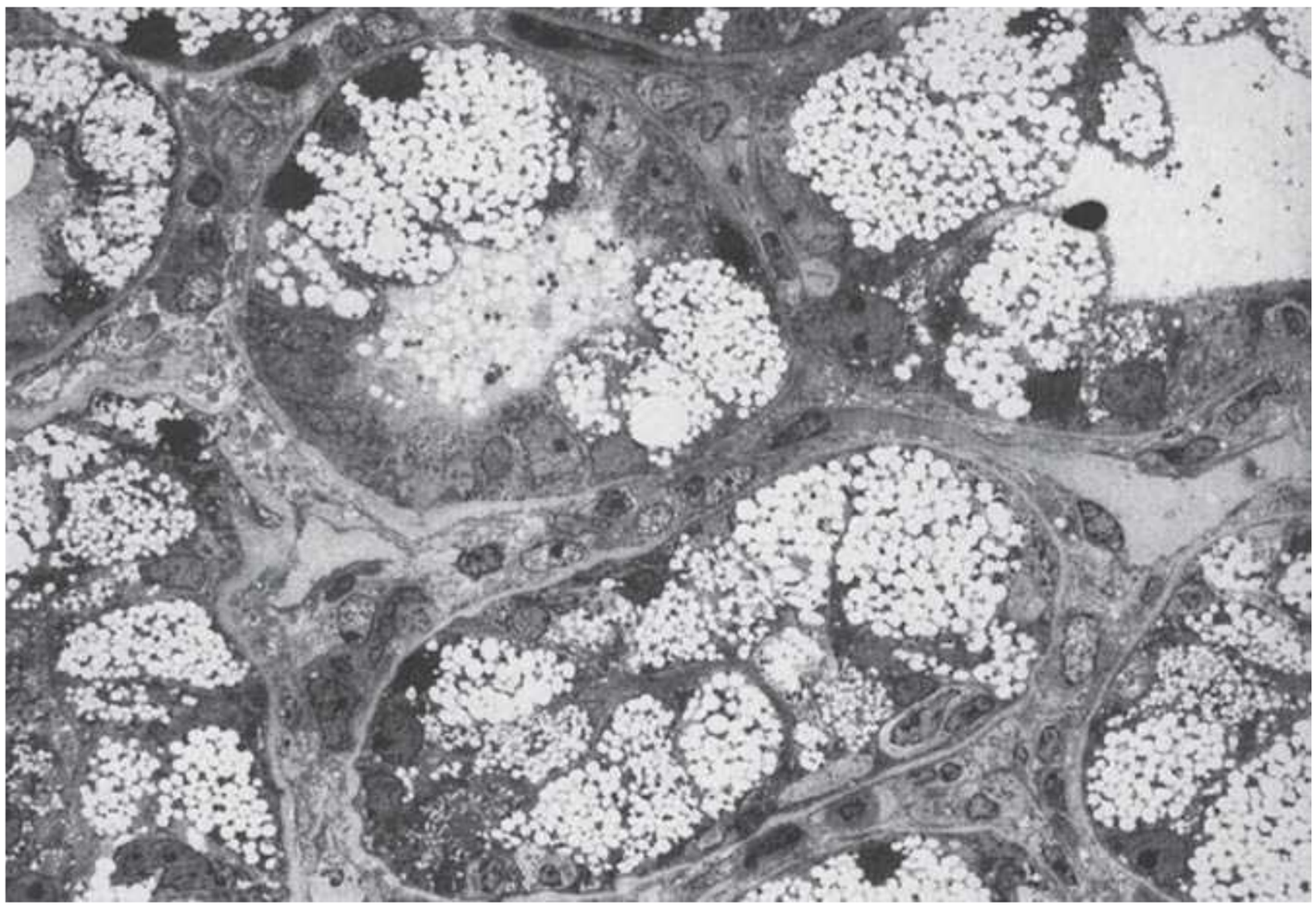


FIGURE 13.15 An electron micrograph illustrating severe tubular vacuolization secondary to calcineurin inhibitor toxicity. The nuclei are pyknotic, and the brush border is often disrupted or lost (magnification ×4,900). (From Tisher CC, Brenner BM, eds. *Renal Pathology with Clinical and Functional Correlations*. Philadelphia: Lippincott; 1989, with permission.)

striking changes, whereas less severe alterations in the form of interstitial inflammation and tubular injury accompany the primary lesion. The interstitium shows the most inflammation in interstitial nephritis, whereas the glomeruli may exhibit secondary involvement. If a process is severe enough and of a sufficient duration, all structures will atrophy and sometimes leave few clues to the etiology of the original disease. One is then left with a diagnosis of end-stage renal disease.

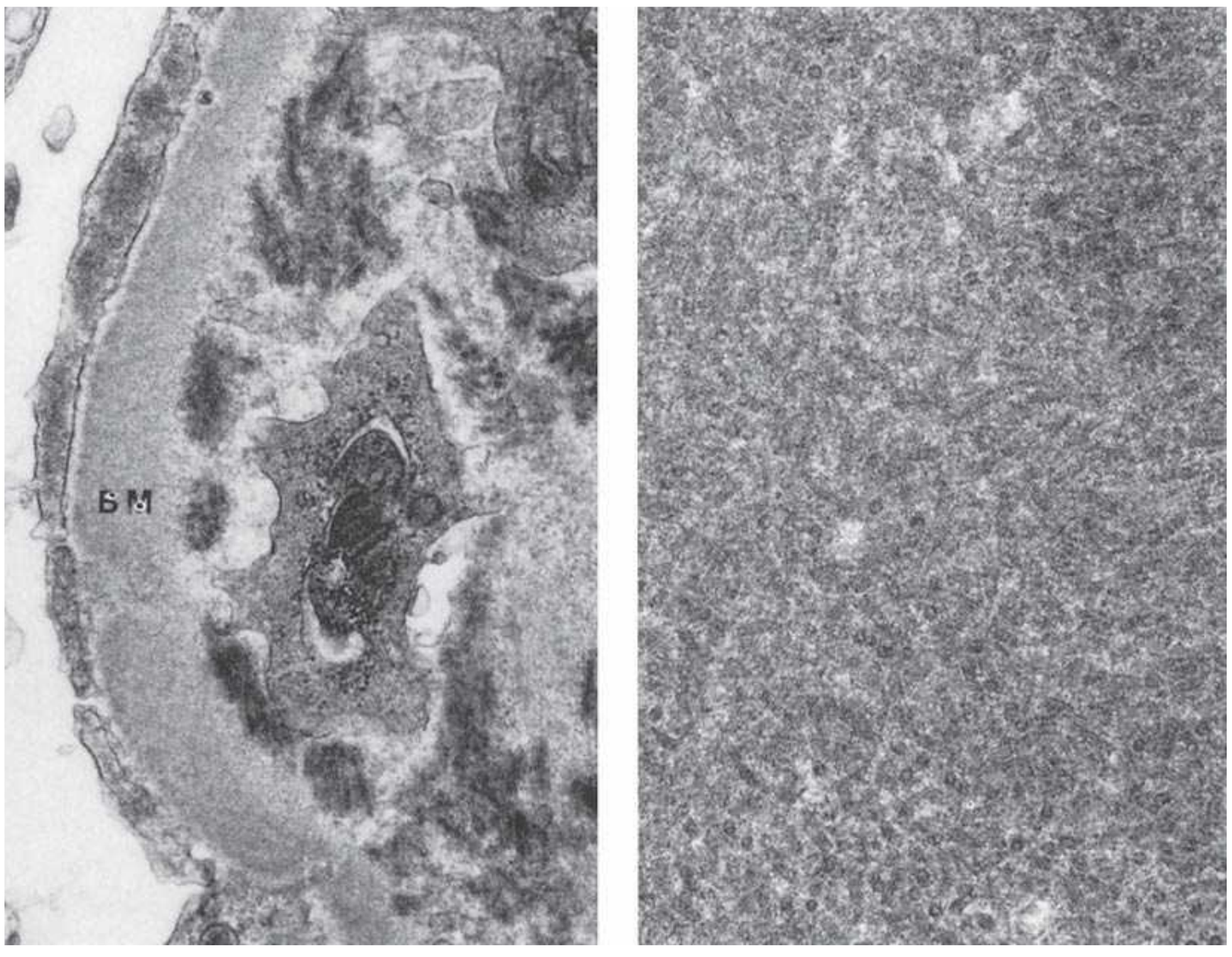
Electron Microscopy

The major advantage of electron microscopy is its greater resolving power when compared with light microscopy. In a kidney biopsy interpretation, electron microscopy is most useful in the examination of glomerular lesions. Immune complexes can be identified by electron microscopy when they are too small to be evident on light microscopy or when the immunofluorescence findings lack specificity. Electron microscopy gives the most definitive localization of immune complex deposits, thus making it possible to subcategorize their location as mesangial, subendothelial, subepithelial, or intramembranous. Some deposits have a characteristic substructure, such as those observed in light-chain disease,[188] amyloidosis,[121] cryoglobulinemic glomerulonephritis (Figs. 13.16A, B),[122] immunotactoid glomerulopathy,[189] or fibrillary glomerulonephritis (Fig. 13.17A, B).[190]

The basement membrane of the glomerular capillary loops may be uniformly thickened, as seen in diabetes mellitus,[191] or may be thin or irregular in appearance, as in Alport hereditary nephritis (Fig. 13.18)[192] or thin basement membrane disease.

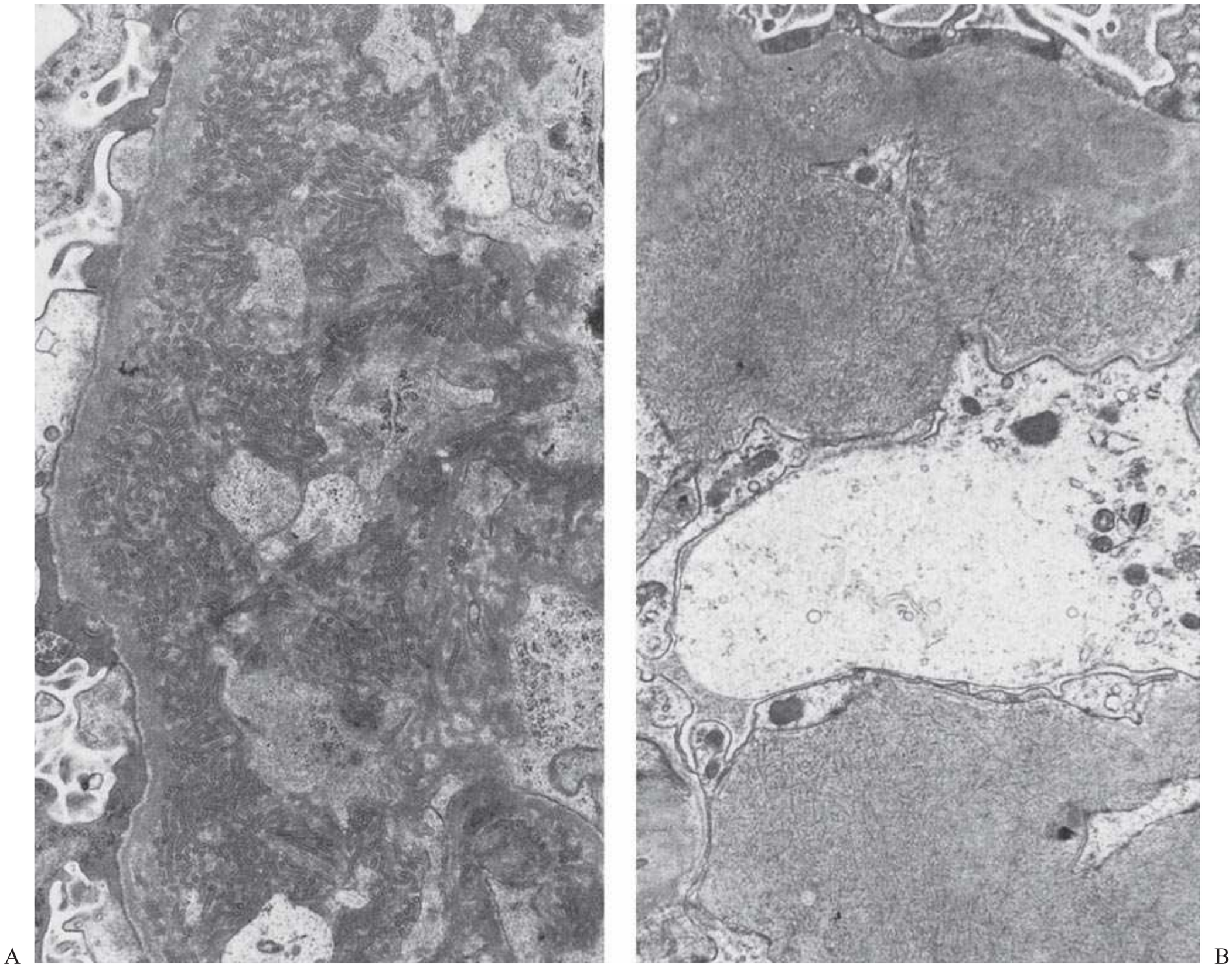
Metabolic abnormalities may be identified by the presence of characteristic accumulations of lipids, such as seen in Gaucher disease.[193] Tubuloreticular arrays may be present in the endoplasmic reticulum of endothelial cells and are characteristic of lupus nephritis when seen in large numbers (Fig. 13.19), although they may be observed in other conditions, such as in HIV-associated nephropathy (Fig. 13.20). Endothelial cell injury is characteristic of thrombotic microangiopathy and vascular allograft rejection. Immune deposits can also be identified along the tubular basement membrane (TBM) in lupus nephritis (Fig. 13.21) and light chain deposition disease.[194]

Recently, the importance of electron microscopy in the evaluation of native kidney biopsies was reaffirmed. In a series of 233 biopsies, Haas[195] found that electron microscopy was necessary to arrive at a final diagnosis in 50 biopsies, representing 21% of the total cases. In another 48 cases, the



A B

FIGURE 13.16 Electron micrographs illustrating cryoglobulin deposits in a glomerular capillary. **A:** A basement membrane with electron-dense subendothelial deposits (magnification ×15,000). **B:** A high-magnification view of the characteristic substructure of cryoglobulin deposits (magnification ×41,000). (Illustrations from Silva F, Eigenbrodt E, with permission.)



A B

FIGURE 13.17 Electron micrographs depicting the characteristic appearance of immunotactoid glomerulopathy (**A:** magnification ×21,000) and fibrillary glomerulonephritis (**B:** magnification ×18,400). (From Alpers CE. Immunotactoid (microtubular) glomerulopathy: an entity distinct from fibrillary glomerulonephritis? *Am J Kidney Dis* 1992;19:185, with permission.)

ultrastructural data were felt to provide important confirmatory information.

One disadvantage of electron microscopy is the limitation in the size of the sample; therefore, ultrastructural findings must be interpreted in the context of other histologic features. In addition, tissue processing for electron microscopy generally takes longer than that for light microscopy. Although rapid processing methods are available, they require special handling and therefore are more costly. Scanning electron microscopy has been used for biopsy investigation, but it is not incorporated into the processing of kidney biopsy specimens for routine clinical evaluation.

Immunohistology

Immunofluorescence and immunoenzyme staining have overlapping but different uses, as discussed previously. Standard immunofluorescence microscopy (as compared to confocal microscopy) is a more rapid but less sensitive technique. It is ideally suited for the detection of immune complex deposits. The major advantages of the immunoperoxidase technique are the greater sensitivity and the ability to examine the tissue with the light microscope, which makes the spatial relationships between tissue structures easier to identify.

There are four major immunoglobulin-staining patterns in glomeruli, which may occur singly or in various combinations (Fig. 13.22). They include linear staining along the basement membrane, granular subepithelial capillary wall staining, granular subendothelial capillary wall staining, and mesangial staining. Paramesangial deposits also may be seen alone, but as a rule, they are observed in association with one of the other granular patterns. Intramembranous deposits also are observed, but their exact location is difficult to determine. The granular deposits may be large and coarse (Fig. 13.22B) or fine (Fig. 13.22C). This difference is readily appreciated when comparing the coarse

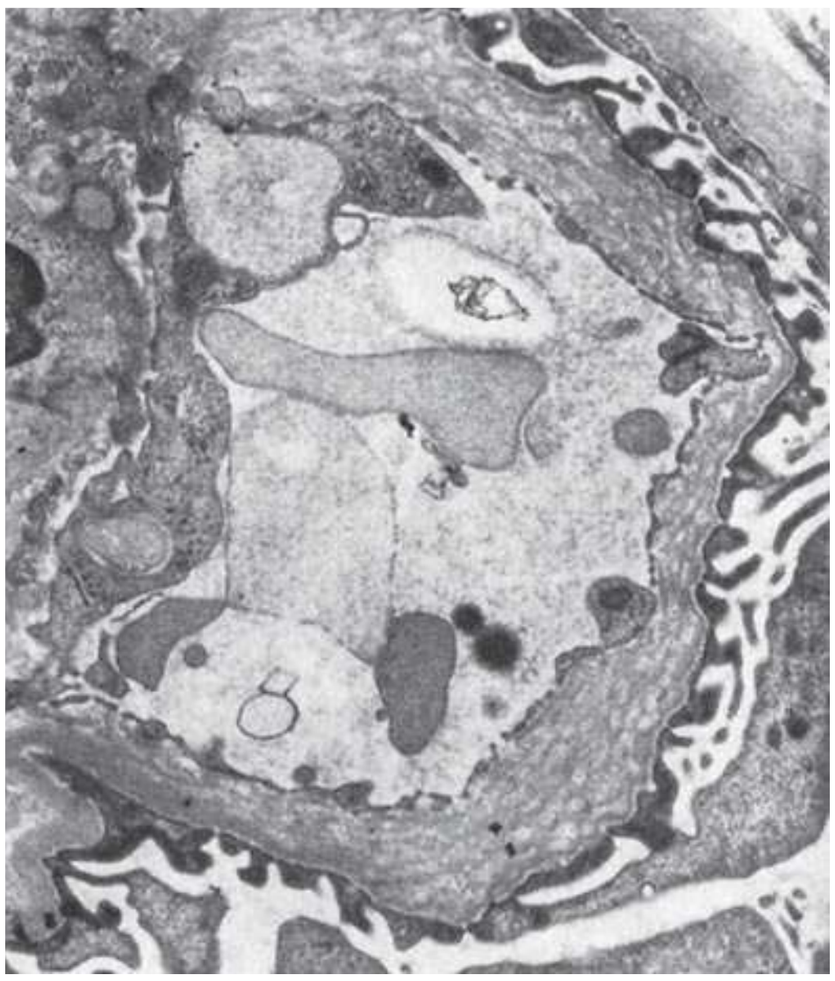


FIGURE 13.18 An electron micrograph of a portion of a glomerular capillary loop from a patient with Alport hereditary nephritis. The typical multilaminated appearance of the thickened basement membrane is evident (magnification ×3,800). (Illustration from Silva F, with permission.)

subepithelial deposits of poststreptococcal glomerulonephritis (Fig. 13.22B) with the subepithelial deposits of an early idiopathic membranous glomerulonephritis (Fig. 13.22C). Immune complex deposits may contain IgG, IgM, or IgA in any combination. C1q or C3 staining may be present in any of the same patterns as seen with immunoglobulins, or they may be observed separately. Linear staining requires careful interpretation. First, finely granular capillary wall staining can appear as a confluence of granules yielding a pseudolinear pattern. Electron microscopy resolves any doubt in this situation. Second, diseases such as diabetes mellitus and most other chronic renal diseases, including chronic allograft nephropathy, cause a thickening of the basement membrane. This is associated with increased staining of the capillary wall for serum proteins, particularly IgG4 and albumin, owing to electrostatic attraction.[196] These situations must be distinguished from the linear capillary basement membrane staining that is specific for IgG as observed in anti-GBM disease (Fig. 13.22A). In anti-GBM disease, the IgG staining clearly exceeds the albumin staining in intensity.

Epithelial cells, especially in the glomerulus and the proximal tubule, commonly have cytoplasmic droplets of protein in proteinuric conditions. Immune complexes also may be seen in the vessels, the interstitium, or along the tubular basement membranes. Vascular or tubular basement membrane staining for C3 in the absence of immunoglobulin is a common and often nonspecific sign of injury.

The use of immunoperoxidase staining with monoclonal antibodies directed against different antigens has made it possible to identify cell populations in tissue sections with great clarity and discrimination.[197] Immunofluorescence microscopy also can be used,[198] but the spatial discrimination is not as good. Most nonspecific inflammatory infiltrates are an approximately equal mixture of B and T cells, with a predominance of T-helper cells (CD4) over T-suppressor or cytotoxic (CD8) cells.[197–199] These cells form nodular aggregates in interstitial areas. Cellular allograft rejection predominantly has T cells (Fig. 13.4F)[197–199] and monocytes (macrophages).

In some cases, a viral cytopathic effect may be seen by standard histology (e.g., nuclear inclusions) and the viral pathogen inferred from the clinical situation or serologic tests. However, the virus can be specifically identified by immunohistochemistry or in situ nucleic acid hybridization. Common examples in the transplant setting are BK virus, cytomegalic virus (CMV), and EBV.

Other enzyme systems can be used to replace peroxidase in immunoenzyme staining. However, immunostaining

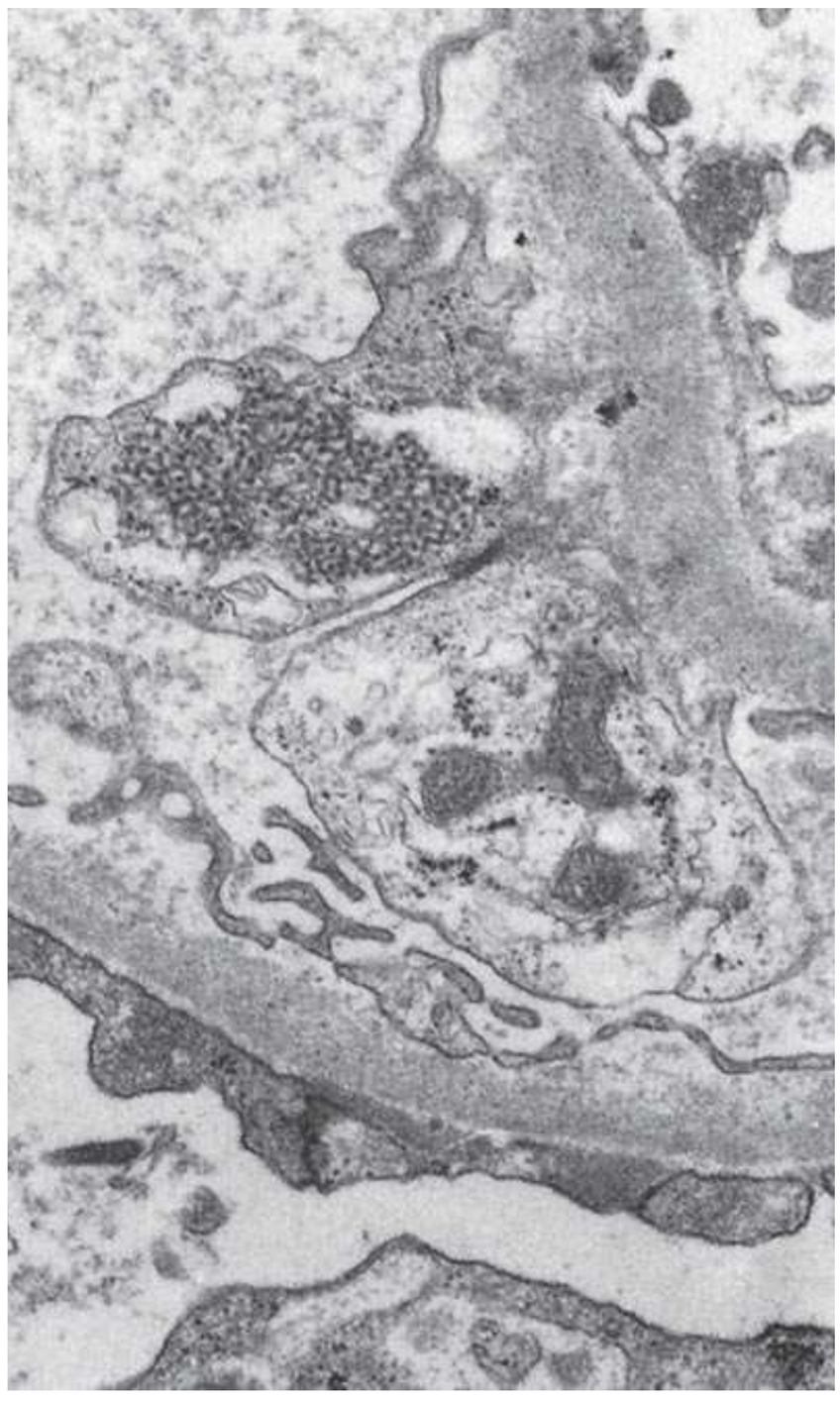


FIGURE 13.19 An electron micrograph depicting tubuloreticular arrays in a smooth-surfaced endoplasmic reticulum of a glomerular capillary endothelium from a patient with lupus nephritis (magnification ×24,000). (From Tisher CC, Kelso HB, Robinson RR, et al. Intraendothelial inclusions in kidneys of patients with systemic lupus erythematosus. *Ann Intern Med* 1971;75:537, with permission.)

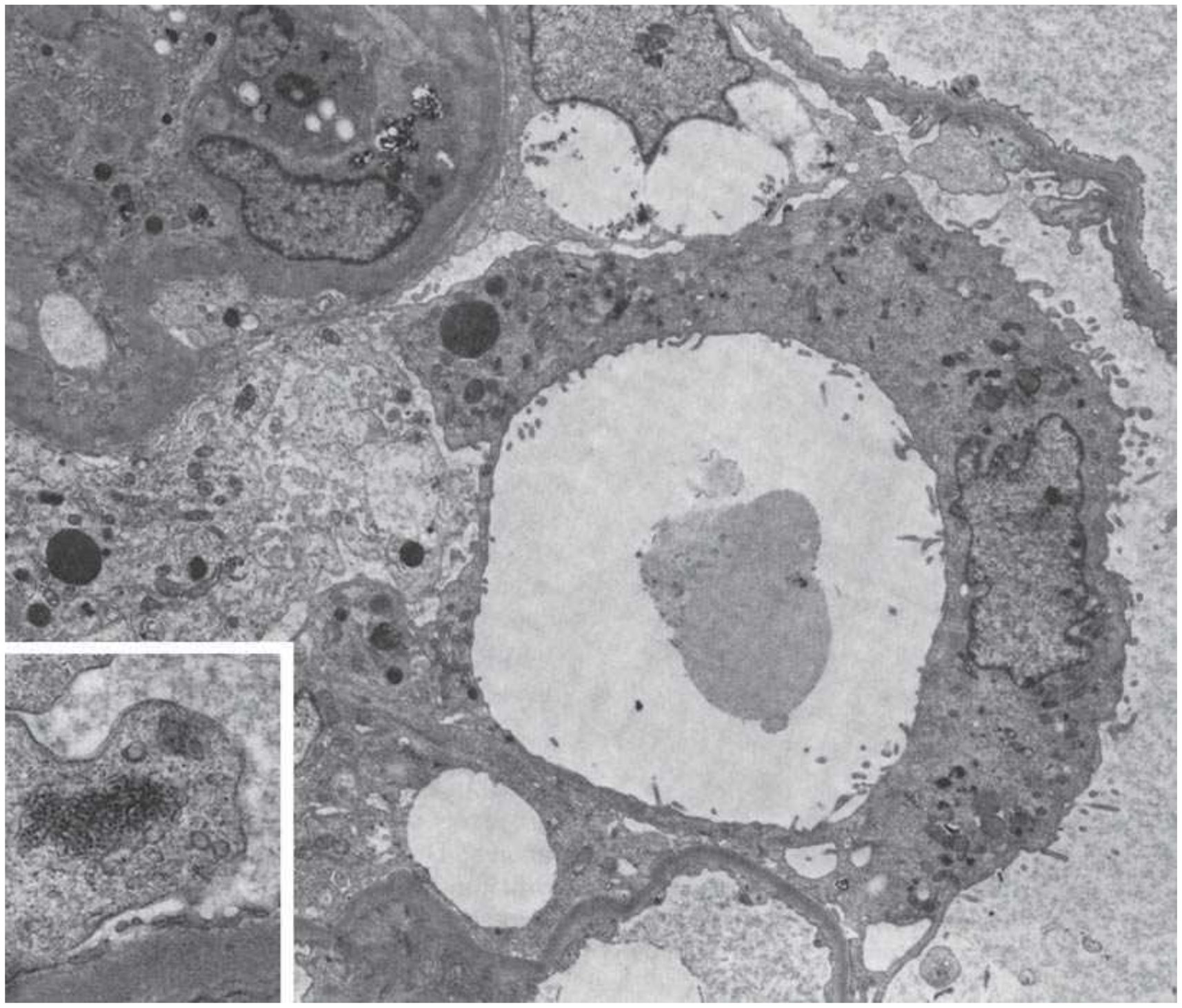


FIGURE 13.20 An electron micrograph illustrating focal and segmental glomerulosclerosis in HIV-associated nephropathy. The inset depicts the characteristic tubuloreticular arrays located within the endoplasmic reticulum of glomerular endothelial cells that are typically observed in this condition (magnification ×4,000; *inset,* magnification ×16,250). (Illustration from Cohen AH, with permission.)

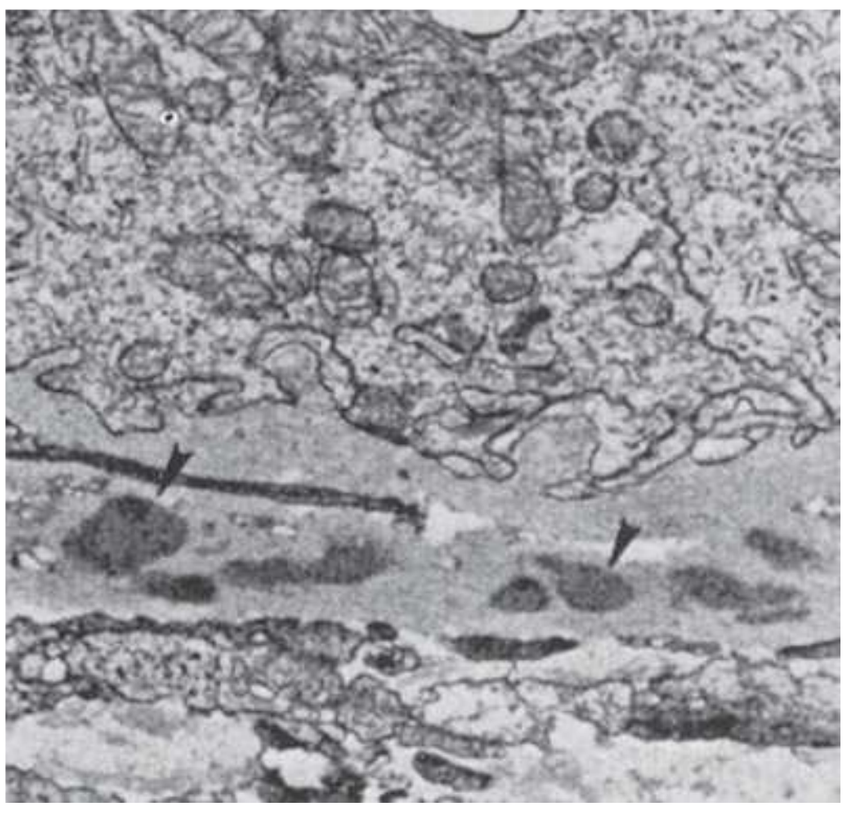


FIGURE 13.21 An electron micrograph illustrating immune complex deposits (*arrowheads*) along a tubular basement membrane in a renal biopsy from a 14-year-old boy with lupus nephritis (magnification ×2,500). (Biopsy specimen from Nash ML, with permission.)

using horseradish peroxidase and diaminobenzidine as the substrate is the best combination currently available because the staining is crisp and the slides are permanent. Diaminobenzidine is a carcinogen and must be handled with caution. Immunohistochemistry has great diagnostic potential, which is limited primarily by the antibody specificity.

The vision of gene expression in kidney disease has progressed in two paradigms for kidney transplant pathology. The first is in "for cause" biopsies where the microarray transcriptiosome signature may help clarify the ambiguous Banff subclassification of "Borderline (Suspicious) for rejection" and various chronic renal allograft pathology states[200] known as chronic rejection, chronic allograft nephropathy, or interstitial fibrosis/tubular atrophy (IFTA).[201,202] The second paradigm is to monitor gene expression in biopsies to evaluate the adequacy of immunosuppresion in terms of determining which gene signature profiles are predictive of chronic progression.[203] Biopsy studies are supplemented by minimally invasive peripheral blood profiles.[204–206]

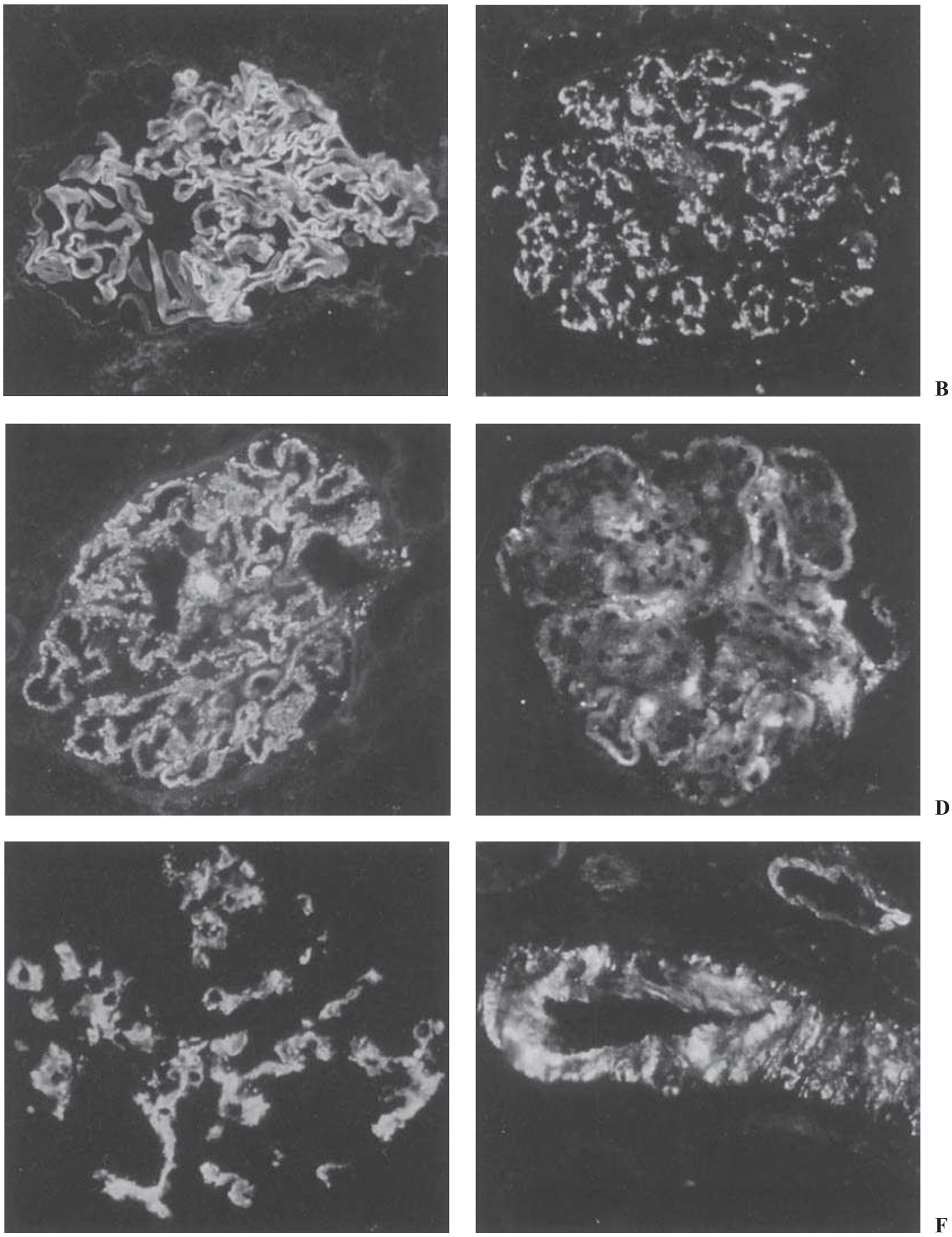


FIGURE 13.22 **A:** A glomerulus with fine, ribbonlike basement membrane staining with IgG indicative of anti-glomerular basement membrane disease (magnification ×200). **B:** A glomerulus exhibiting coarse granular deposits of IgG distributed irregularly along the glomerular basement membranes. This pattern is typical of poststreptococcal glomerulonephritis. C3 is usually present in the same pattern (magnification ×200). **C:** A glomerulus with fine IgG granules evenly deposited along the glomerular basement membrane. This pattern is typical of the subepithelial deposits of idiopathic membranous glomerulonephritis (magnification ×200). **D:** A glomerulus demonstrating the typical pattern of subendothelial immune complex deposits. The outer edge of the deposits abuts the inner surface of the glomerular basement membrane and is relatively smooth. The inner aspect of the deposits is shaggy and irregular and may merge with the mesangium, which is expanded. This pattern is characteristic of membranoproliferative glomerulonephritis (IgM; magnification ×200). **E:** A glomerulus exhibiting the typical pattern of IgA mesangial immune deposits. Note the absence of basement membrane localization. The dark spaces in the midst of the deposits represent mesangial cells, not capillary lumina. This pattern is typical of IgA nephrology, Henoch-Schönlein purpura, and mesangiopathic lupus nephritis (magnification ×200). **F:** An arteriole with transmural staining for IgM. This pattern is characteristic of relatively mild, acute vascular lesions of vascular rejection. In more severe lesions, there is a greater disruption of the vessel wall, which also can be seen by light microscopy. This pattern should be distinguished from the subintimal glossy deposits of hyaline that are seen in chronic vascular disease (magnification ×400). (See Color Plate.)

CLINICOPATHOLOGIC CORRELATIONS

Once the light, immunohistologic, and electron microscopic findings are completed, they should be integrated to derive a histologic diagnosis that is indicative of the disease process. The histologic diagnosis then is related to the clinical findings to give a clinicopathologic diagnosis that can be used to plan a course of therapy, establish the prognosis, or both. We do not try to describe all of the many histologic patterns of kidney disease in this section, because they are discussed in considerable detail in other chapters of this book. Instead, we briefly discuss selected examples in which the approach we have outlined is used.

Several renal diseases fail to reveal significant changes or only nonspecific changes on a histologic examination. For instance, in MCNS, the findings are principally the result of proteinuria and include foot-process simplification in the glomerulus and evidence of increased protein resorption by the proximal tubule. The only abnormality in benign recurrent hematuria may be the presence of red blood cells in the tubules. The differential diagnosis should include Alport disease early in its course and thin basement membrane disease. Differentiation requires a thorough electron microscopic examination of the specimen and the appropriate clinical studies.

Mesangial Expansion

In many conditions, mesangial expansion may be the only abnormality observed on light microscopy. The immunofluorescence findings separate a group of immune complex diseases that exhibit mesangial involvement. For instance, if IgA is the predominant immunoglobulin that localizes to the mesangium (Fig. 13.22E), the differential diagnosis should include IgA nephropathy and Henoch-Schönlein disease. If IgG is the principal immunoglobulin, then lupus nephritis should be considered and a careful search should be made by electron microscopy for subendothelial and subepithelial deposits or fibrillary glomerulonephritis. If the predominant immunoglobulin is IgM, then the differential diagnosis should include IgM nephropathy and the mesangiopathic form of lupus nephritis. Mesangial localization of C3 in the absence of immunoglobulins may represent a resolving immune complex disease. Evidence of resolving immune complexes may be seen by electron microscopy. The mesangial expansion may represent early diabetic nephropathy, arteriolonephrosclerosis, or FSGS if no complexes are noted on electron microscopy. If the material responsible for the mesangial expansion is negative or only weakly positive with the PAMS and PAS stains, it is important to examine additional sections after staining with Congo red in search of evidence of amyloid. On light microscopy, the most specific indication of amyloid is a green birefringence that occurs when the Congo red stain is viewed under polarized light (Fig. 13.4D). Occasionally, electron microscopy may be necessary to establish the diagnosis because it is the most sensitive technique to detect amyloid. Other, less common immune deposits that are also demonstrated easily with electron microscopy include κ light-chain disease, cryoglobulinemic glomerulonephritis, fibrillary glomerulonephritis, and immunotactoid glomerulopathy (Figs. 13.16A,B and 13.17A,B).

Neutrophilic Exudates

A neutrophilic exudate may be observed in a variety of renal diseases. The most prominent neutrophilic exudate is typically seen in poststreptococcal glomerulonephritis (Fig. 13.5),[207] a disease that does not usually require a biopsy for diagnosis. Occasionally, the typical clinical features are obscured, however, and a kidney biopsy is required. The patient may not seek medical attention until later in the disease course, when the light microscopic and immunofluorescence microscopic findings of coarse granular IgG deposits (Fig. 13.22B) may not be present. Often, however, C3 deposits remain. Again, electron microscopy may demonstrate typical large humplike subepithelial deposits that are in various stages of resolution. Numerous neutrophils also may be seen in other forms of postinfectious glomerulonephritis, in MPGN (Fig. 13.23),

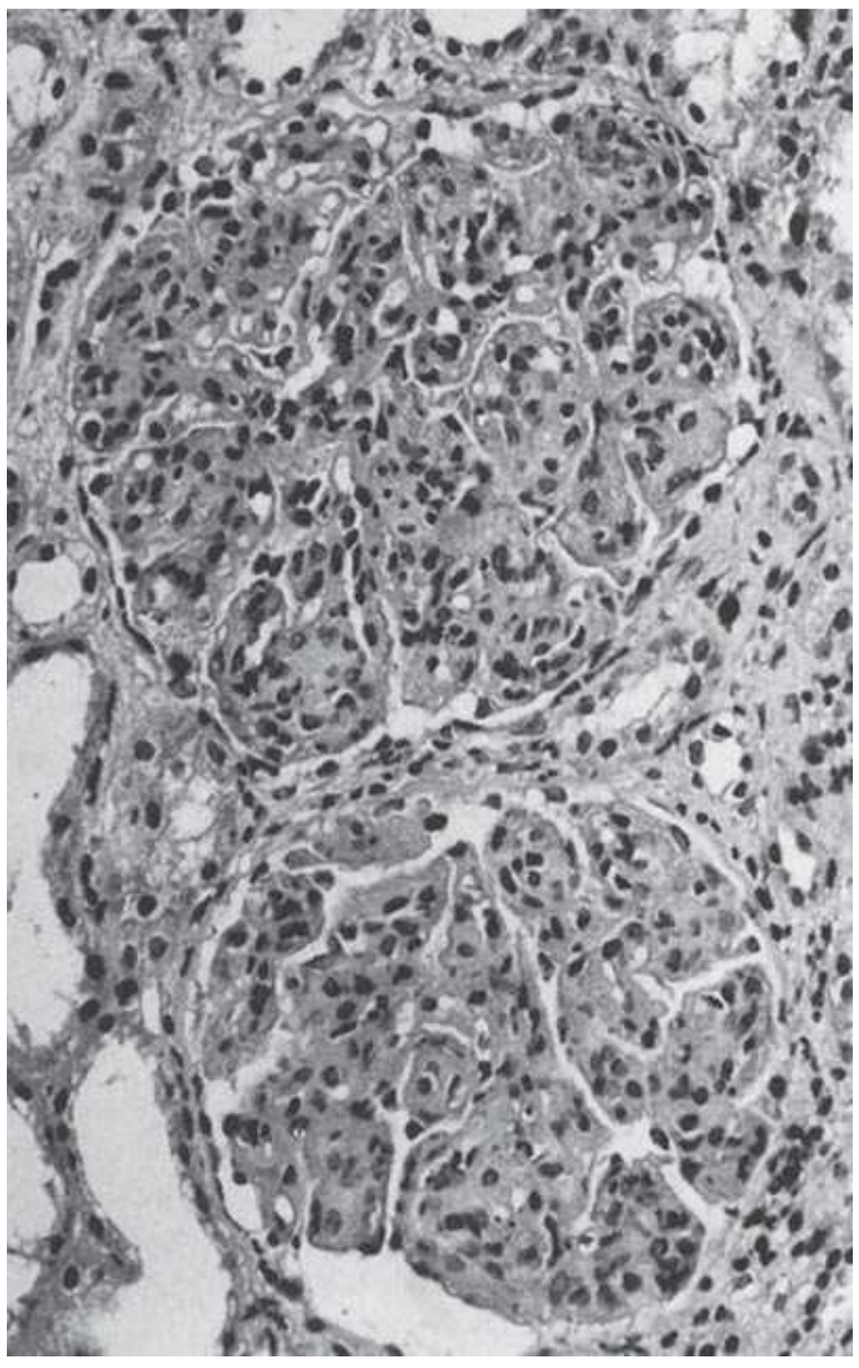


FIGURE 13.23 A photomicrograph demonstrating a mesangial hypercellularity and neutrophilic infiltrates in two glomeruli from a patient with type II membranoproliferative glomerulonephritis (H&E; magnification ×300). (From Lamb V, Tisher CC, McCoy RC, et al. Membranoproliferative glomerulonephritis with dense intramembranous alterations. A clinicopathologic study. *Lab Invest* 1977;36:607, with permission.)

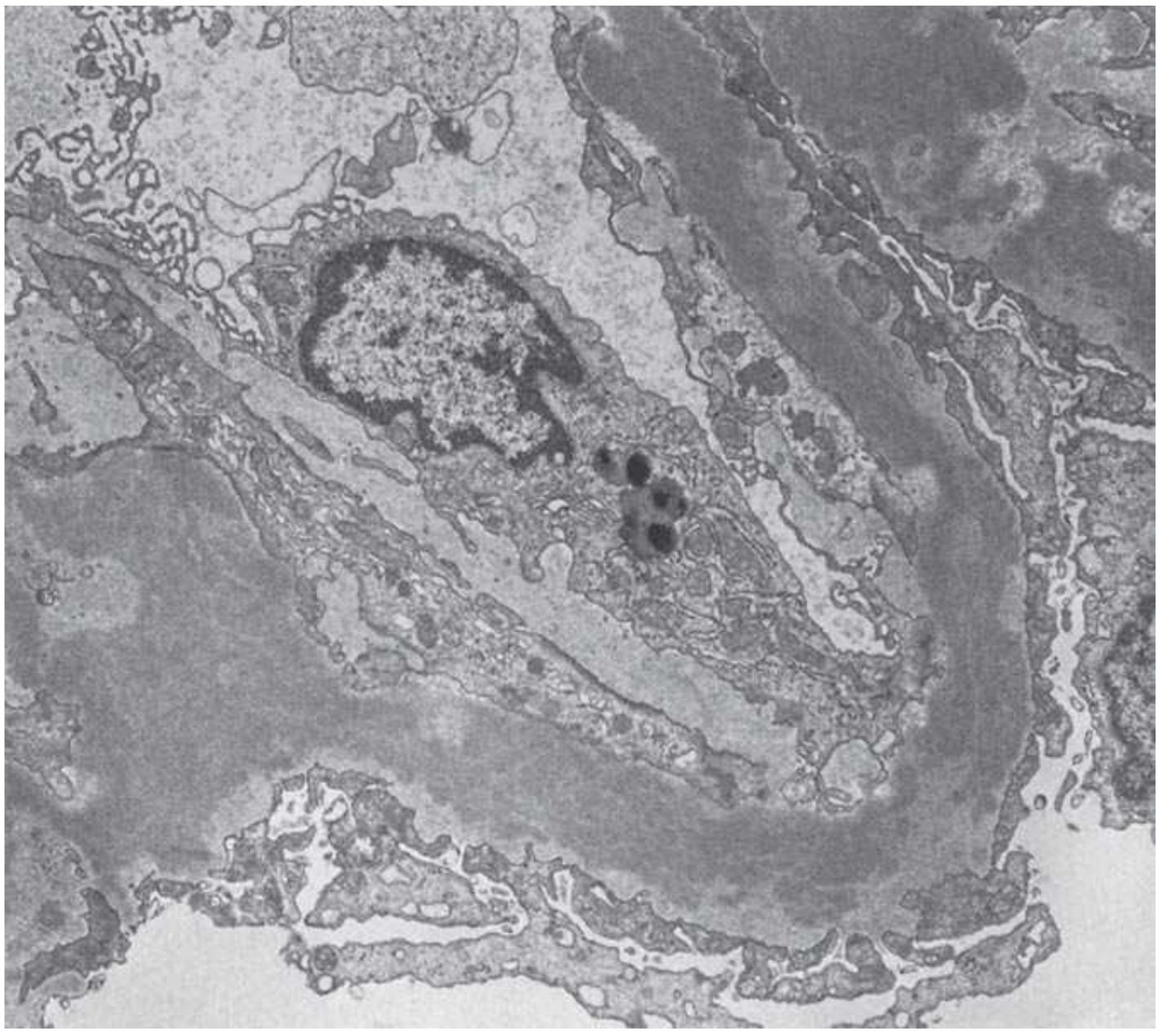


FIGURE 13.24 An electron micrograph of a peripheral capillary loop from the glomerulus of a patient with type II membranoproliferative glomerulonephritis. Intramembranous electron-dense deposits are present throughout the widened basement membrane (magnification ×9,650). (From Lamb V, Tisher CC, McCoy RC, et al. Membranoproliferative glomerulonephritis with dense intramembranous alterations. A clinicopathologic study. *Lab Invest* 1977;36:607, with permission.)

and in the proliferative forms of lupus nephritis. Membranoproliferative glomerulonephritis may be identified by its characteristic pattern of mesangial interposition and the electron-dense deposits that are primarily subendothelial in location in type I disease and intramembranous in location in type II disease (Fig. 13.24) when observed by electron microscopy. The proliferative forms of lupus nephritis are often characterized by the prominent variability in their histologic appearance from one part of a glomerular tuft to another and from one glomerulus to another.

Crescents

Crescents may be present in every type of immune-mediated glomerulonephritis; therefore, they are not diagnostic. Most types of glomerulonephritis associated with crescents can be identified by their characteristic patterns of immunoglobulin localization with immunofluorescence microscopy. These include the more severe forms of IgA nephropathy and Henoch-Schönlein purpura (IgA deposits), lupus nephritis, anti-GBM disease, and MPGN. Serologic studies for antineutrophil cytoplasmic antibodies (ANCA) have helped clarify the diseases with sparse immune deposits (pauci-immune glomerulonephritis) and fibrinoid necrosis or crescents.[208] These glomerular changes are indistinguishable from the glomerular involvement observed in the microscopic form of polyarteritis nodosa or Wegener granulomatosis. In the absence of a vasculitis involving the muscular arteries in the biopsy specimen, these conditions can be separated by the presence or absence of other systemic organ involvement.[209]

The association of crescents and the clinical outcome is typified by the findings in anti-GBM disease. Several authors have noted the generally benign course of anti-GBM disease in those few patients who do not develop crescents over the course of their disease even with minimal therapy.[210–212] A close follow-up is prudent in this group because, rarely, a patient has been shown to progress from noncrescentic to crescentic glomerulonephritis.[211] A graded response in renal and patient survival is dependent on the percentage of crescents. Five studies in the literature[210,212–215] had comparable results that could be combined to evaluate the relationship between the percentage of crescents in the biopsy and renal survival in a total of 133 patients. If the percentage of crescents was less than 85%, most patients (48 of 61) had independent renal function, that is, did not require renal replacement therapy, at follow-up (78%). If the percentage of crescents was 85% or greater, most patients (61 of 72) progressed to renal failure (85%) (Table 13.1). Similar results are found correlating serum creatinine values at presentation with renal survival in anti-GBM disease (Table 13.1).[210,212,214–216] There also is a correlation between serum creatinine and the percentage of crescents at presentation.[212]

In general, the presence of crescents in glomeruli is associated with a worse prognosis. Exceptions include poststreptococcal glomerulonephritis in children where the crescents may resolve without adverse sequelae.[217,218] ANCA-associated glomerulonephritis does not have as clear an association between crescents and renal survival.[208] Therefore, it is preferable to separate the various causes of crescentic glomerulonephritis for the determination of prognosis and treatment.

13.1 Survival in Anti-GBM Disease[241]

	Patient Survival		Renal Survival[a]	
	1 year	5 year	1 year	5 year
Initial creatinine < 5.66 mg/dL (500 μmol/L)	100%	94%	95%	94%
Creatinine > 5.66 mg/dL (500 μmol/L) but no dialysis	85%	80%	69%	50%
Dialysis dependent	67%	44%	5%	13%

[a]Renal survival is defined as the absence of the need for dialysis or renal transplantation.

Glomerulosclerosis

Glomerulosclerosis may be the result of scarring from a prior proliferative or immune complex lesion, or it can be primary in nature.[219] Two common examples of the latter are diabetes mellitus and FSGS. FSGS is commonly associated with hyalinosis and foam cells within the glomerular tuft and hyperplasia of the parietal epithelium in the area adjacent to the sclerosis (Fig. 13.8); however, these features may be absent in a given biopsy specimen. In the early stages of FSGS, only a few glomeruli are affected and these are usually located deep in the cortex.[220] Not infrequently, a biopsy may miss glomeruli with segmental lesions and the histology will resemble MCNS. With time, the segmental sclerosis progresses to global sclerosis and involves a greater number of glomeruli throughout the cortex. Although diabetic nephropathy and FSGS are not considered immune complex diseases, the globally or segmentally sclerotic glomeruli usually have staining for IgM and C3. Segmental glomerulosclerosis may also be the end result of any disease that progresses toward chronic renal failure. Therefore, FSGS as a primary disease must be differentiated from the end-stage process of focal glomerulosclerosis. To make a diagnosis of FSGS, one must exclude other causes of segmental sclerosis that produce hyperfiltration and secondary changes of glomerular sclerosis. The diagnosis cannot be made with certainty in the presence of advanced nephron destruction or severe vascular disease.

Capillary Wall Thickening

Thickening of the glomerular capillary walls can be seen in a variety of renal diseases. In its early stages, diabetes is characterized by thickening of the lamina densa, which may be visualized by electron microscopy. It may occur as a consequence of glomerulosclerosis and take on a wrinkled, ribbonlike appearance in association with other evidence of glomerular tuft ischemia or atrophy. Localized thickening can be seen in postinfectious proliferative glomerulonephritis because of the presence of subepithelial, humplike, immune-complex deposits. In idiopathic membranous glomerulonephritis, discrete granular immune-complex deposits are localized to the subepithelial surface of the capillary wall and are associated with subepithelial extensions of basement membrane material, referred to as spikes (Fig. 13.4C). These progress to form bridges to completely enclose the subepithelial deposits, yielding a tram-track configuration. In type I MPGN, subendothelial immune-complex deposits are often seen in association with a reduplication of the peripheral glomerular basement membrane (mesangial interposition, Fig. 13.25). Similar changes can be seen in type II MPGN, in which electron-dense material expands the original basement membrane of the peripheral capillary loop and is also associated with mesangial interposition (Fig. 13.24). In both type I and II MPGN, this combination of histologic changes can give rise to a double contouring of the peripheral portion of the glomerular capillary wall.

Regardless of the particular glomerular alteration, it is often important for a diagnostic and prognostic evaluation to note whether the lesion is segmental versus global or focal versus generalized. An example of this distinction is emphasized in the most recent classification of lupus nephritis,[221] which divided the World Health Organization class IV into segmental (class IV-S, Fig. 13.26) and global (class IV-G) subdivisions. There is a presumption or hypothesis that the segmental subclass may have a different pathogenesis, and one published study[222] shows a difference in outcome when compared with the global subclass.

Interstitial Inflammation

The differential diagnosis of interstitial inflammatory infiltrates poses some interesting diagnostic problems for the nephropathologist. Kidneys with nephron atrophy may have fibrosis and associated dense nodular lymphocytic infiltrates composed of mixtures of B cells and T cells. Interstitial infiltrates in the absence of nephron atrophy or acute glomerulonephritis suggest a primary interstitial

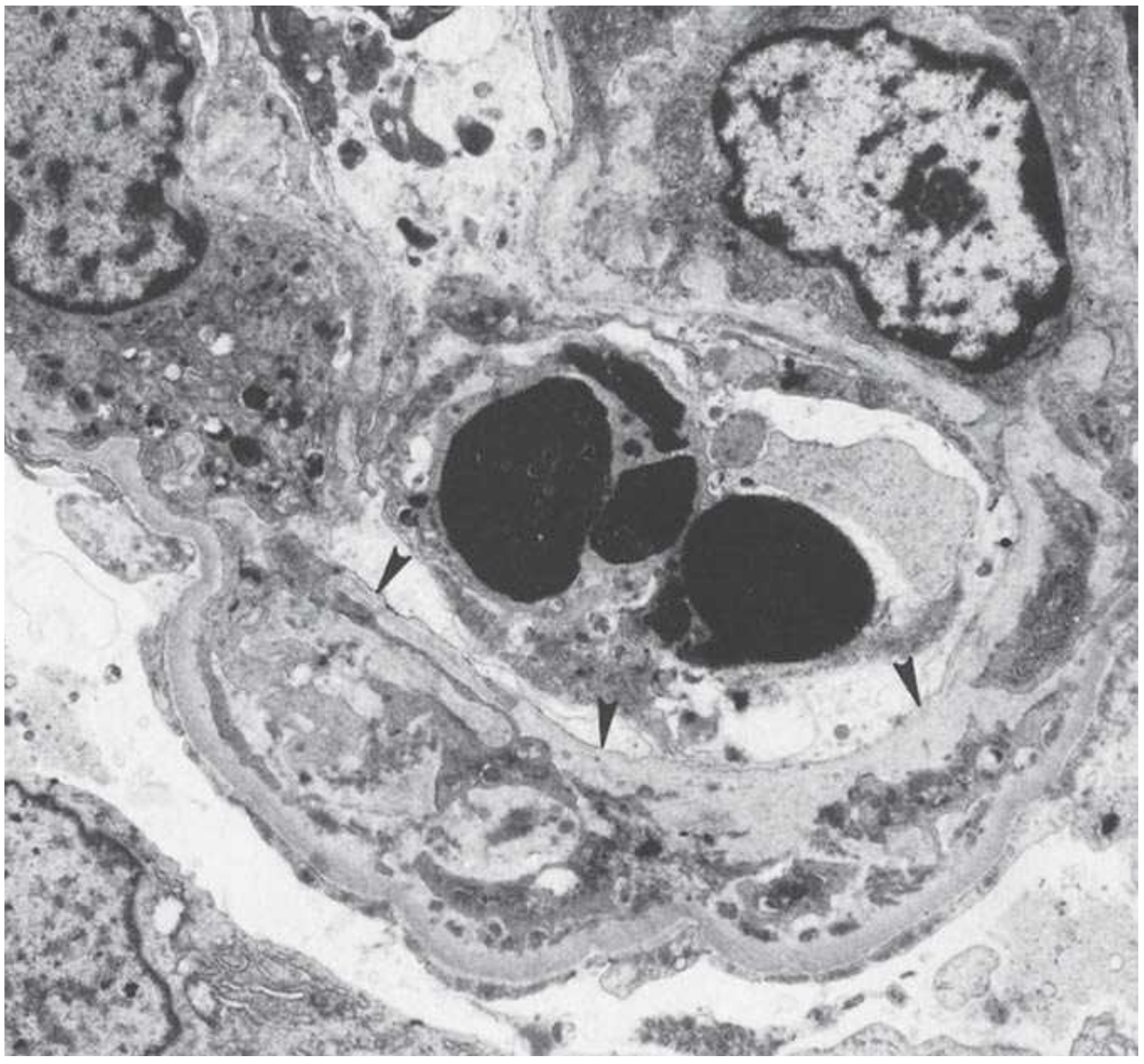


FIGURE 13.25 An electron micrograph illustrating typical mesangial interposition (*arrowheads*) in type I membranoproliferative glomerulonephritis (magnification ×3,200). (Illustration from Silva F, with permission.)

nephritis. The presence of large numbers of neutrophils in the tubules (neutrophilic tubulitis), the Bowman space, and the interstitium suggests a diagnosis of acute bacterial interstitial nephritis, whereas an occasional tubule with neutrophils may be seen in ATN. Cellular infiltrates composed of lymphocytes and plasma cells, with or without eosinophilia, commonly are the result of a drug allergy or are idiopathic in nature. Urinary outflow obstruction and renal vein thrombosis may cause interstitial edema and mild inflammation, but these changes are relatively nonspecific. Acute tubular necrosis is also associated with mild interstitial infiltrates in the region of the straight portion of the proximal tubule. A more chronic interstitial nephritis can result from an adverse response to nonsteroidal anti-inflammatory drugs. A pattern of nephron atrophy that affects the tubules before the glomeruli and vessels is consistent with, but is not pathognomonic of, chronic interstitial nephritis. Rarely, a lymphocytic lymphoma or leukemia may be seen on a kidney biopsy.

Vascular Lesions

Arteriosclerosis is the most common vascular lesion observed in kidney biopsies. It may represent a primary vascular disease such as that seen with long-standing hypertension, or it may be observed in association with progressive nephron loss that occurs with end-stage renal disease of any etiology. Extensive hyaline arteriolosclerosis suggests the presence of diabetes mellitus (Fig. 13.10). Milder hyaline lesions are seen in FSGS and, sporadically, in hypertension and other conditions. Necrotizing vasculitis is rarely seen in kidney biopsies in the absence of the glomerular lesions of polyarteritis, lupus nephritis, or Henoch-Schönlein purpura. Thrombotic microangiopathy is a distinctive lesion of small arteries and glomerular capillaries. It is characterized by an insudation of fibrin and RBCs, the latter of which is often in the form of schistocytes, into the walls of the small arteries and glomerular capillaries. The latter may appear as mesangiolysis. These acute changes often progress to renal ischemia and atrophy. This lesion can be seen in varying degrees of severity in several clinical settings, including the hemolytic–uremic syndrome, malignant hypertension, progressive systemic sclerosis, lupus nephritis, and anti-GBM disease.

Renal Allograft Pathology

Percutaneous biopsies of renal allografts introduce another set of diagnostic challenges for the pathologist. Virtually any lesion that occurs in the native kidney also can be found in the allograft in addition to the histologic picture of rejection.[137] An absence of function in the immediate postoperative period, especially in a cadaveric kidney, is usually due to ischemic tubular damage or rejection. A pathogenetic classification of rejection is given in Table 13.2, which incorporates features of immunologic mechanisms and the clinical setting. A hyperacute rejection may occur in a small percentage of patients and has a very distinctive morphology

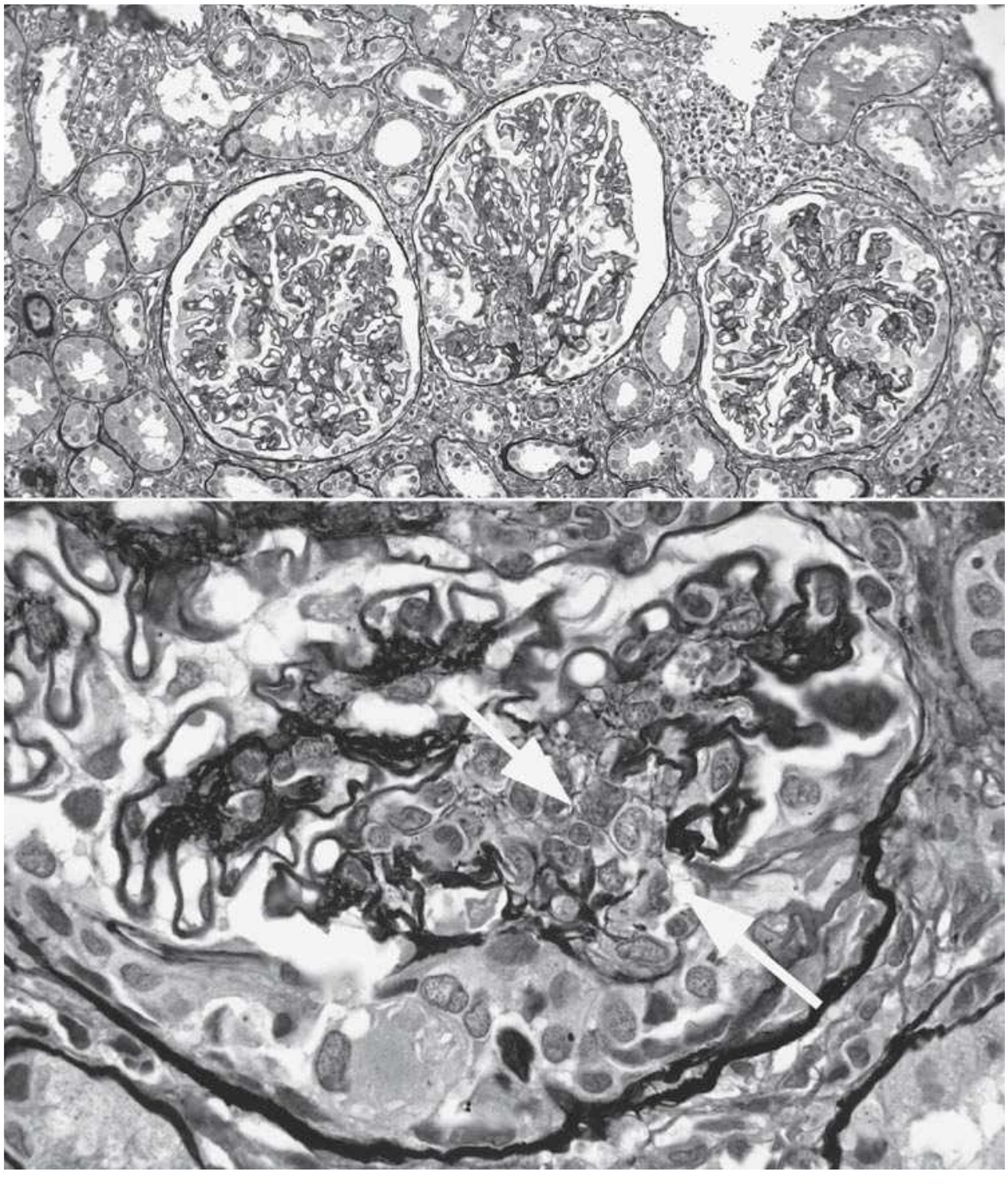


FIGURE 13.26 A segmental class IV lupus nephritis. **A:** This figure shows three glomeruli with segmental involvement (PAMS; magnification ×200). **B:** This is a higher magnification (×500) of a segmental lesion with increased cellularity and fragmentation and disruption of the GBM between the arrows (PAMS).

characterized by the presence of extensive fibrin thrombi and neutrophils in glomerular capillaries. This form of rejection is thought to be secondary to the presence of a preformed circulating antibody in the recipient that was not detected with the usual screening procedures.

Acute vascular rejection can occur from the first week of engraftment. It may be antibody or cell-mediated. It resembles the histologic picture of a low-grade vasculitis, with intimal proliferation or disruption and exudation in the walls of arteries and glomerular capillaries (Color Fig. 13.4E). The vascular lesions usually are patchy and are more frequently missed by light microscopy in biopsies with few arteries. The immunofluorescence findings of IgM, C1q, and C3 in the walls of vessels are more sensitive indicators of vascular rejection (Fig. 13.22F). Immunoperoxidase staining for the complement component, C4d (Fig. 13.27), is a marker for antibody-mediated rejection and is more widely distributed.[223] Electron microscopy reveals a swelling of the glomerular endothelium and the lamina rara interna.

Tubulointerstitial rejection is another type of acute rejection and is a form of acute interstitial nephritis. In most cases, the infiltrate is composed of large active cytotoxic T cells.[224] The cytotoxic T cells identified by immunohistochemical staining (Fig. 13.4F) infiltrate the tubular cytoplasm, a process termed emperipolesis, to produce lymphocytic tubulitis.[225] The differentiation of acute cellular rejection from other forms of interstitial nephritis can be difficult in certain settings, especially when characterized by a delayed-type hypersensitivity (DTH) response with CD4 T cells and macrophages.[198,199,225]

It is also important to note the distribution of the cellular infiltrate. Both native and allograft kidneys may have nodular infiltrates. These are often located in the adventitia of blood vessels and are composed predominantly of T4 cells and B cells. This type of cellular infiltrate is nonspecific.

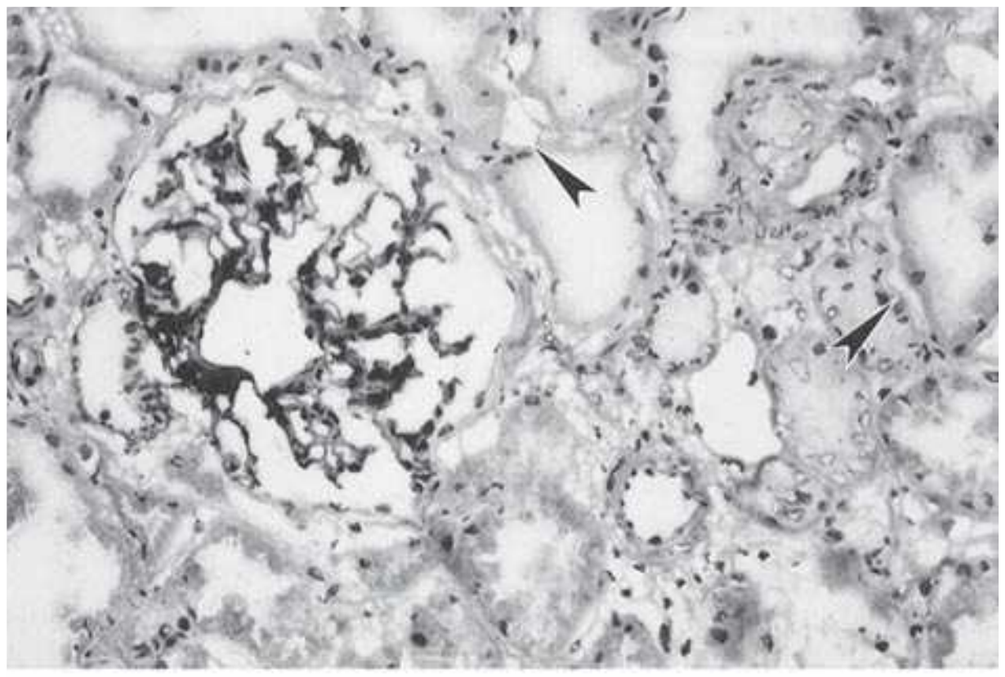
13.2 Immunopathogenic Mechanisms Associated with Renal Allograft Rejection

Type	Onset of Clinical Manifestation	Morphology	Mechanism
Acute Rejection			
Antibody-mediated (Vascular)			
Hyperacute	0–72 hr	Intracapillary PMN inflammation and thrombosis	Preformed antibody with complement and fibrinogen activation
Accelerated acute	3–7 d	Endovascular inflammation similar to above but histologically less intense	Memory antibody response
Acute rejection	7 d or more	Endovasculitis with PMN but without lymphocytes	De novo antibody response
Cell-mediated			
Vascular	7 d to 3 mo	Endovasculitis with varying proportions of CD4 + CD8 lymphocytes and macrophages	May be any of several cell-mediated mechanisms
Tubular		Lymphocytic tubulitis with predominately CD8 cells	Cytotoxic lymphocytes
Interstitial		Mononuclear interstitial inflammation with predominately CD4 lymphocytes and macrophages	Delayed type hypersensitivity
Chronic rejection (3 mo or longer)			
Vascular		Intimal proliferation	Antibody
Cell-mediated		Interstitial nephritis	Continued DTH and cytotoxic responses
Innate response			
Acute		Intrinsic cell activation	Oxidative and other stress injury
Chronic		Chronic interstitial nephritis	Macrophage activation and fibrogenesis

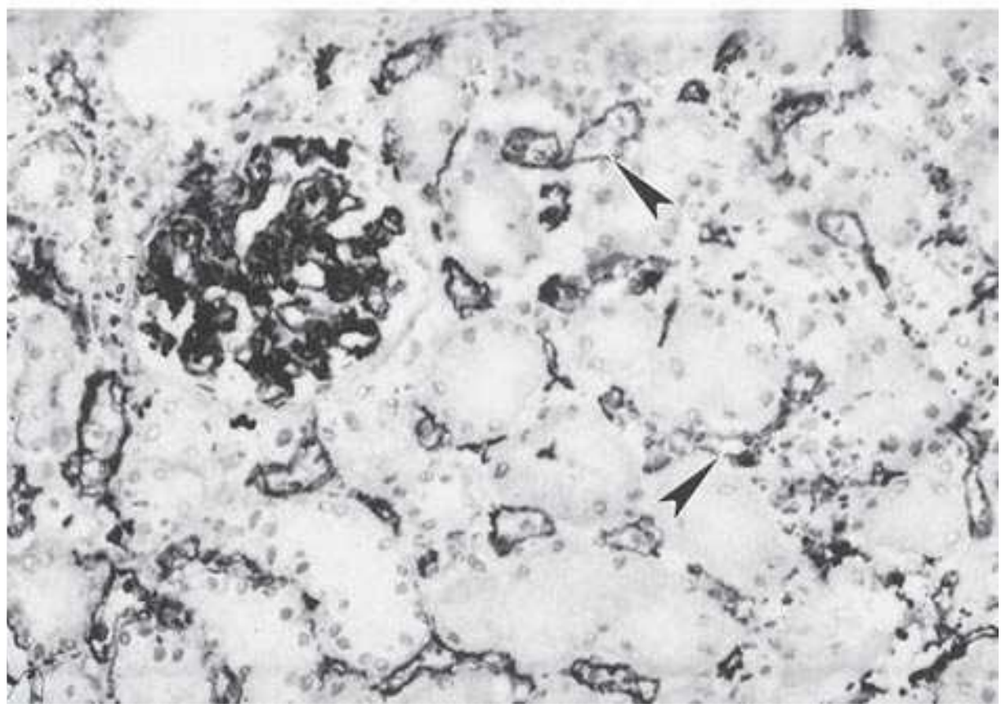
PMN, polymorphonuclear leukocyte; DTH, delayed type hypersensitivity.

In cellular rejection, allografts often exhibit a superimposed diffuse interstitial and tubular cytotoxic T-cell infiltrate.[225] An increase in cytotoxic T cells during cellular rejection has been confirmed in these infiltrates by fine-needle aspiration.[226] B-cell infiltrates suggest PTLD, particularly in the presence of EBV and destructive nodules. The BK virus also produces interstitial nephritis and is indicated by nuclear inclusions and is confirmed by electron microscopy, immunostaining, or molecular studies (Fig. 13.28).[227]

An immunohistochemical examination of the renal biopsy is also beneficial. In particular, the presence of alloantibodies, which classically localize to peritubular and glomerular capillaries, suggests the presence of antibody-mediated rejection.[126] The most commonly employed tests evaluate the presence of C4d deposition in renal transplant biopsies. Feucht et al.[228] initially showed that peritubular capillary C4d deposition predicated a worse prognosis. This was followed by numerous studies demonstrating



A



B

FIGURE 13.27 This figure shows C4d staining. **A:** An allograft with acute tubular necrosis. Peritubular capillaries (*arrowheads*) are unstained (immunoperoxidase, C4d; magnification ×210). **B:** An allograft with antibody-mediated rejection. Peritubular capillaries (*arrowheads*) are staining (immunoperoxidase, C4d; magnification ×210).

an interrelationship between C4d deposition, circulating donor-specific antibodies, and renal histopathologic findings.[126,127] Activation of the classic complement pathway generates C4b, of which C4d is a biologic fragment; C4d does not have a known biologic function, but because it is tightly bound to tissue, it serves as an excellent marker of complement-fixing, circulating antiendothelial antibodies. Peritubular capillary C4d deposition can be seen in hyperacute rejection, acute humoral rejection, and chronic humoral rejection.[126,127] In addition, C4d deposition can be observed in the kidneys of patients who received transplantation across ABO barriers, using special protocols to deplete naturally occurring anti–blood group antibodies, in whom circulating anti–blood group antibodies return but do not cause obvious graft rejection,[126] a state termed accommodation.[229] In addition, C4d deposition can occur in a number of different conditions, including I/R injury, necrosis, and lupus nephritis.[129–133] Correlating findings of C4d deposition with the clinical presentation, the renal histology, and the serologic evidence of anti-donor HLA antibodies is essential to guide appropriate therapy.

Calcineurin inhibitor toxicity is an important cause of decreased renal function in the allograft. The tubular changes were noted in an earlier section (Fig. 13.14). The most widely accepted demonstrations of calcineurin inhibitor-related renal vascular changes have been observed in the setting of transplantation of solid organs other than the kidney (e.g., the heart)[230] or inflammatory diseases of other organs (e.g., type I diabetes).[231]

Although acute changes in renal function are established in many systems,[232] the current data suggest that morphologic changes associated with long-term calcineurin inhibitor use are characteristic, but are not pathognomonic, of this class of drugs.[233] Those features include hyalinosis of arterioles (Fig. 13.29), interstitial fibrosis, and tubular atrophy.[230,231,234] When these lesions are noted in the allograft biopsy, calcineurin inhibitor toxicity should be considered in the differential diagnosis.

Systemic cytomegalovirus infection continues to be a significant cause of morbidity and mortality among recipients of renal transplants.[157] Cytomegalovirus may be detected in the biopsy, whereas viremia is determined best by peripheral blood studies.

Traditionally, rejection has been defined as an immune response directed against the graft antigens or alloimmune response. In more recent years, it has become evident that a

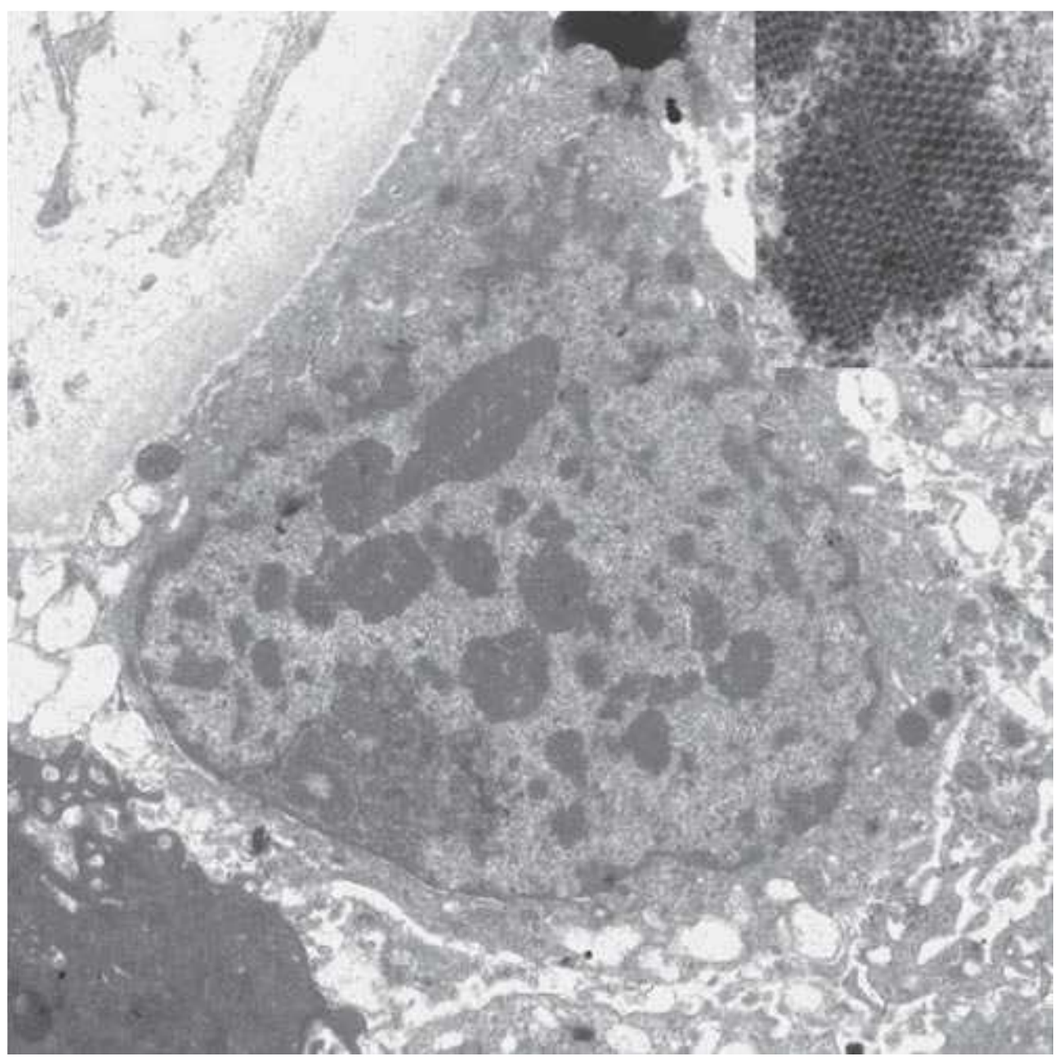


FIGURE 13.28 An electron micrograph of a tubular epithelial cell showing coarse clusters of nuclear material. The identification of the virus is not obvious at this magnification (magnification ×7,500). **Inset:** A higher magnification of nuclear densities (magnification ×33,000) showing the polyoma virus in a crystalloid array.

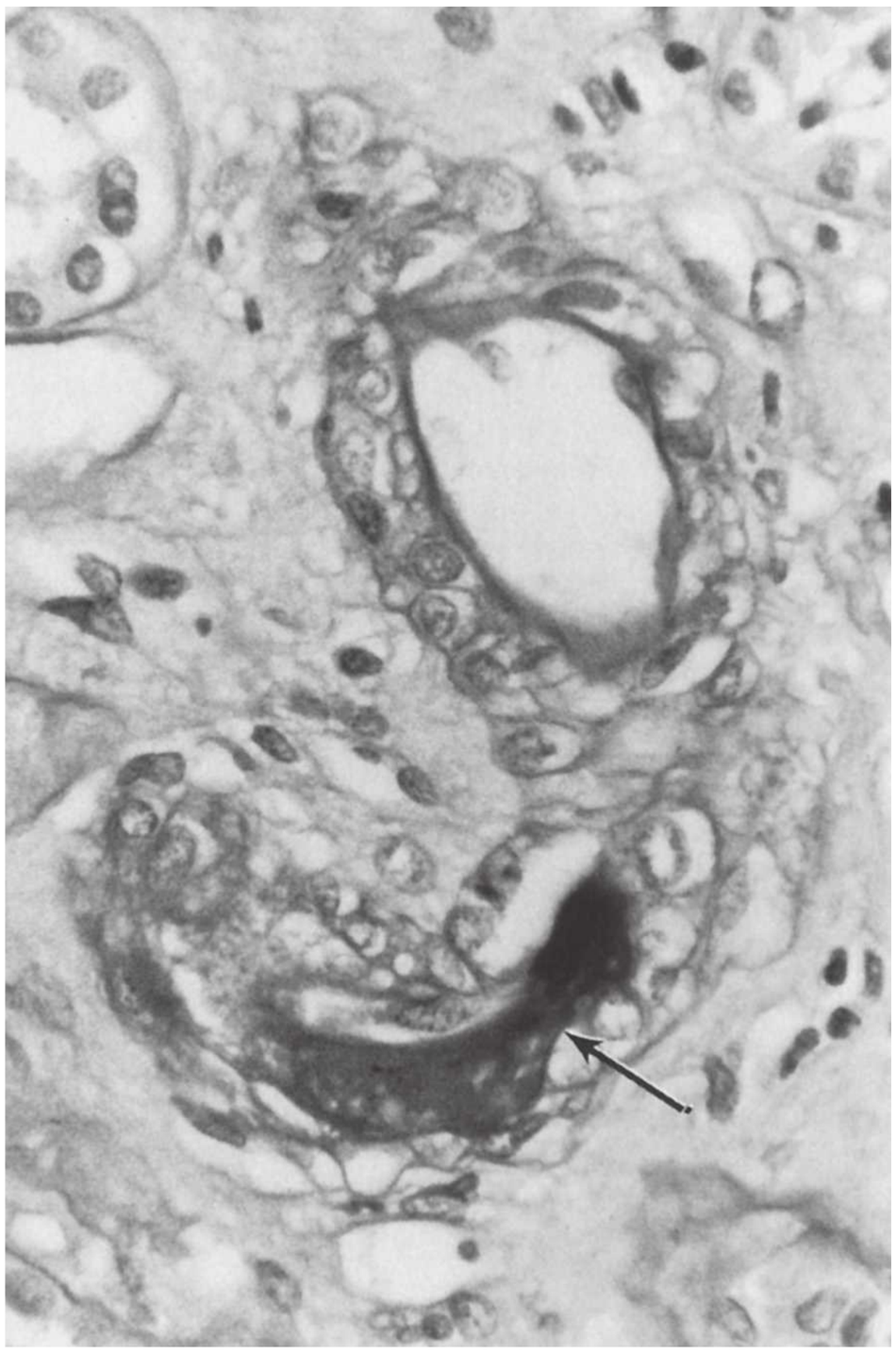


FIGURE 13.29 A photomicrograph illustrating calcineurin inhibitor toxicity in a terminal interlobular artery. Degenerative changes are evident in the muscularis in association with a large dense hyaline deposit (*arrow*) (PAS; magnification ×750).

variety of factors affect graft survival that are unrelated to traditional antigen driven B- and T-cell responses. They include donor factors such as age and gender, and events coincident to organ procurement such as cadaveric donation and ischemia time.[235,236] The kidney response in these settings recapitulates the responses of innate immunity (Table 13.2).

Finally, late irreversible changes in the graft that are associated with chronic azotemia may represent the sequelae of chronic rejection. This is not necessarily a separate entity, but may be the result of repeated episodes of acute rejection and innate immunity. Therefore, the term chronic transplant nephropathy is preferred, rather than chronic rejection, to identify the nonspecific pathologic changes. The morphologic features are those of severe tubular atrophy, glomerulosclerosis, interstitial fibrosis, and arteriosclerosis with varying degrees of chronic inflammation.

In addition to the pathogenetic classification (Table 13.2) noted in the preceding sections, several schemes for the histologic classification of rejection have been developed to facilitate interinstitutional studies and therapeutic trials. The Banff schema[237] was specifically designed toward this end and is based on light microscopic features with readily available histologic stains. With the experience of experimental validation and clinical trials, the original Banff schema was modified in 1995,[238] 1997,[239] and 2003.[128] The National Institute of Health-sponsored Combined Clinical Trials in Transplantation (CCTT) classification[240] is simpler but similar in structure for acute rejection. Clinical correlation in the CCTT study indicated that mononuclear cell margination of the vascular endothelium was an indicator for clinical severity. Although more complex, the strength of the Banff schema is that it captures fundamental histologic data. Although acute rejection continues to be a significant problem, a greater problem (as noted in the preceding section) is chronic rejection and chronic allograft nephropathy. Structural relationships remain the cornerstone for understanding these processes and developing the molecular genomic, the functional proteomic, and the genetic basis for graft failure versus survival continues. Using a classification that incorporates these elements is essential for understanding and categorizing the many features of renal allograft pathology. Any classification must also be flexible to accommodate morphologic changes that may be associated with new therapeutic measures in this rapidly changing field.

CONCLUSION

We have presented a time-tested, step-by-step approach to the use and evaluation of the kidney biopsy. An in-depth discussion of specific diseases that affect the kidney can be found in other chapters of this text. We have selected some common diseases that are amenable to a diagnosis on a kidney biopsy in an effort to demonstrate the necessity and advantage of using a combination of light, electron, and immunohistologic microscopy to obtain the maximum amount of information from a biopsy specimen to aid in the clinical management of the patient. We expect that molecular genetic, functional genomic, and proteomic studies will eventually augment the classic structural and functional approach outlined in this chapter.

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CHAPTER 14

Introduction to Genetic Renal Disease

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GENETIC RENAL DISEASE

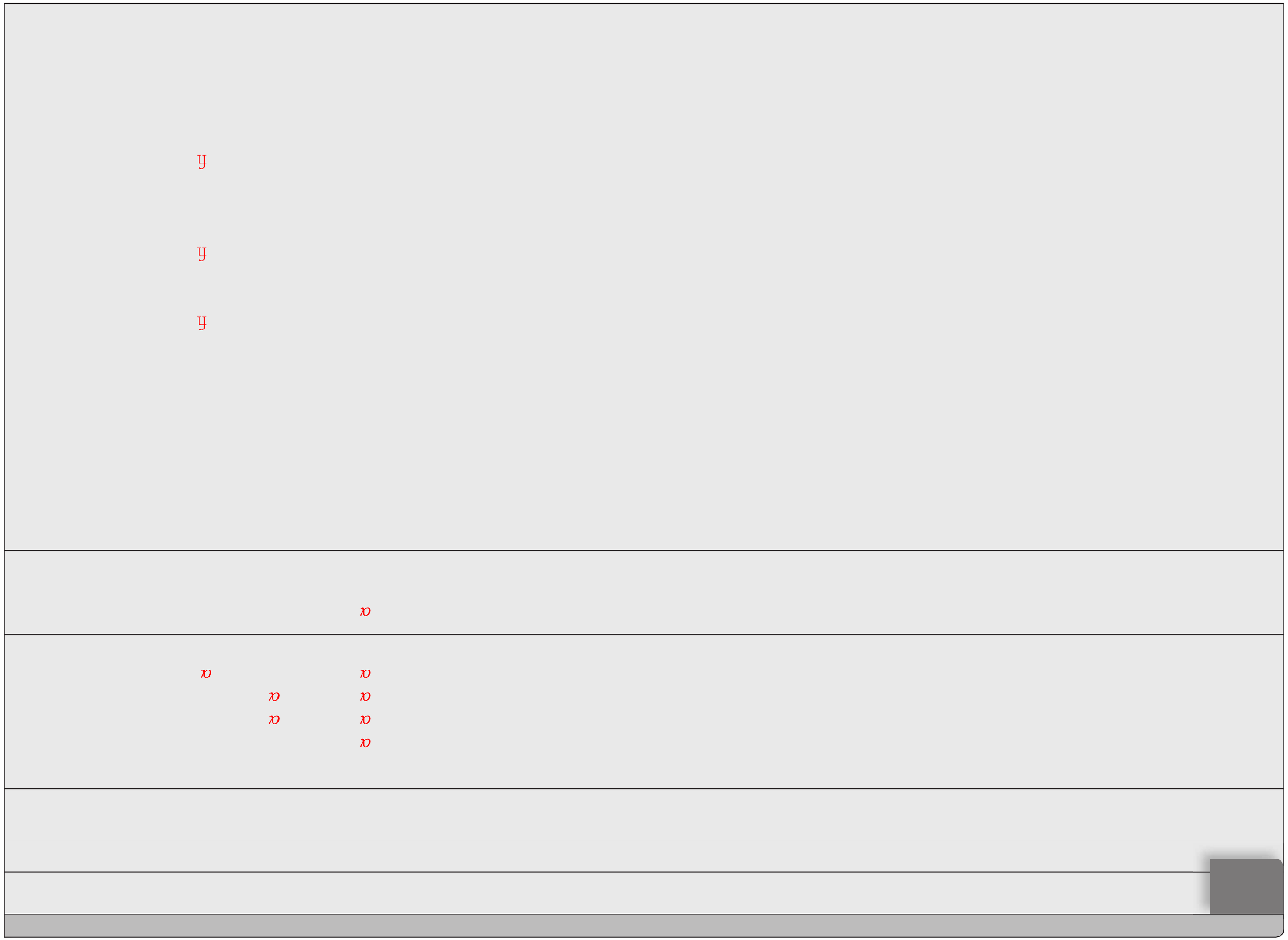
The success of the human genome project has resulted in dramatic advances in our understanding of inherited renal diseases. There has been an explosion in the number of disease-causing genes that have been identified and in our understanding of the pathogenic mechanisms that underlie these disorders (Tables 14.1 to 14.8). The spectrum of physiologic and developmental pathways that are disrupted is broad and includes defects in isolated transport mechanisms (e.g., cystinuria, primary hypomagnesemia, pseudohypoaldosteronism), defects in complex developmental pathways (e.g., autosomal dominant polycystic kidney disease [ADPKD] and renal coloboma syndrome), and defects in structural proteins (e.g., Alport syndrome and congenital nephrotic syndrome of the Finnish type). The tremendous progress in the field over the past 5 years has made an exhaustive discussion of the topic of genetic renal disease far beyond the scope of this chapter. The interested reader is referred to the Online Mendelian Inheritance of Man (OMIM) for a more complete description. This Web-based database provides a complete catalogue of diseases, their clinical features, and their molecular genetics (http://www.ncbi.nlm.nih.gov/Omim/). Instead, we will describe the process of gene discovery and how that process has evolved over the past several years. We will then review some of the scientific tools that have been applied in the postcloning stages of gene discovery in order to understand important aspects of renal biology. Finally, we will consider the clinical implications of these insights and how they may ultimately be applied in patient care.

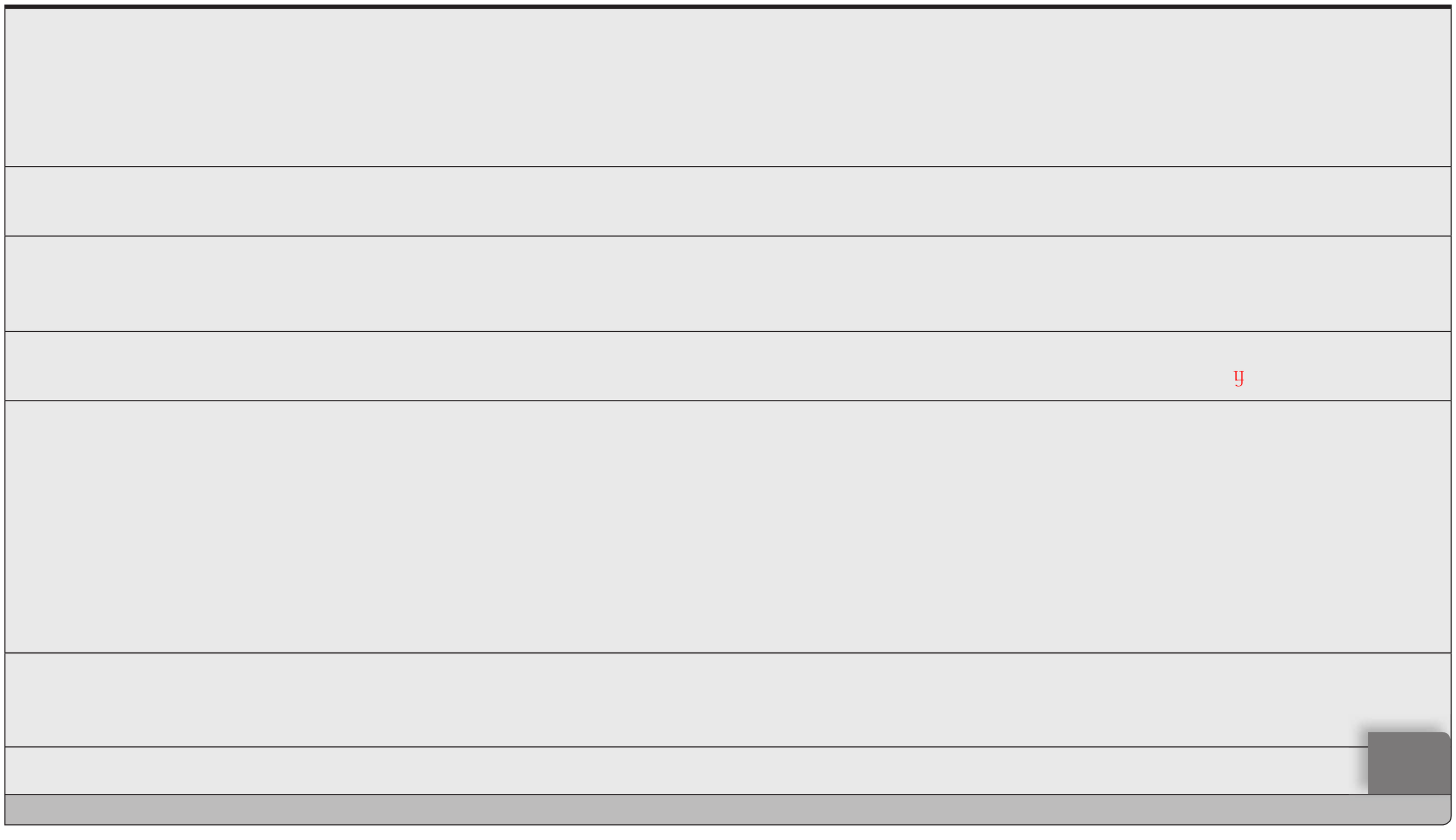
GENE IDENTIFICATION

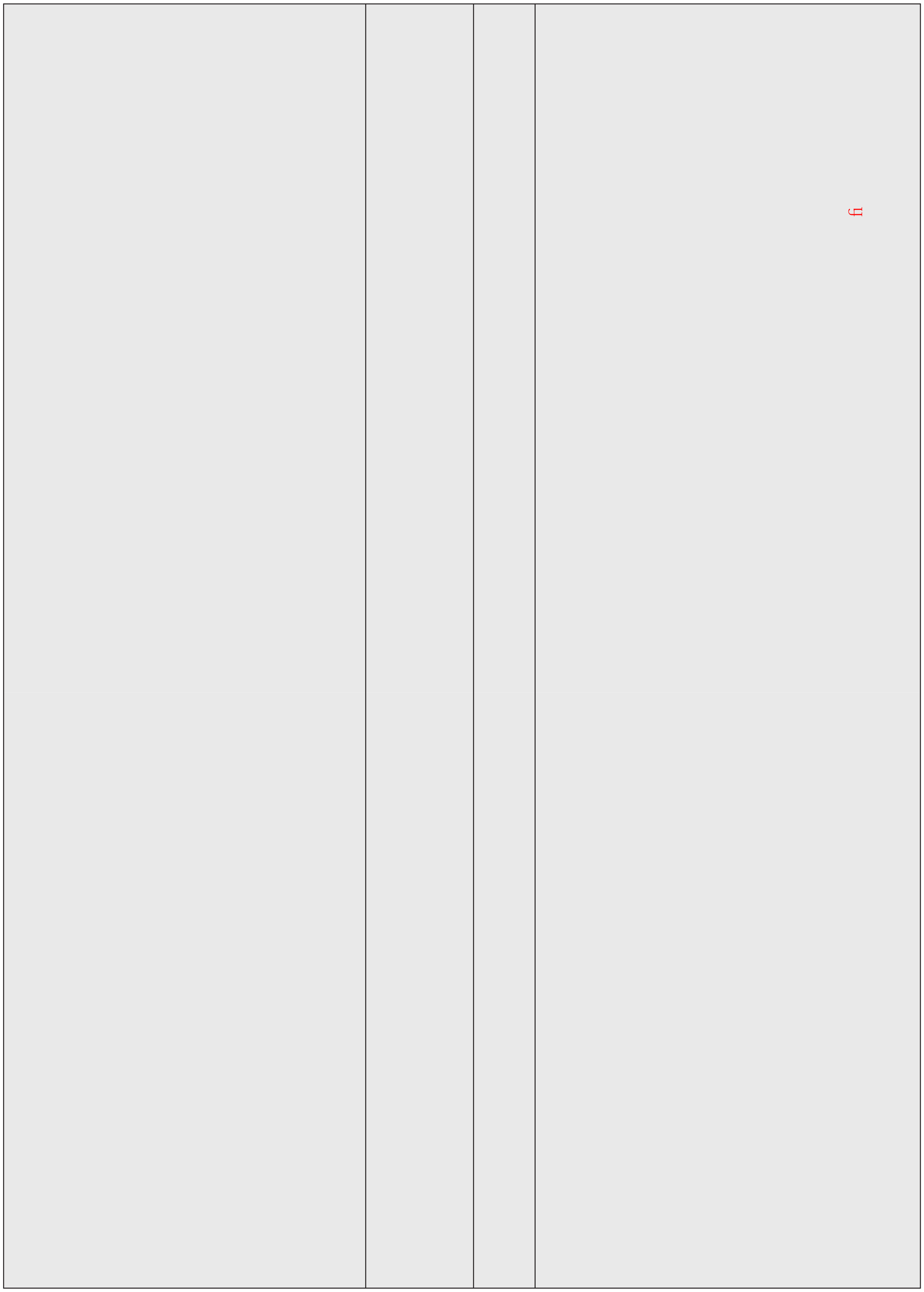
The basis of any inherited disease is an underlying alteration in genomic DNA that is transmitted from parent to offspring. Theoretically, one could compare the entire genomic sequence of an individual affected with a particular disease to that of unaffected individuals in order to identify the pathogenic difference. As simple as this approach sounds, it has, until recently, been a daunting challenge due to the size of the diploid human genome (6×10^9 base pairs [bp]), the limited throughput of traditional DNA sequencing methods (10^6 to 10^7 bp per day), and the high degree of normal variation in human populations. Important technical and bioinformatic developments have greatly changed the genetic disease discovery landscape, however. Whole genome sequencing is now a reality, and the <$1,000 genome will soon be here. Enigmatic clinical disorders that were once deemed too difficult to study using conventional positional cloning approaches because of their rarity are now giving up their secrets. In this section, we will briefly review the history of renal disease gene discovery, highlighting some of the most illustrative examples, and then discuss the impact of next generation sequencing on this field.

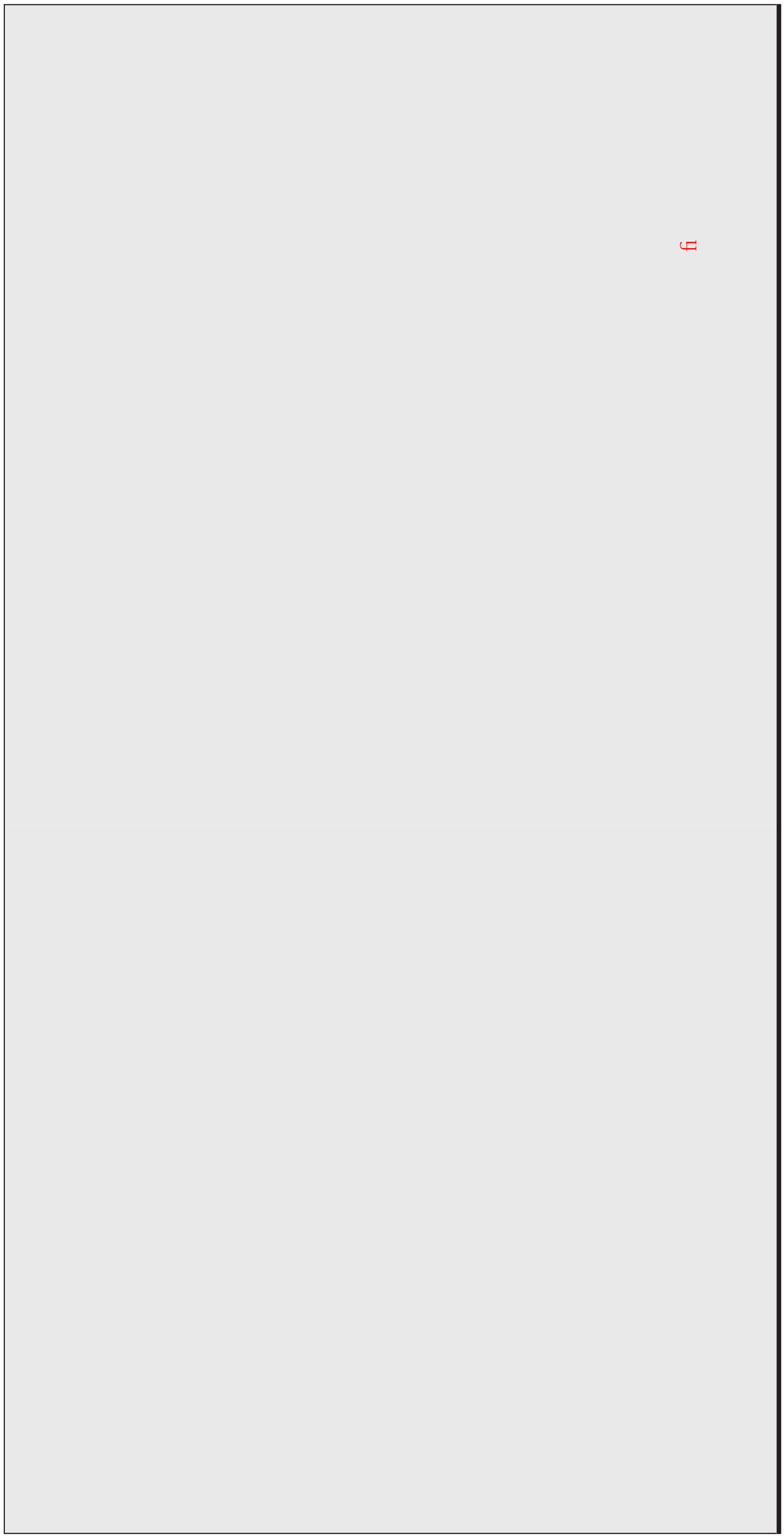
Prior to the easy availability of inexpensive DNA sequencing, multiple approaches had been developed to facilitate disease gene discovery. With some disease entities, a broader understanding of underlying pathogenic mechanisms had enabled the identification of potential "candidate genes." Alport syndrome is an example of the successful application of this approach.[1] Biochemical analysis of the Alport glomerular basement membrane (GBM) identified a set of α chains of type IV collagen that were missing.[2–5] Molecular techniques were then used to clone the type IV collagen genes, and mutation analyses revealed that sequence variants in a subset of these genes segregated with the disease. In a similar manner, the recognition that individuals suffering from the infantile form of Bartter syndrome have a clinical presentation similar to that of patients on loop diuretics prompted investigators to evaluate the drug's target, the Na-K-2Cl cotransporter (SLC12A1), as a probable candidate gene. As predicted for this recessive disease, inactivating mutations were found in both alleles in a subset of families.[6]

For other disorders, the underlying gene defect was identified by expression cloning of candidate genes. In this approach, one identifies genes responsible for a particular function by the transfer of genetic material into cells that lack that function and then screening for activity. Typically, multiple pools of genes are used for the initial screening and then the search is focused on only those pools that demonstrate the









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desired activity. By using a reiterative process of serial dilutions and functional testing, one can ultimately identify the gene or genes responsible for the observed activity. Finally, the cloned candidate genes are scanned for sequence differences (mutations) that segregate with disease. This approach has been termed expression cloning and has been used most successfully to identify various transporters. The genes implicated in cystinuria, SLC3A1 and SLC7A9, were identified by this approach.[7–11] Likewise, the three subunits that comprise the epithelial sodium channel, ENaC, were isolated in this manner.[12] Inactivating mutations of each of the subunits have been associated with recessive forms of pseudohypoaldosteronism type I,[13,14] whereas activating mutations of either the β or γ subunit have been found in Liddle syndrome, an autosomal dominant form of hypertension.[15,16]

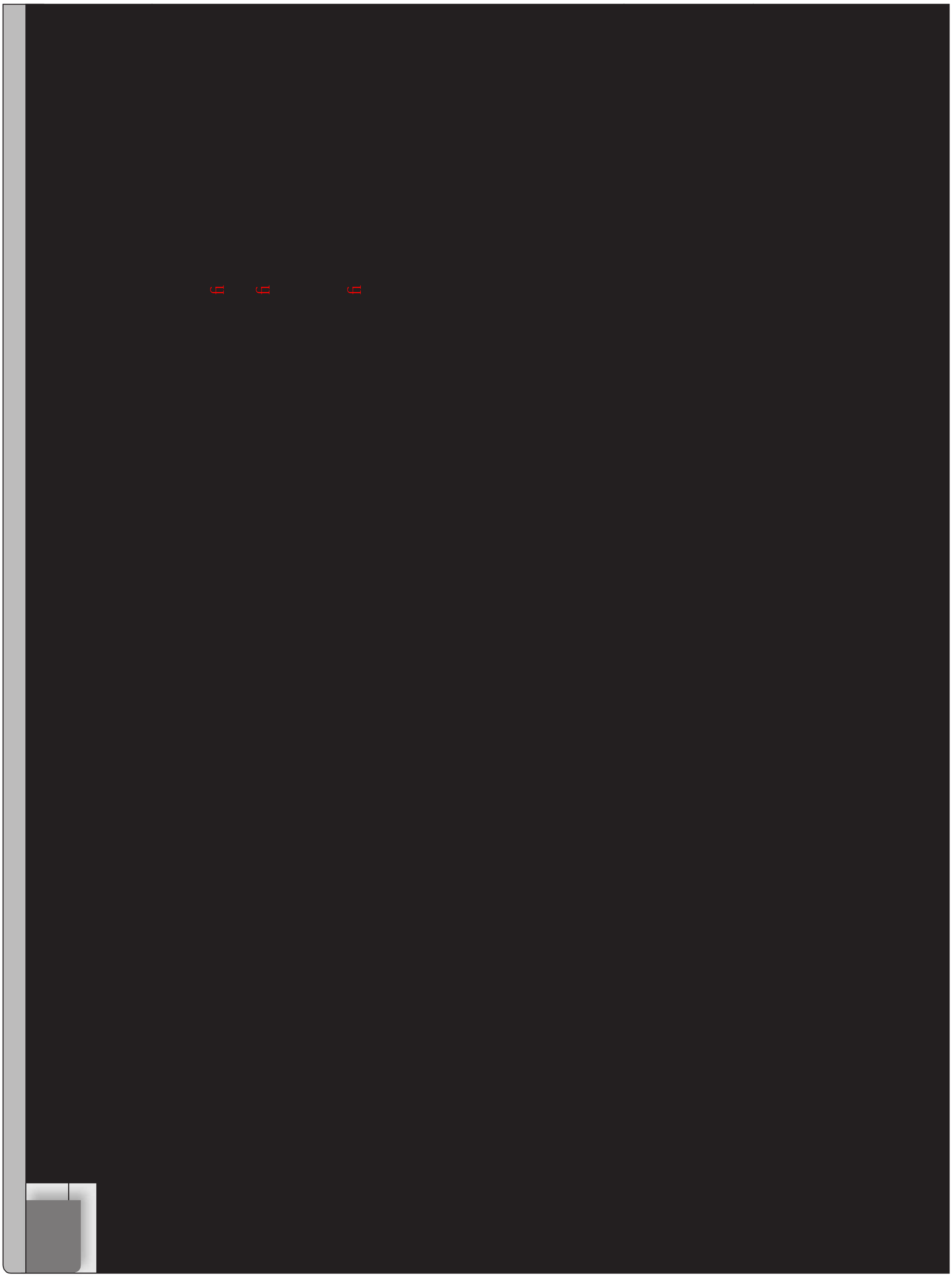
Each of the prior approaches requires an in-depth understanding of the underlying pathobiology of the disease for its success. Unfortunately, we lack this information for most diseases. This necessitated the use of a strictly molecular genetic approach, termed positional cloning, which seeks to identify a disease gene solely on the basis of its chromosomal location.[17]

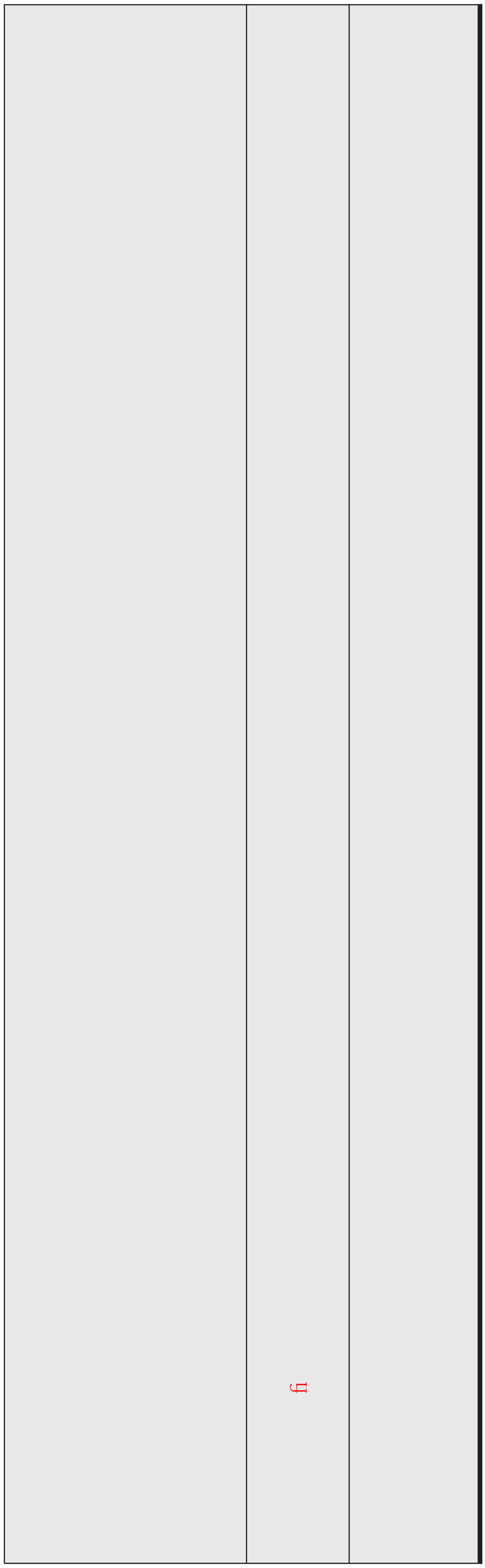
In some cases, important positional clues were provided by a cytogenetic analysis of affected individuals. Several of the loci involved in the origin of renal tumors were identified by this approach (Table 14.4). The gene responsible for the major form of Wilms tumor, WT1, was initially discovered because of its involvement in WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation).[18–20] Individuals affected by this disorder were found to have constitutional deletions of 11p13 involving a zinc finger transcription factor, WT1, a paired box transcription factor (Pax6), and adjacent DNA sequences. Fine mapping proved that WT1 was responsible for the genetic susceptibility to Wilms tumor, whereas Pax6 was responsible for the aniridia phenotype.[21] One of the familial forms of nonpapillary renal cell carcinoma (RCC) also was identified on the basis of its underlying chromosomal rearrangement. A translocation between chromosomes 3p and 8q (t[3;8][p14.2;q24.1]) was found to segregate with renal cell carcinoma.[22] Nearly 2 decades later, investigators identified the genes disrupted by the translocation. They determined that the chromosomal rearrangement resulted in a novel gene that consisted of 5′ elements of a gene called FHIT (fragile histidine triad gene) fused to the coding sequence of TRC8.[23] The protein product of TRC8 has high homology to the basal cell carcinoma/segment polarity gene product, Patched, a signaling receptor, suggesting that it may have a similar function. Since that time, 11 further chromosome 3 translocations have been associated with a susceptibility to RCC, including several that disrupt candidate tumor suppressor genes such as LSAMP and NORE1.[24]

Perhaps one of the most striking examples of the power of cytogenetic abnormalities to expedite gene discovery is that provided by the search for PKD1, the gene responsible for the most common form of ADPKD. A combination of molecular and genetic techniques had rapidly localized the gene to a 500 kilobase (kb) gene-rich segment, but the lack of known chromosome rearrangements or deletions coupled with the large number of potential candidate genes greatly complicated the search.[25,26] Several years of mutation screening had failed to determine which one of the many candidates was in fact PKD1 when an astute clinician identified an unusual family that had individuals with classic ADPKD as well as a child with both tuberous sclerosis and renal cysts. Because it was known that a major form of tuberous sclerosis (TSC2) was located near the PKD1 gene,[27] cytogenetic studies of the family were undertaken. This revealed two individuals in the family with balanced translocations between chromosomes 16 and 22 (t[16;22][p13.3;q11.21]).[28] The child with TSC2 had an unbalanced karyotype and was missing a portion of chromosome 22 as well as the telomeric portion of chromosome 16 (45XY/-16-22+der[16][16qter-16p13.3::22q11.21-22qter]). It was correctly speculated that the TSC2 gene was located in the portion of chromosome 16 that was lost while PKD1 was likely to be the gene bisected by the translocation breakpoint. This was confirmed by additional studies and resulted in the identification of both TSC2 and PKD1.[28,29]

Although chromosomal rearrangements are incredibly helpful when associated with disease, they are uncommon. Therefore, most gene searches began using linkage-based methods. Linkage analysis requires both well-characterized pedigrees and an array of genetic markers. Genetic markers are DNA variants that differ within the normal population in their length or sequence and can be used to trace inheritance of parental chromosomes within families. The principle underlying this approach is as follows: a genetic disease is assumed to be the clinical manifestation of a DNA mutation. Therefore, one can identify the location of the mutant gene by comparing the segregation of the disease phenotype with a battery of genetic markers. Alleles of loci (a specific chromosomal address of a DNA segment) on different chromosomes will appear to segregate randomly, whereas alleles of loci physically close on the same chromosome are inherited together and are linked. Meiotic recombination produces novel haplotypes by exchanging alleles between homologous chromosomes and the frequency with which this occurs depends in part on the distance separating them. In other words, if the alleles of two genes are adjacent to each other on the same chromosome segment, there will be no recombination between them. Statistical programs are used to score the probability that an observed association has not happened by chance. The most common approach determines the ratio of the probability of the observed associations assuming linkage to that of no linkage. The LOD score is the decimal logarithm of this ratio and is considered significant when greater than 3.

Until recently, it was necessary to clone the chromosomal interval in question, identify its genes, and then screen them for mutations. These steps often took many years to complete. The human genome project revolutionized this

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process by virtually providing the complete, annotated sequence of the human genome in public databases and producing dense genetic maps that included over a million single nucleotide polymorphisms (SNPs). SNP-chips (which query hundreds of thousands of SNPs in a single hybridization) have facilitated rapid genetic localization of disease loci, and the genomic maps have provided a comprehensive set of all potential candidate genes. Despite these advances, the task of identifying a disease-associated gene still remained a time-consuming endeavor prior to the widespread availability of high-throughput sequencing. The reason for this is that the resolution of genetic mapping is typically on the order of 500,000 to 1,000,000 bp. With an average gene density of one gene per 30,000 bp in gene-dense regions, a segment of this length could harbor between 30 to 40 genes! For diseases that are uncommon, the small number of family members available for testing often limited the resolution of genetic mapping to an area millions of base pairs in length.

Several shortcuts were used to minimize the level of effort. The first strategy was to use publicly available databases to determine the identity and likely function of genes within one's target region. One could use a modest understanding of the pathobiology of a disease to narrow the field of candidates to a set whose functions were consistent with the underlying defect. For example, linkage studies revealed that one of the loci responsible for hereditary papillary renal carcinoma (HPRC) mapped to an interval on 7q31 that included the MET proto-oncogene. The well-established relationship between MET and other carcinomas prompted the investigators to focus their search on this gene, leading to the rapid discovery of pathogenic mutations.[30] In another example, Dent disease was known to be an X-linked disorder often associated with microdeletions of Xp11.[22.31] Fisher et al.[32] initiated their search for the gene responsible for Dent disease by screening for expressed sequences that were encoded by the deleted segment. They identified a novel chloride channel family member that was deleted in many patients with Dent disease, that had a restricted pattern of expression, and whose function was consistent with the pathophysiology of the disease. They subsequently showed that mutations of CLCN5 were responsible for this disease.[33] In the case of ADPKD, investigators seeking the identity of PKD2 determined that one of the candidate genes in their genetic interval had homology to the gene product of PKD1, polycystin-1. This gene was an obvious candidate for PKD2, and mutation analysis quickly confirmed this suspicion.[34]

One of the most interesting examples of this approach was its use to identify a novel locus for Bardet-Biedl syndrome (BBS). Investigators had determined that this rare, autosomal recessive disorder, which is characterized by obesity, mental retardation, anosmia, fibrocystic renal disease, congenital hepatic fibrosis, and left/right axis defects, was genetically heterogeneous. A number of loci were known, and investigators had found that their respective gene products were localized to either the basal body and/or the primary cilium. The overlap in clinical features between BBS and other diseases that result from dysfunction of ciliary proteins gave rise to the idea that candidate genes for BBS and other similar diseases could be identified based on this property. Scientists compared the complete genomic sequence of multiple species that have cilia to those that do not, and identified a set of genes exclusively present in organisms with cilia and basal bodies. Two of the genes mapped to a previously defined interval for BBS5 that contained 230 predicted genes. Molecular testing identified mutations in one of the two genes in BBS5 families.[35] The locus encodes a novel protein of unknown function that would have otherwise been a low priority candidate for further study.

A second approach that had been used to speed the pace of gene discovery was to search for disease-associated microscopic chromosomal abnormalities that were below the level of resolution of standard cytogenetic analyses. The genes responsible for the most common form of nephronophthisis (NPHP1) and for cystinosis (CTNS) were identified in this manner. In the case of NPHP1, large-scale rearrangements were detected in 80% of the patients belonging to inbred or multiplex NPHP1 families and in 65% of the sporadic cases.[36] Most of the time, large homozygous deletions of approximately 250 kb involving a 100-kb inverted duplication were discovered to disrupt the gene. In a small number of individuals, oligo-base pair mutations were identified, proving that NPHP1 was the specific gene responsible for the disorder.[37,38] CTNS was identified in a similar manner. Investigators found that one of the genetic markers used in their study was homozygously deleted in 23 out of 70 patients.[39] They quickly focused their search on the minimal deleted region and identified a ubiquitously expressed transcript that was disrupted in all patients with deletions involving this segment. They subsequently found single or oligo-base pair mutations in many of the remaining patients, thus proving that this gene and not one of its neighbors was in fact responsible for the disease.

A third strategy had been to determine the expression pattern of the various candidates and see if any were consistent with the clinical features of the disorder. Fuchshuber and colleagues[40] had localized a form of steroid-resistant idiopathic nephrotic syndrome (NPHS2) to a 2.5 million-base pair interval on chromosome 1. They had identified multiple putative candidates but focused their search on one whose expression by Northern blot was detectable only in fetal and adult renal tissues. They subsequently discovered recessive, inactivating mutations in NPHS2, and further showed that its expression was restricted to glomerular podocytes.[41] In a similar manner, the kidney-restricted pattern of expression of PCLN1 helped to identify it as a probable candidate gene for primary hypomagnesemia.[42]

In a number of diseases, gene discovery resulted more from good luck than from the pursuit of a particular strategy. The ability to manipulate the murine genome through gene targeting (described in more detail later) has allowed investigators to generate a lengthy list of murine models of human diseases. In most cases, scientists first identified the

disease gene and then created a mutant phenotype in the mouse with the intention of modeling the human disease state (see the subsequent text). In some cases, however, the genes targeted for study had not been previously implicated in a genetic disorder; rather, they had been selected for study because the investigators had a fundamental interest in their biologic properties. Careful analysis of the murine phenotypes revealed surprising similarity to human diseases, leading investigators to test for mutations in their human homologues.

It was in this way that LMX1B, which encodes LIM homeobox transcription factor 1β, was found mutated in Nail-Patella syndrome (NPS). Investigators with an interest in basic developmental processes had targeted this gene for inactivation and discovered limb and kidney defects in Lmx1b mutant mice that were remarkably similar to those observed in human NPS.[43] They quickly identified three independent NPS patients with de novo heterozygous mutations of LMX1B.[44] The identification of CD2AP (CD2-associated protein) as a cause of steroid-resistant nephrotic syndrome is another example.[45,46] CD2AP was thought to be an adapter protein critical for stabilizing contacts between T cells and antigen presenting cells. Mice that were null for Cd2ap had compromised immune function but died unexpectedly at 6 to 7 weeks from renal failure. The investigators showed that homozygotes developed proteinuria associated with defects in epithelial cell foot processes and eventual glomerulosclerosis. CD2AP was found expressed in podocytes where it associates with nephrin, the primary component of the slit diaphragm. Subsequent studies in humans discovered CD2AP mutations in patients with focal segmental glomerulosclerosis.[47–49]

It is likely that additional fortuitous relationships will be established, as the list of murine genes that are inactivated by gene targeting becomes more complete. The National Institutes of Health (NIH)-sponsored Knockout Mouse Project (http://www.komp.org/), in collaboration with the International Knockout Mouse Consortium (http://www.knockoutmouse.org/), is aiming to mutate all protein-coding genes in the mouse and then perform broad, standardized phenotyping on a large subset (https://commonfund.nih.gov/KOMP2/overview.aspx). These studies will likely identify additional, unsuspected candidate genes for human disorders.

Unfortunately, these approaches could not be successfully used for most genetic diseases. In these cases, one had to resort to the use of sequence-based strategies to identify the disease gene. As the reader can understand from the previous discussion, this was often a tedious, time-consuming, and expensive process. For rare diseases where the genetic interval defined by genetic linkage was on the order of millions of base pairs, gene discovery was stalled.

Breakthroughs in DNA sequencing technology have revolutionized this process. As indicated in the introduction, there has been an explosion of new high-throughput methods for determining DNA sequence. A comprehensive review of the subject is beyond the scope of this chapter and surely would be outdated by the time this volume is published. Common to all of the methods is the use of massively parallel systems that determine 10^6 to 10^8 sequence reads of ~30 to 400 bp per read in a single experiment. This is in sharp contrast to standard Sanger sequencing machines that maximally determine up to 384 sequence reads of 600 to 1,000 bp per read. Although the new methods, commonly called next generation sequencing, dramatically increase throughput, they also generally have higher error rates and require much greater levels of redundancy to maximize accuracy. Depths of coverage are routinely greater than 20×, and even with this degree of redundancy, gaps and sequence errors can occur. Most laboratories confirm variants of interest with direct Sanger sequencing because of its greater reliability.

There presently are three different approaches for using next generation sequencing for disease gene discovery. In situations where linkage studies have already localized a gene to a chromosome region, investigators use a variety of methods to "capture" the genomic interval and then subject it to next generation sequencing. When linkage is either not possible or when the investigator prefers to use a generally applicable method, he or she pursues either whole genome or whole exome sequencing. As the respective names imply, whole genome sequencing (WGS) determines the sequence of the entire genome, whereas whole exome sequencing (WES, also known as targeted exome capture) restricts its analysis to the DNA sequence of exonic sequences and flanking intron/exon boundaries for all genes. Although WGS is the most comprehensive approach because it includes all regulatory and intronic sequences, its cost is still limiting and the sheer quantity of data it produces presents significant bioinformatic challenges. WES is currently the preferred option because the exome is less than 5% of the size of the entire genome but is estimated to harbor over 85% of all disease-causing mutations.

WES has been successfully used to identify a rapidly growing list of disease genes. In the renal community, this approach was recently used to identify MYO1E (myosin 1E, a podocyte cytoskeletal protein) and NEIL1 (endonuclease VIII-like 1, a base-excision DNA repair enzyme) as new candidate genes for human autosomal recessive steroid-resistant nephrotic syndrome.[50] In another example, this approach helped to identify NPHP1 mutations as the cause of disease in two families where consanguinity mapping localized the gene to an interval of almost 30×10^6 bp, far too large to tackle with conventional Sanger methods.[51] Using a variation of this approach, Otto et al.[52] identified mutations in SDCCAG8 (serologically defined colon cancer antigen 8) as the cause of a nephronophthisis-related ciliopathy (Fig. 14.1). As the cost drops even further and the technique becomes universally accessible, there is little question that WES is destined to accelerate gene discovery for hundreds of Mendelian disorders.[53]

In summary, the combination of multiple avenues of biologic data with genetic map position has proved to be a

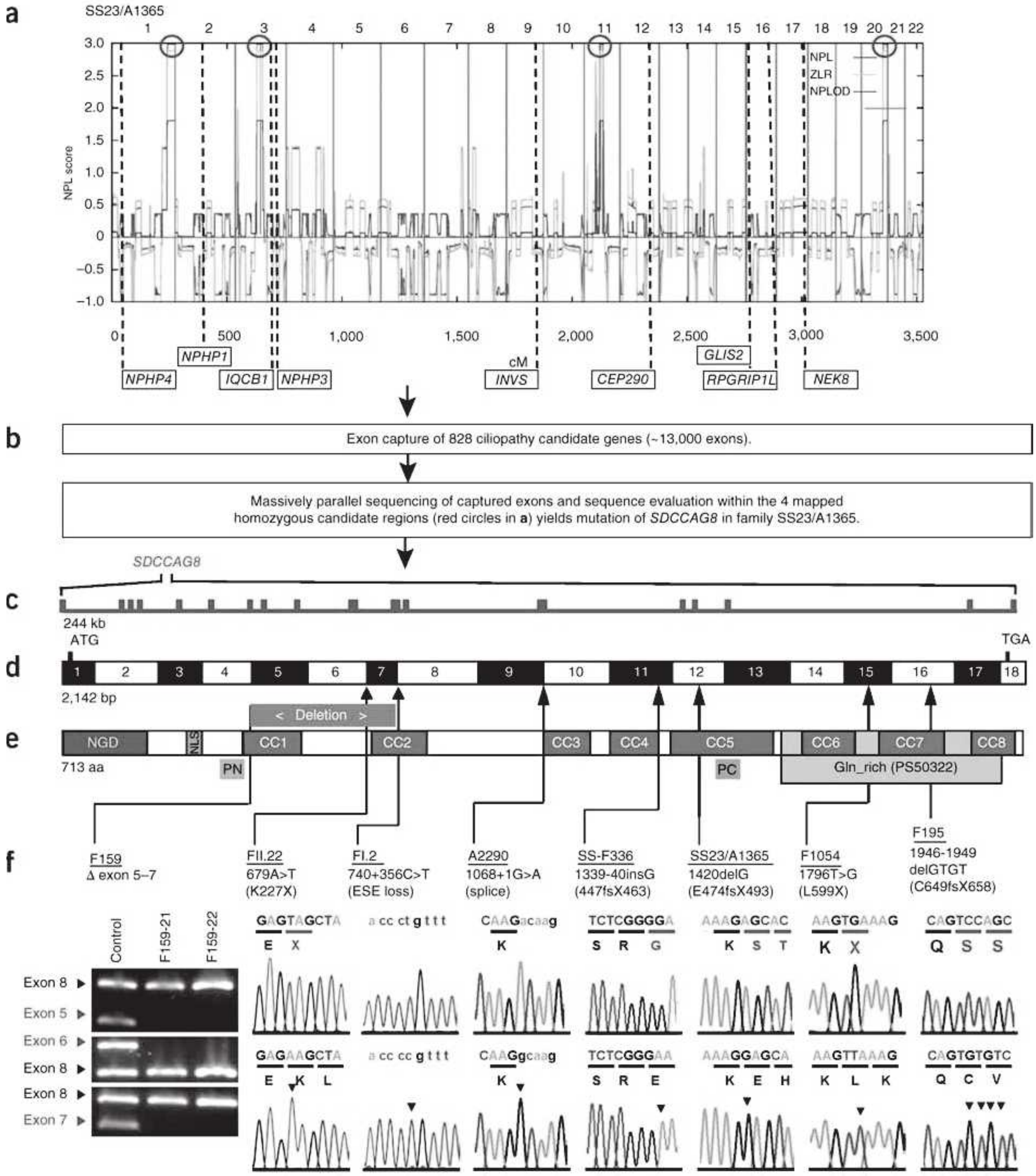


FIGURE 14.1 Homozygosity mapping, exon capture, and massively parallel sequencing identifies *SDCCAG8* mutations as causing nephronophthisis with retinal degeneration. **A:** Nonparametric log of odds (NPL) scores across the human genome in two siblings with nephronophthisis and retinal degeneration of consanguineous family SS23/A1365. The *x*-axis shows Affymetrix 250K *StyI* array single nucleotide polymorphism (SNP) positions on human chromosomes concatenated from *p*-ter (*left*) to *q*-ter (*right*). Genetic distance is given in centimorgans. Four maximum NPL peaks (*red circles*) indicate candidate regions of homozygosity by descent. **B:** Exon capture of 828 ciliopathy candidate genes with consecutive massively parallel sequencing and sequence evaluation within the four mapped homozygous candidate regions (*red circles* in image **A**) identifies mutation of *SDCCAG8* in SS23/A1365. **C:** The *SDCCAG8* gene extends over 244 kb and contains 18 exons (vertical hatches). **D:** Exon structure of human *SDCCAG8* cDNA. Positions of start codon (ATG) and of stop codon (TGA) are indicated. For mutations detected, *arrows* indicate positions relative to exons and protein domains. **E:** Domain structure of the SDCCAG8 protein. NGD, N-terminal globular domain, NLS, nuclear localization domain, CC, coiled-coil domains, and Gln_rich, glutamine-rich region. PN and PC denote peptides used for antibody generation. **F:** Eight homozygous *SDCCAG8* mutations detected in eight families with nephronophthisis and retinal degeneration. Family number, mutation, and predicted translational changes are indicated. A homozygous deletion covering exons 5 through 7 is demonstrated by agarose gel electrophoresis (see *gray bar* in image **E**). Sequence traces are shown for mutations that are above normal controls. Mutated nucleotides are indicated by *arrowheads* in traces of normal controls. (From Otto EA, Hurd TW, Airik R, et al. Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. *Nat Genet*. 2010 Oct;42(10):840–850. Reprinted by permission from Macmillan Publishers Ltd.) (See Color Plate.)

powerful strategy for finding disease genes. Hence, we have witnessed a remarkable increase in the number of inherited diseases the genetic bases of which have been elucidated.

POSTCLONING PHASE OF GENE DISCOVERY

Using Databases

Identification of a gene is often when the work of defining the biology of a disease begins. This is especially true in diseases such as ADPKD or tuberous sclerosis where a complex phenotype exists and a unifying biochemical defect is not apparent. The first step is usually to perform a series of database analyses, looking for sequence similarities, functional motifs, and structural features that might be used to generate a variety of testable hypotheses regarding a gene's function. In some cases, one finds that a segment of one's query has a high degree of similarity to a family of proteins of known function. In primary hypomagnesemia, the protein encoded by PCLN1 was found to have a sequence and structural similarity to members of the claudin family.[42] All other members of this family localize to tight junctions and appear to bridge the intercellular space by homo- or heterotypic interactions, suggesting a similar function for PCLN1. This result was particularly intriguing in that renal magnesium ion (Mg^{2+}) resorption occurs predominantly through a paracellular conductance in the thick ascending limb of Henle (TAL).

In other situations, one may identify homologous genes in other organisms, vertebrate or invertebrate, that have already been studied and for which a function may be known. The potential power of this strategy to help expedite the study of disease genes is highlighted by the fact that over 60% of human disease genes have a homologue in the common fruit fly, Drosophila melanogaster, and a surprisingly high fraction is even conserved in yeasts.[54–58] The TSC1 and TSC2 genes, mutated in tuberous sclerosis, are representative examples. The sequence of each gene provided little in the way of functional insight. Tuberin, the gene product of TSC2, was found to have a region of homology to the GTPase-activating protein GAP3,[28] whereas hamartin, the TSC1 protein, was homologous to a yeast protein of unknown function.[59] Given that other GTPase activating proteins had previously been identified as tumor suppressors, it was presumed that tuberin would have a similar function but the mechanism by which it would do so was obscure. Slow progress was made in defining the respective function of each TSC protein until the discovery and functional characterization of their homologues in Drosophila (discussed later). As saturation mutagenesis strategies of simpler organisms help to reveal the function of currently unknown genes, these insights will increasingly help us to better understand the human homologues.

Conservation between species is also helpful because it can be used to identify essential functional components that are hidden in otherwise featureless sequences. In the case of PKD1, for example, a comparison of the sequence of human polycystin-1 to that of the puffer fish, Fugu rubripes, two species separated by nearly 400 million years of vertebrate evolution, identified several highly conserved regions with no known sequence homologies.[60] One of these was subsequently identified as a putative 20-amino-acid heterotrimeric G-protein activation sequence.[61] Sequence conservation can also be used to help resolve disputes regarding the putative structure of a protein. In the case of polycystin-1, comparison of the human and Fugu sequences led to the identification of a set of 11 conserved probable transmembrane spanning elements,[60] subsequently confirmed by biochemical methods.[62]

Several databases use specific amino acid patterns culled from an analysis of many proteins to find certain motifs. This can be an especially powerful tool because it can identify patterns otherwise missed by direct sequence comparisons. One example of the successful use of this approach is provided by the identification of a GPS domain (G-protein coupled receptor proteolytic site) in polycystin-1.[63] This domain was first identified as the internal cleavage site for the neuronal G-protein coupled receptor, latrophilin, the putative receptor for α-latrotoxin and a member of the LNB-TM7 family of proteins.[64–66] The GPS site is a feature common to all members of the family, positioned in the extracellular N-terminus approximately 30 amino acids before the first transmembrane (TM) spanning element of each protein. In the case of latrophilin, cleavage at the site yields two fragments: a C-terminal portion that includes a short extracellular stalk followed by the 7 TM-spanning elements and an N-terminal portion that is tethered to the extracellular stalk. It is presumed that cleavage and tethering is essential for creating the active ligand binding site for α-latrotoxin or its endogenous ligands. Subsequent studies by Qian et al.[67] confirmed that the GPS site in polycystin-1 similarly undergoes proteolytic cleavage, and that mice with targeted mutation of the cleavage site (Pkd1 [V/V]) develop rapid cystic dilatation of distal nephron segments, biliary duct dilatation, and uremia.[68] Interestingly, in contrast to Pkd1 knockouts (Pkd1 -/-), Pkd1 (V/V) mice do not demonstrate embryonic lethality, proximal tubular cysts, or severe cystic expansion of the kidneys and pancreas. This finding reveals important differences in the developmental and functional roles between the uncleaved full-length and the GPS-cleaved forms of polycystin-1.[68]

Finally, a variety of algorithms can be used to identify functional protein domains. For example, there are a number of programs that can be used to predict hydrophobic areas in a protein that may signify transmembrane regions. This type of information can be extremely important in deciphering the function of a protein. In the case of PKD2, this type of analysis suggested that polycystin-2 had six transmembrane domains and resembled a known class of cation channels.[69] This hypothesis was testable, and it was

ultimately confirmed that polycystin-2 could function as a nonselective cation channel in appropriate conditions.[69]

MUTATION ANALYSIS AND THE MOLECULAR MECHANISM OF DISEASE

An important step in determining the molecular basis of disease is defining how disease mutations alter the function of the gene product. In recessive disorders, the fact that a mutation of both copies of a gene is required to cause the disease suggests that the mutations must impair or result in a complete loss of the protein's normal function. In dominant disorders, the mechanism by which mutations cause disease is less straightforward because they can act in one of three ways: (1) by reducing the amount of functional protein (via a hypomorphic allele, haploinsufficiency, or by a combination of germ line and somatic mutations); (2) by producing a protein with increased activity or a new function (dominant gain-of-function); (3) or by producing proteins that inhibit the function of the normal allele (dominant negative).

In the case of ADPKD, it was certain clinical features of the disease that provided the clue to unraveling the molecular mechanism of the disease. One of the hallmarks of ADPKD is its focal presentation.[70] Pathologic examination of cystic kidneys has shown that cysts arise as focal outgrowths of normal tubules. The logical question was that if all cells are genetically identical and carry the same germ line mutation, then why don't all cells form cysts? Considered among the many different potential explanations was the possibility that each cyst might be genetically distinct, forming as the consequence of a second, possibly rate-limiting step. By examining epithelial cells lining single cysts, it was demonstrated that renal cysts originate from one cell and thus can be termed monoclonal.[71] Furthermore, somatic mutations affecting the normal allele can be detected in a significant fraction of cysts.[72–79] This model is depicted in Figure 14.2 and suggests that the first step in cyst formation is the functional inactivation of the normal PKD allele. This model implies that PKD is recessive on a molecular level because both copies of PKD are mutated in each cyst. A small minority of cysts from kidneys with a germ line mutation in PKD2 may instead have as their somatic hit an inactivating PKD1 mutation and vice versa.[76,80] This finding has several clinical implications. First, any curative therapy must be directed at replacing the function of the gene product in cells where a "second hit" has actually occurred. Second, these findings explain at least some of the observed clinical variability because the rate at which second hits occur may determine disease severity. Clearly, an understanding of the factors that determine the rate of second hits might be used to slow disease progression. These might include processes that cause DNA instability or increase cellular proliferation.

Mutation analysis of a large number of pedigrees may also be used to establish genotype–phenotype correlations. One may discover that certain patterns of mutation are associated with specific clinical characteristics. For example, some mutations may lead to an unstable message that is never made into protein. This may have more severe functional consequences than a protein that is made but has only partially reduced activity.

Von Hippel-Lindau syndrome (VHL) provides an excellent example of the use of this approach. VHL is a dominantly inherited familial cancer syndrome caused by mutations in the VHL gene on chromosome 3.[81,82] It is characterized by a predisposition to develop renal cell carcinomas (RCC) and hemangioblastomas of the retina and central nervous system. The VHL gene is predicted to function as a tumor suppressor, and somatic mutation of the wild-type VHL allele has been detected in renal tumors from families with germ line VHL mutations.[83] In addition, up to 91% of sporadic clear cell carcinomas demonstrate somatic mutation or inactivating epigenetic changes of the VHL gene.[84] One of the most important functions of the VHL protein is in the regulation of proteasomal degradation of the hypoxia-inducible factors (HIFs),[85] which act as transcriptional regulators for a wide variety of genes important in tumorigenesis.[86,87] The VHL protein targets HIFs for degradation by binding with elongins B and C as part of a multimeric complex.[88–92]

Clinically, VHL falls broadly into two categories differentiated by their risk of pheochromocytoma, which is low in VHL type 1 (VHL1) and high in VHL type 2 (VHL2).[93–95] It turns out that these clinical phenotypes are closely correlated with the class of mutation that is detected. VHL1 is produced by mutations that completely disrupt VHL protein structure (e.g., frameshift mutations, stop codons, and gene deletions). On the other hand, VHL2 appears to be caused by missense mutations in residues that would be predicted to be in contact with elongin B.[94,96,97] Further studies in genotype–phenotype correlations have also been able to stratify risks for other manifestations of the syndrome such as RCC and hemangioblastomas based on variations in the type of amino acid change,[98] the size of the gene deletion,[99] or the presence of contiguous gene deletion.[100,101] Therefore, mutation analysis in this disease can be used both to establish a presymptomatic diagnosis and to provide specific information with respect to the disease phenotype.

Genotype–phenotype studies have recently revealed surprising relationships between three disorders previously thought to be distinct. Nephronophthisis (NPHP) is an autosomal recessive form of fibrocystic kidney disease that is one of the most common inherited causes of pediatric end stage kidney disease. Tubular atrophy and tubulointerstitial fibrosis are dominant features and cysts, if present, are mostly localized to the corticomedullary junction. Retinitis pigmentosa is present in some families. Meckel-Gruber syndrome (MGS) is a much rarer autosomal recessive, neonatal lethal disorder that classically presents with cystic kidneys, occipital encephalocele, and congenital hepatic fibrosis. Joubert syndrome (JBTS) in its typical form has little in common with either NPHP or MGS except being another rare autosomal recessive condition. Affected individuals

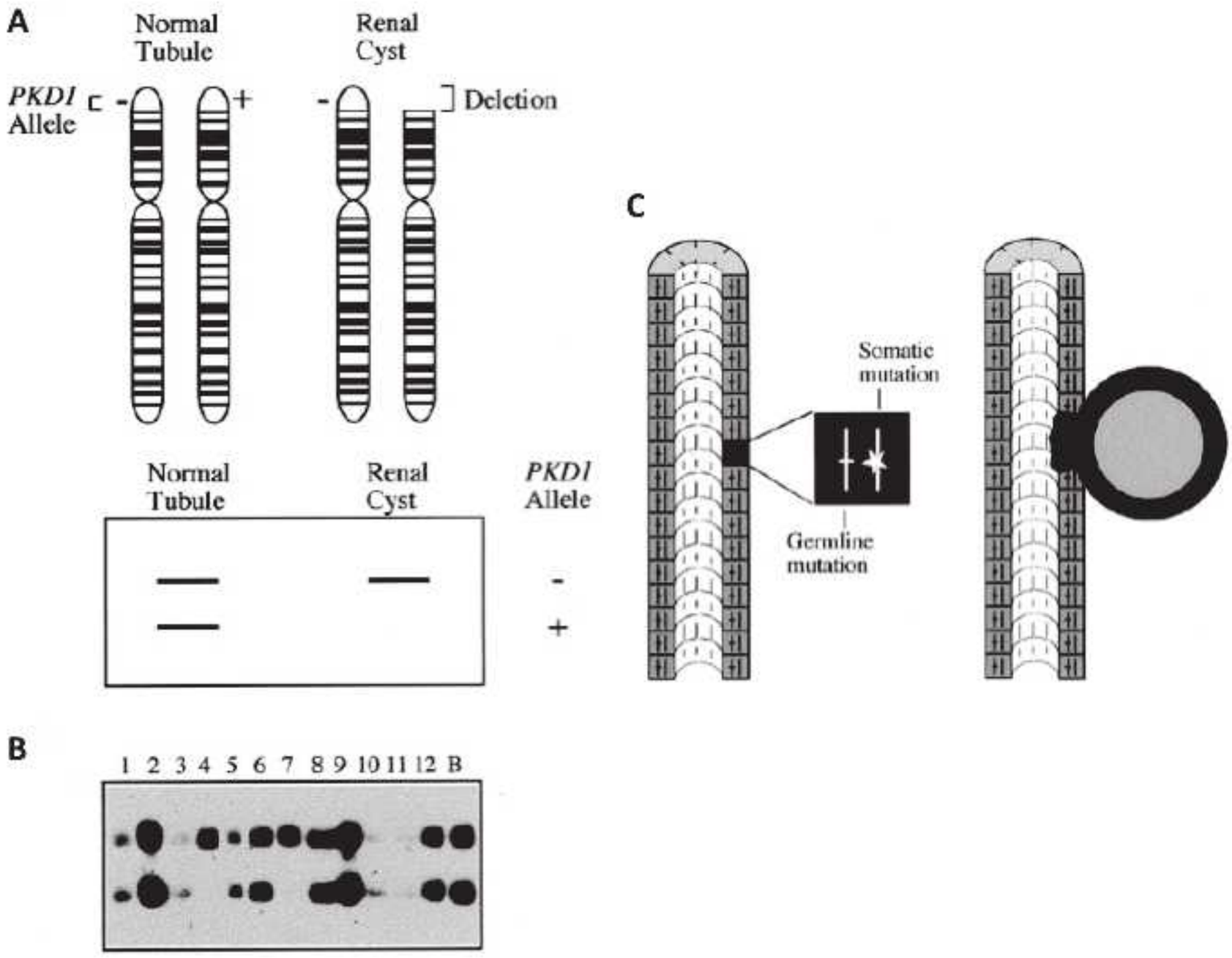


FIGURE 14.2 Loss of heterozygosity (LOH) for the normal *PKD1* allele in renal cystic tissue. **A:** Each renal tubular cell in an individual with ADPKD contains two *PKD1* alleles, one mutant copy (indicated by -) and one normal copy (indicated by +). The two alleles can be distinguished using polymerase chain reaction-based (PCR) assays if the allele-specific PCR products differ in size. Deletions of chromosome 16 that remove the normal *PKD1* locus will lead to LOH, as evidenced by the disappearance of the PCR product derived from this allele. As a result, only one band remains. **B:** Testing for LOH of the *PKD1* locus in 12 renal cysts of an individual with ADPKD using the marker KG8, an intragenic PKD1 marker. Most of the cysts have two *PKD1* alleles, but cysts #4 and #7 have only one band because each has lost the normal *PKD1* allele (*bottom band*). **C:** "Two-hit" model of cyst formation in ADPKD. Each epithelial cell in a renal tubule has the same germ line mutation of one *PKD1* allele. The normal allele presumably produces sufficient quantities of polycystin to permit a normal differentiation and the maintenance of tubular integrity. When a cell acquires a somatic mutation in the normal allele (indicated by a *star*), the level of polycystin falls below a critical threshold. The cell harboring the somatic mutation undergoes clonal expansion and forms a cyst via pathways that not yet have been identified. (From Qian F, Watnick TJ. Somatic mutation as mechanism for cyst formation in autosomal dominant polycystic kidney disease. *Mol Genet Metab.* 1999 Oct;68(2):237–242. Used with permission.)

typically present with midbrain and hindbrain anomalies and a distinctive "molar-tooth sign" on radiologic imaging of the brain.

Each of these conditions is genetically heterogeneous and, in its classic form, results from mutations at distinct loci. There is a subset of patients, however, that presents with an overlapping constellation of features of JBTS, NPHP, and MKS that includes NPHP, retinitis pigmentosa, cystic dysplasia, congenital hepatic fibrosis, or a small encephalocele. Mutations have been found in loci responsible for causing JBTS, NPHP, and MKS.[102] The diseases are now thought to represent a spectrum of disorders, which also includes BBS and orofacial digital syndrome, with phenotypic expression in part determined by the nature of the mutation.[52,103–106] The overlap of identified genetic loci and phenotypic features of these disorders is illustrated in Figure 14.3.

Mutation studies of affected individuals may also provide a database of allelic variants that can be used to guide the study of important functional domains. Missense mutations that have phenotypic consequences are particularly instructive because they identify essential amino acid residues and critical functional elements. Perhaps the best example of this is a mutation in the mineralocorticoid receptor (MR), S810L, which causes early onset hypertension in heterozygous carriers.[107] This mutation affects an amino acid that lies in the MR hormone-binding domain (HBD), which is conserved in MRs across a wide evolutionary span. In order to test the functional significance of this change, investigators expressed wild-type and mutant MRs in Cos-7 cells and were able to show that in the absence of added steroid, the mutant MR retained a significant level (27% of maximal) of activity. This suggested that the mutant receptor was constitutively

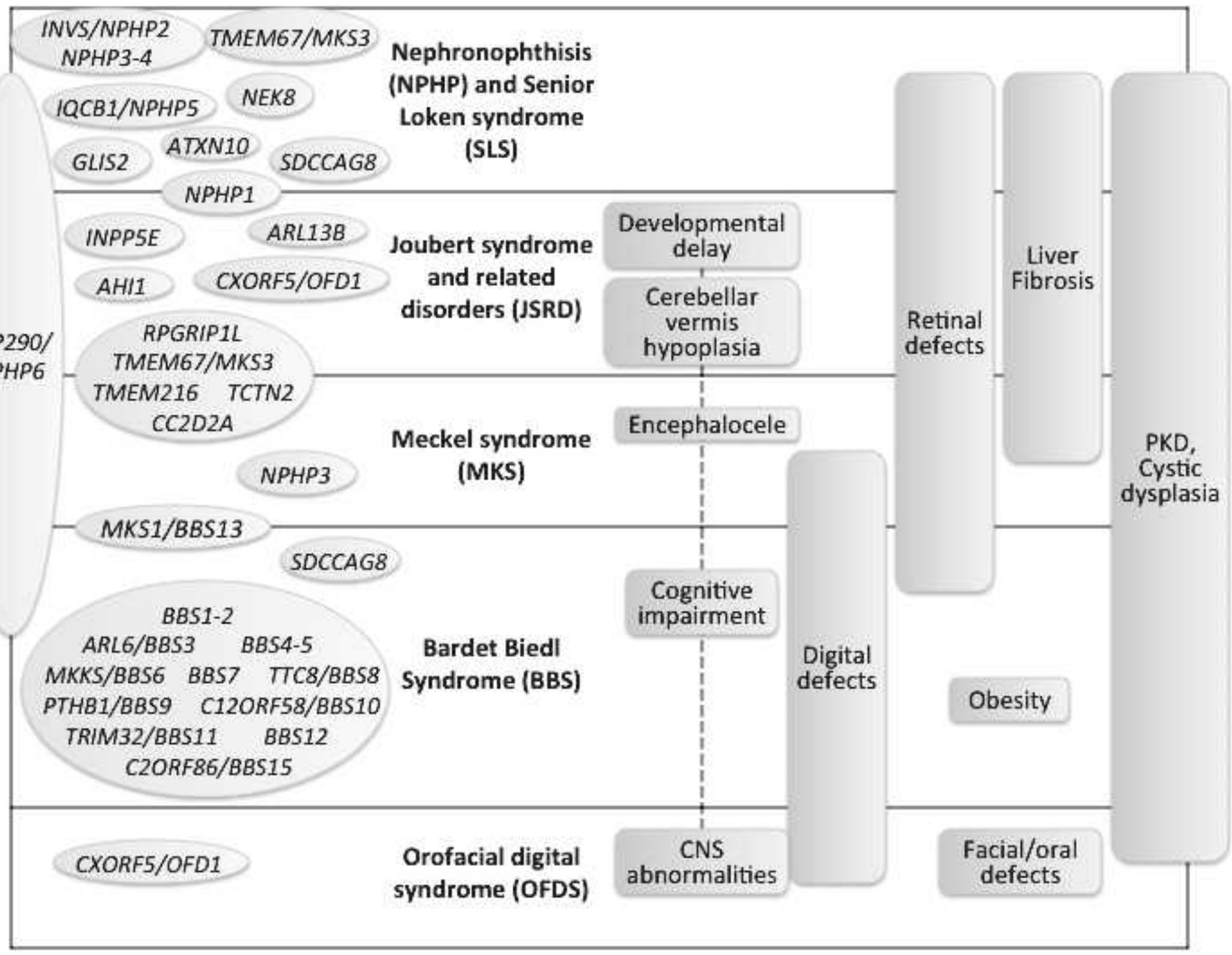


FIGURE 14.3 The genes and phenotypic abnormalities associated with syndromic forms of cystic kidney disease. Diseases are listed in the center; genes associated with them are shown on the left (*gray ovals*). On the right are phenotypes found in these different disorders (*blue boxes*). Note the high degree of overlap in genes and phenotypes between the different disorders. (Adapted from Harris PC, Torres VE. Polycystic kidney disease. *Annu Rev Med.* 2009;60:321–337. Reprinted with permission from the Annual Review of Medicine, Volume 60. © 2009 by Annual Reviews [www.annualreviews.org].)

active. In an elegant series of experiments, they then showed that steroids that normally bind but do not activate wild-type MRs were able to activate the mutant receptor. Steroids with 21-hydroxyl groups such as aldosterone were able to bind and activate both proteins, whereas steroids with 17-keto groups (i.e., estradiol and testosterone) activated neither. Steroids such as progesterone, which lacked both modifications, were found to be potent activators of the mutant MR, leading to the conclusion that activation no longer required a steroid 21-hydroxyl group. Using computer manipulation of an established structural model of the steroid HBD, the authors were able to understand that the mutation resulted in increased van der Waals interactions between helix 5 and helix 3 of the HBD (Fig. 14.4). This essentially substituted for the interaction of the steroid 21-hydroxyl group with helix 3 in the wild-type receptor.

These analyses result in several important insights. First, these findings explained why several affected women in families with the S810L mutation developed severe hypertension during pregnancy, a state that is associated with a drastic increase in progesterone levels. Second, it provided a critical understanding of the general basis for steroid hormone activation because the helix 5–helix 3 interaction is highly conserved among diverse nuclear hormone receptors. Last, this type of in-depth structural understanding is often a prerequisite for rational drug therapy.

DEFINING THE EXPRESSION PATTERN OF A GENE AND ITS PROTEIN PRODUCT

The expression pattern of a gene may provide valuable information about its function. Northern blots of tissue are commonly used as a screening tool but are very insensitive and provide little information regarding the specific cell types that express the gene of interest. For example, NPHS1 and PCLN1 have a similar pattern on Northern blot—each detects a kidney-specific signal—though they have very different functions.[42,108] More precise localization can be achieved using in situ hybridization techniques (Fig. 14.5A,B). Using this method, one can readily distinguish the podocyte-specific expression of NPHS1 from the TAL-specific expression of PCLN1. The temporal and spatial distribution of gene expression is invaluable in defining developmental pathways and identifying cofactors of a gene of interest. RNA-based studies are particularly useful in tracking the expression of genes that encode secreted products (i.e., growth factors

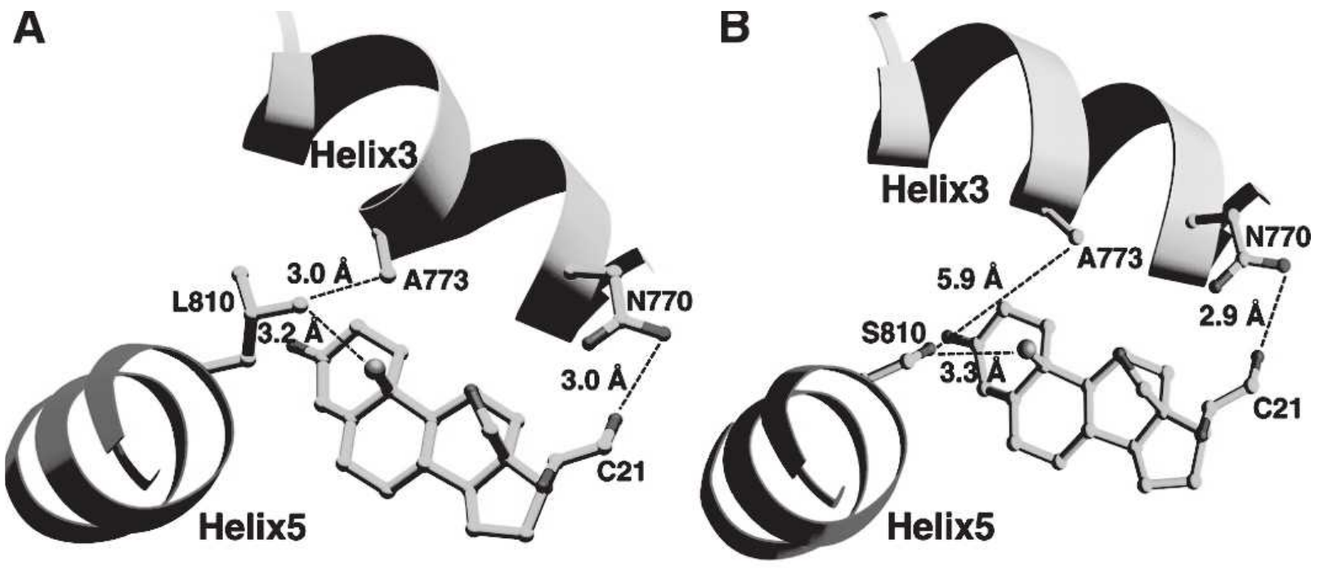


FIGURE 14.4 Helix 3–Helix 5 interaction in a progesterone-mediated activation of mutant mineralocorticoid receptor, MR_{L810}. **A:** The structural model of a portion of the hormone-binding domain of MR_{L810} bound to aldosterone, based on the crystal structure of progesterone receptor. This model predicts that the side chain of L810 lies sufficiently close to A773 and the C19 methyl group of the steroid to form van der Waals interactions. **B:** A model of $MR_{wild\ type}$. The side chain of S810 does not interact with A773 because the distance between them is too great. **C:** The activity of mineralocorticoid receptors (MRs) with various amino acid substitutions at residues 810 and 773. Mutant receptors containing the indicated substitutions at the two positions were assayed using a luciferase reporter assay in the presence of 1 nM aldosterone or 19-NP (19-norprogesterone, a progesterone derivative). MRs with amino acid substitutions that contained larger side chains were activated by 19-NP, as predicted by the model in **A** and **B**. A, alanine; G, glycine; L, leucine; M, methionine; S, serine; V, valine. (From Geller DS, Farhi A, Pinkerton N, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science*. 2000 Jul 7;289(5476):119–123. Used with permission.)

and cytokines) that are difficult to identify using immunohistochemical methods. In situ techniques also have the advantage that they can be used in cases where the specificity of an antibody for one's target has not been adequately demonstrated.

One important limitation of RNA-based studies is that they reveal nothing about the fate of the protein that they encode. Therefore, immunolocalization studies aimed at providing detailed characterization of a protein's cellular and subcellular distribution are key steps in its initial evaluation. In many cases, the protein structure provides clues as to where it is likely to be positioned within the cell. Nuclear localization signals, transmembrane-spanning elements, and homologies to other known proteins offer hints, but each of these predictions must be confirmed in cells and tissues. For membrane proteins, one must determine the specific membrane compartment in which they reside because this could have profound implications for understanding their function. Costaining studies using antisera that recognize known proteins are often helpful.

For novel proteins, one must first develop a set of antibodies that have a specific recognition for their target. The strategy used to generate antibodies is straightforward: immunize rabbits, chickens, or other species with either synthetic peptides or recombinant polypeptides derived from the gene of interest; screen for immunoreactivity; and then affinity-purify the reactive antibodies using the antigen against which it was raised. Antibodies are generally thought to be specific if they recognize the epitope against which they were raised and if the immunoreactivity was the direct result of the immunization process (i.e., preimmune sera lacked the activity).

In many cases, the results are unambiguous and provide important functional insights. As noted previously, nephrin, the gene product of NPHS1, the gene mutated in congenital nephrotic syndrome of the Finnish type, was found to be exclusively expressed in the glomerular podocyte.[108] An immunohistochemical analysis of renal tissue showed that the protein was confined to the slit diaphragm in the pore region of the glomerular basement membrane (Fig. 14.5C).[109–113] This invaluable information is unlikely to have been obtained by any other method, and has important implications for understanding the protein's function. This result also helped to explain why a loss of this protein could result in the massive nephrotic syndrome observed in those affected with this disease. In a similar manner, immunolocalization

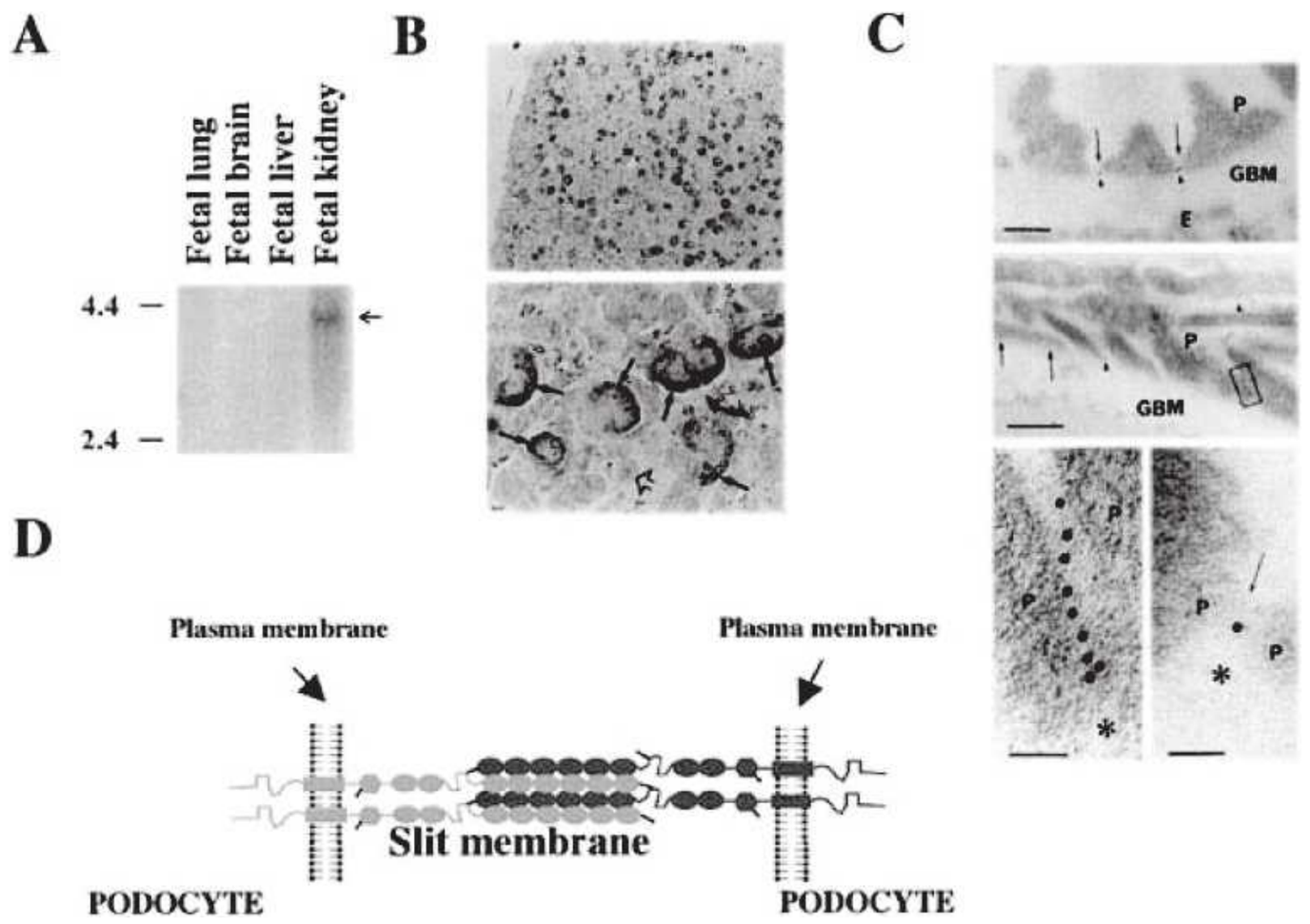


FIGURE 14.5 The expression of *NPHS1* and its protein product, nephrin. **A:** A Northern blot of poly(A) RNA from four fetal human tissues, hybridized with a fragment of *NPHS1* cDNA probe. A specific signal can be seen only with the fetal kidney RNA (*arrow*). **B:** The expression of *NPHS1* in the human fetal kidney by in situ hybridization. The *top panel* shows intense expression in the glomeruli throughout the renal cortex with little expression in other structures. A higher magnification is shown in the *lower panel*, revealing intense expression in the periphery of the glomeruli and little or no expression in the Bowman capsule (*bent arrow*), the proximal tubules (*open arrow*), or the endothelial cells of vessel walls. **C:** Nephrin is localized to slit membranes. Immunoelectron microscopic localization of nephrin in human renal glomeruli. In the *top panel*, gold label (*arrowheads*) can be seen between foot processes of podocytes (*P*). The label is located in the central area of the slit, between the glomerular basement membrane (*GBM*) and the barely visible slit diaphragm (*arrows*). The endothelium is unlabeled. Bar = 200 nm. In the *middle panel*, several gold particles can be seen lying in a row (*box*) between tangentially sectioned podocytes (*P*) foot processes. The *lower left panel* presents a close-up of the boxed section, whereas the *lower right* shows a gold particle between the slit diaphragm (*arrow)* joining two podocytes (*P*) and the GBM (*asterisk*) in cross-section. Bar = 50 nm. **D:** A hypothetical model of how nephrin molecules assemble to form the slit diaphragm. In this example, four nephrin molecules are shown to associate in the slit between two foot processes. The interdigitation is mediated by immunoglobulin (Ig) repeats 1 through 6 (*ovals*) and disulfide bridges that cross-link the molecules. (**Panels A and B** from Kestila M, Lenkkeri U, Mannikko M, et al. Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell.* 1998 Mar;1(4):575–582. **Panels C and D** from Ruotsalainen V, Patrakka J, Tissari P, et al. Role of nephrin in cell junction formation in human nephrogenesis. *Am J Pathol.* 2000 Dec;157(6):1905–1916. Used with permission.)

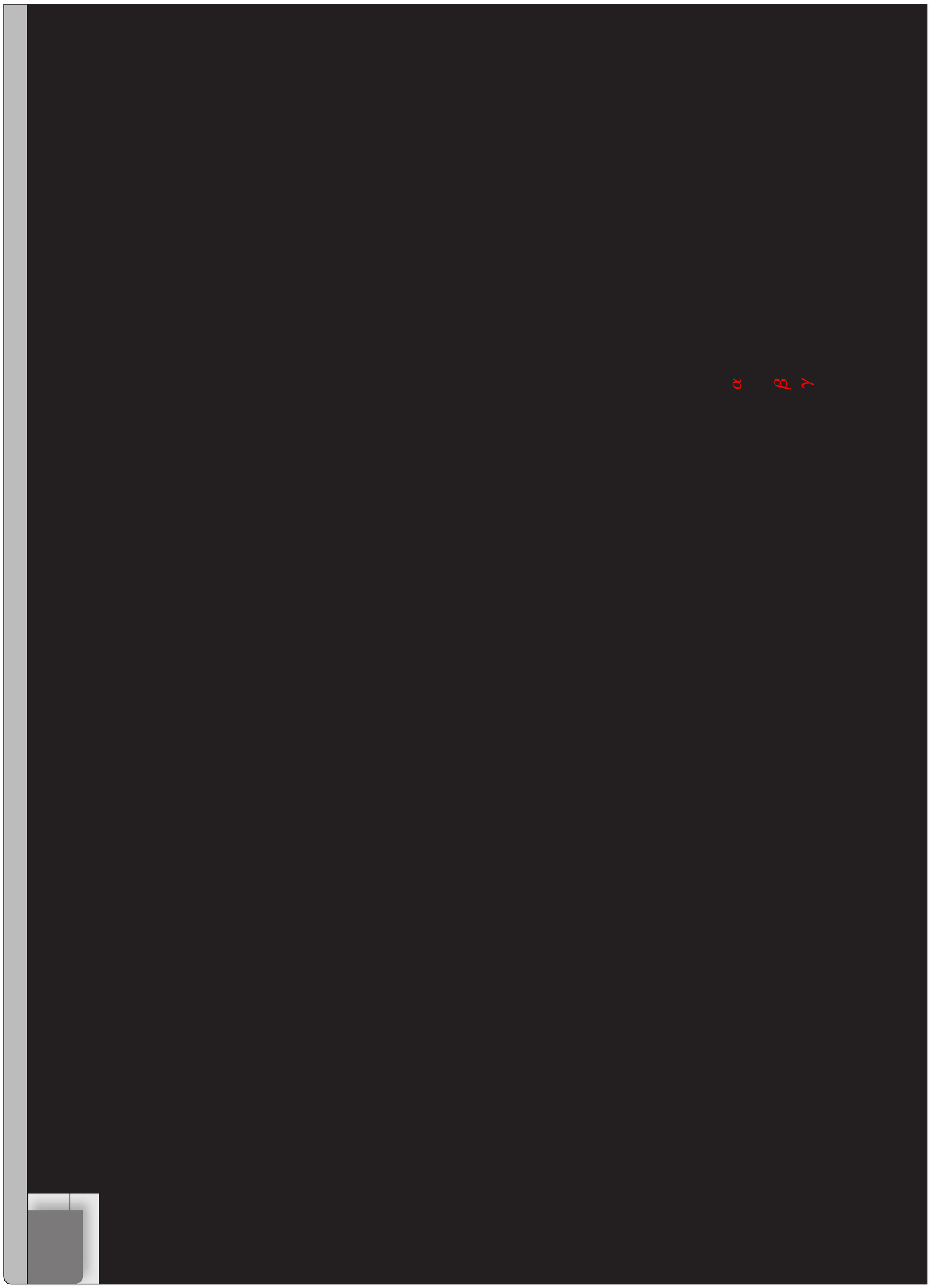
studies of PCLN1, the protein that is defective in primary hypomagnesemia, revealed that it is located in tight junctions of the thick ascending limb of Henle.[42]

Unfortunately, the process of antisera characterization is far less straightforward than it appears, and the commonly accepted standards are insufficiently stringent to ensure that one's antiserum is truly specific exclusively for one's target. A further complication is that many proteins are present in very low abundance, so even highly specific antisera have difficulty detecting them. These problems often result in conflicting data and uncertainty about the true nature of the disease gene product, and they likely account for the confusion regarding the detailed characterization of polycystin-1 (PC1).[69,114–136]

Antibodies specific for one's protein of interest are extremely useful for other essential steps in its characterization. For example, many proteins are subject to a number of functionally important posttranslational modifications such as proteolytic cleavage, glycosylation, and phosphorylation. These properties are often best assessed by immunoblot and immunoprecipitation studies, and they often are disrupted by missense mutations. As will be discussed in more detail in the next section, most proteins also act in concert with others via pathways, and antibodies are useful tools for identifying these protein networks.

FUNCTIONAL ANALYSES

Once the initial description of a new gene is completed, the next goal is to begin its functional characterization. For many of the genes listed in Tables 14.1 to 14.8, their roles have been obvious because they were identified based on their functional properties. For example, each of the transporters listed in Tables 14.5 and 14.6 had been cloned and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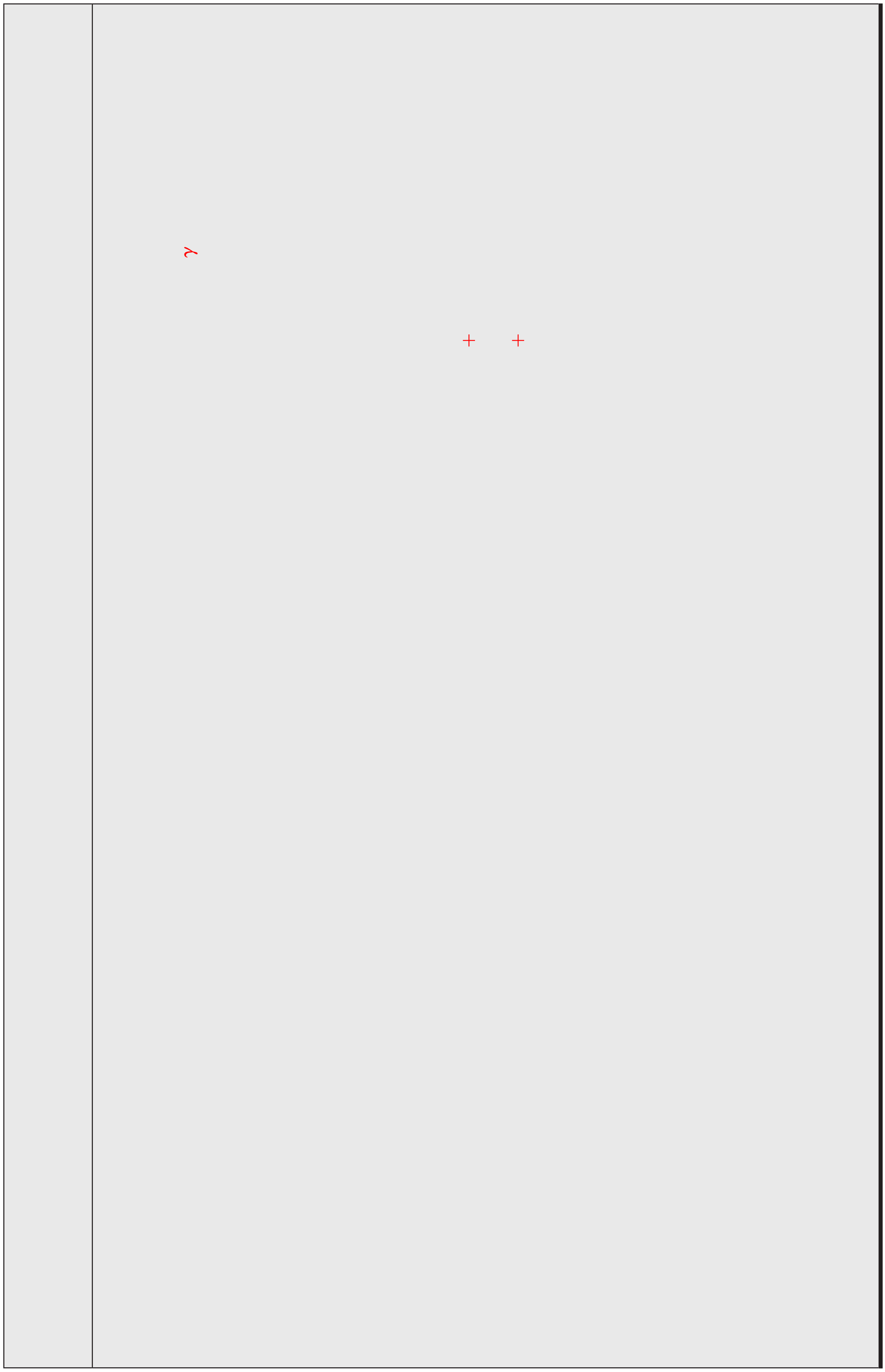

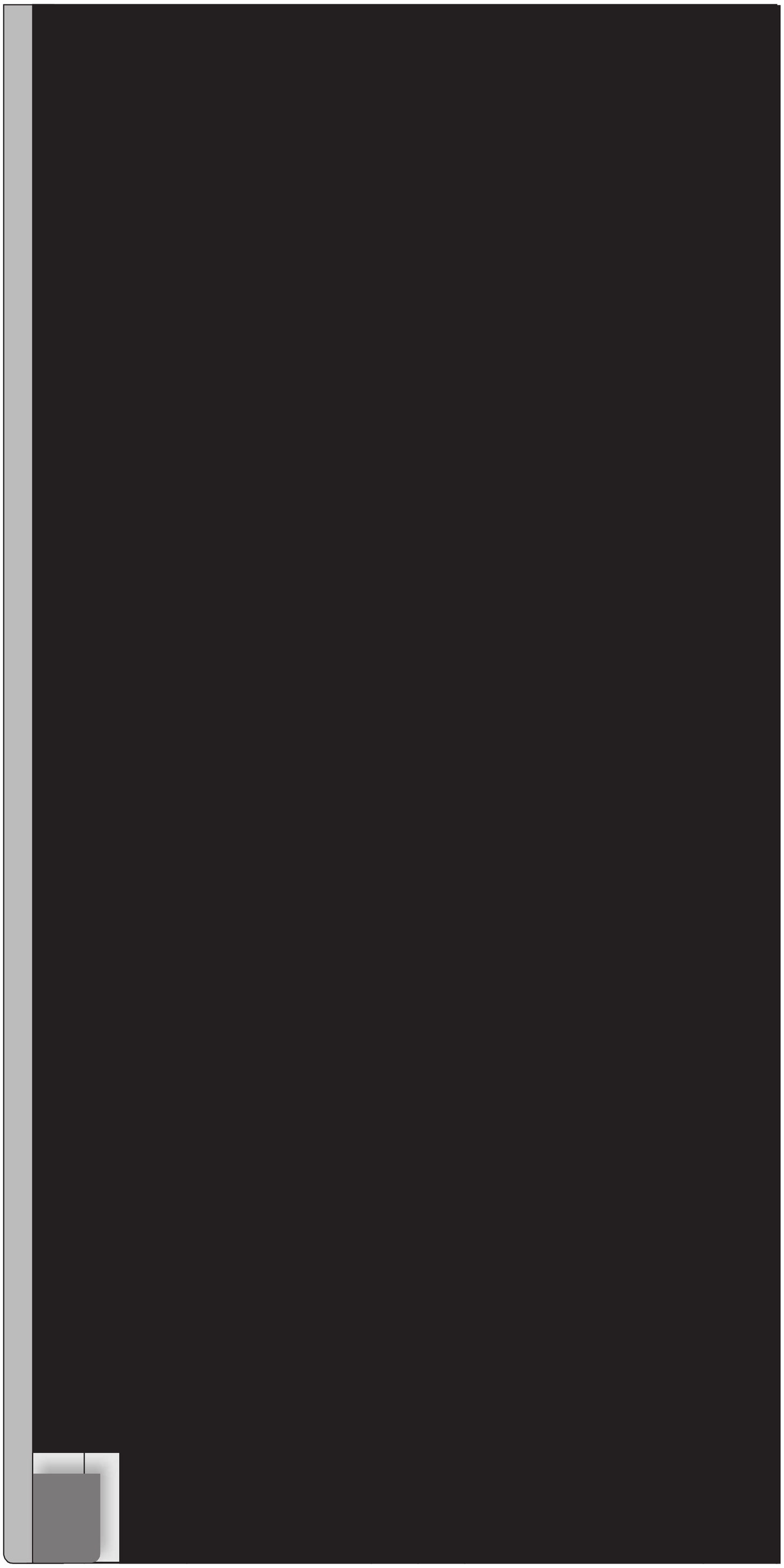

fig

characterized prior to the discovery of its mutation in the disorder with which it is associated. In these conditions, the focus is on determining how mutations alter the protein's function and how these abnormalities result in the observed pathology. A subset of the genes listed in the tables was discovered to be homologues of known genes that had not been previously associated with the human disease phenotype. For example, mutation of INVS (NPHP2), one of the genes associated with nephronophthisis, had been previously found to result in situs inversus and severe polycystic disease in mice.[137,138] Its subsequent association with human NPHP provided a critical insight into the functional properties of NPHP-related proteins.[139,140] Members of this group are now thought to comprise essential components of the primary cilia and the basal body. A similar approach is applied to the study of novel genes, like PKD2, that have a predicted but unproven function based upon their sequence. A final subset of genes, like VHL and NPHP1, encode novel proteins of unknown function, the sequence of which provides few clues.

It is obvious that the particular set of experiments that one uses to characterize a gene depends on the nature of the protein that it encodes. The strategy for studying a putative transporter is very different than the one used to examine a transcription factor. Likewise, one will approach the study of a known gene product like the Na-K-2Cl cotransporter differently than one would a more enigmatic one like nephrocystin-1, the NPHP1 gene product. In this section, we will provide both a brief review of some of the general methodologies used to determine a protein's functional properties and several illustrative examples.

The Identification of Interacting Partners

The specific nature of interactions between proteins is often an important determinant of their functional characteristics. One paradigm of functional protein–protein interactions is that of receptor-based signaling. According to this schema, the receptor, often situated in a membrane, binds to one or more ligands. This binding usually leads to a cascade of signaling events that may begin, for example, with phosphorylation of the receptor by itself or some other protein. This event may recruit other proteins such as adapter molecules with src homology 2 or src homology 3 domains to the receptor-ligand complex. These proteins may also be phosphorylated, and when activated in this way, they move to the nucleus, ultimately leading to the transcription of new genes. Frequently, one receptor may participate in a number of signaling pathways that "talk to one another." In many cases, these factors serve to amplify the signal, whereas parallel or negative feedback systems act to dampen or balance it. In either case, each step in this pathway critically depends on the interaction of proteins capable of transmitting the signal.

Proteins involved in receptor-based signaling are by no means the only types of proteins whose activity depends on protein–protein interactions for their normal function. Many channels or transporters are formed by multimeric complexes of a single subunit (in the case of potassium channels) or multiple independent subunits (e.g., H^+ ATPase). Moreover, the activity of most channels is regulated by the direct binding of other non-channel proteins. Likewise, transcription factors depend on interactions with specific cofactors, transactivators, or inhibitors for their activation or repression. It is fair to say that most proteins critically depend on protein–protein interactions for their functional activity, and identifying the partners of one's protein of interest can provide important clues into its function. This is especially true if the partners are discovered to be factors that participate in well-defined pathways or regulatory systems. Given the tremendous impact such studies can have on one's understanding of the pathobiology of a new protein, much effort is often directed at identifying the protein partners of a gene product of interest.

Several strategies can be used. The first is guided by an understanding of the disease process. A growing body of literature suggests that inherited disorders that are characterized by genetic heterogeneity often result from mutations of genes the products of which either form a multimeric complex (such as a transporter) or function in a common pathway. This property is exploited in Drosophila research as a tool to define the functional pathways of novel genes. In the case of human renal diseases, there are numerous examples that fall into this group: ADPKD, NPHP, tuberous sclerosis (TSC), distal RTA (OMIM# 267300 and 602722), Liddle syndrome, PHA type I (recessive forms), and cystinuria. In some of these examples, like Liddle syndrome, the genes associated with the disease had been known to encode interacting subunits prior to their discovery as disease-causing loci.[12] In others, like ADPKD, NPHP, and TSC, the functional interactions of their respective gene products were discovered solely because of a focused search.[69,139,141–146]

Sometimes it is possible to make an "informed guess" about potential partners based on the known properties of the protein of interest. For example, a protein that had been shown in immunolocalization studies to be located in focal adhesion plaques might be expected to associate with other components of that complex. One would then pursue a directed search to look for functional associations between the protein and a battery of pertinent interacting factors. Other clues might be provided by homology to known proteins. The identification of a putative G-protein binding site in polycystin-1 resulted from such an analysis.[61] In other cases, a protein may have domains that are known to be generalized sites of protein–protein interaction. Ankyrin repeats and coiled-coil motifs are examples of such structures and are good places to begin to look for interacting partners.

Finally, in many cases, a protein is truly novel and there is no way to predict to what it might bind. This is common when a gene has been identified using a strictly positional approach or when there is no sequence homology to serve as a guide. The VHL gene product is an example of this situation. Truly novel proteins require the use of methods that make few prior assumptions about the nature of interacting partners. In such situations, an investigator can purify the protein (either the endogenous protein or a recombinant,

epitope-tagged version purified from tissue or cells) and determine the peptide sequence of any factors that copurify. As one might predict, the rate of progress in understanding the function of proteins that fall into this class is usually far slower than it is for others.

The strategies used to determine the functions of the genes linked to BBS and NPHP nicely illustrate some of these principles. Multiple loci have been linked to each disease, and it was suspected that many if not all of their respective gene products likely formed multimeric protein complexes. In the case of BBS, investigators identified a complex they called the "BBSome" that is composed of seven highly conserved BBS proteins and at least one novel subunit that, when depleted in zebra fish, results in a BBS phenotype.[147,148] They subsequently showed that the BBSome is recruited onto membranes by another BBS protein (Arl6, encoded by BBS3) that is not part of the BBSome to form a coat complex that sorts membrane proteins to primary cilia.[149] These findings help to explain the ciliopathy phenotype common to the genetically distinct forms of the disease.

A similar strategy was used to investigate the relationships between NPHP, JBTS, and MKS proteins. Investigators purified nine disease gene products from a kidney cell line and identified the set of proteins interacting with each by mass spectrometry. They identified 850 interacters, performed proteomic network analysis, and discovered three connected modules: one functioned at the apical surface, a second at centrosomes, and a third that was linked to Hedgehog signaling. Reasoning that some of the interacting proteins may represent unrecognized disease loci, they screened 38 of the genes for mutations and identified two novel loci as causes of NPHP-JBTS (ATXN10, TCTN2).[150] In sum, this approach not only elucidated some of the functional properties of the NPHP-JBST-MKS protein family, but it also helped to identify new causes of the disease.

Taken together, these examples highlight the tremendous insights that a search for protein interactions can provide.

MODEL SYSTEMS

Cell Culture Systems

The biochemical approaches described in the preceding section are limited by the nature of in vitro studies. They do not generally provide information about the physiologic consequences of activation or disruption of a particular pathway. It has become increasingly obvious that cell culture systems can be especially valuable in this regard. Cell culture systems have the advantage that they often can be easily scaled up to perform "bucket biochemistry," they can be used under carefully controlled and well-defined conditions, and they can be scaled down to the single-cell level to study molecular processes. They are invaluable tools for studying a molecule's transport properties, its growth-regulatory characteristics, or the signaling pathways in which it participates. If one transfects a cell with a cDNA for a putative ion channel, one can demonstrate and characterize its channel activity using patch clamp methods or calcium photometry for calcium fluxes. Likewise, one can test for the transport of other molecules by simply modifying the approach to look for the movement of the molecules in question. Cell transfection experiments also can be used to quantitate the growth effects of a protein of interest. Once an effect is defined, one can perform cell-cycle analysis to determine whether it acts through a cell-cycle dependent process, by altering the rate of programmed cell death, or through some other mechanism. Regardless of which mechanism is implicated, there is a series of well-defined steps that can be followed to define in a precise manner how the protein affects its activities. In the case of transcription factors, cell culture systems may be used to identify potential targets.

A complete review of the many insights that have resulted from the use of cell-culture systems is far beyond the scope of this chapter. Several select examples will serve to illustrate the use of this approach.

Pseudohypoaldosteronism type II (PHA2, Gordon syndrome/familial hypertensive hyperkalemia) is a rare autosomal dominant disease that is distinct from most other Mendelian genetic forms of hypertension in that affected individuals have both hypertension and hyperkalemia.[151] Mutations in WNK1 and WNK4, two related members of the serine threonine kinase superfamily, were found to cause the disease. A series of studies performed using recombinant proteins and cell culture systems demonstrated that the proteins act as a molecular switch that allows the kidney to alternate between NaCl-reabsorbing and K^+-secreting states through their inhibitory effects on the thiazide-sensitive NaCl cotransporter and the renal outer medullary K^+ (ROMK) channel. The observation that the proteins had been localized to the tight junctions in renal epithelia prompted Kahle et al.[151] to investigate whether WNKs also function to regulate paracellular ion flux using canine renal epithelial cells (MDCKII cell line). They generated stable cell lines that had inducible expression of either wild-type or mutant WNK4 and showed that the recombinant proteins localized to tight junctions by immunofluorescence. They then showed that WNK4 decreased transepithelial resistance in MDCK cells grown in monolayers by selectively increasing the paracellular conductance of chloride. They ruled out gross structural changes of the tight-junction complex as a trivial explanation for their findings by transmission and freeze-fracture electron microscopy. The authors concluded that the WNKs likely help coordinate transcellular and paracellular flux to achieve NaCl and K^+ homeostasis.

A different kind of cell culture system has been developed to study the function of polycystin-1. This protein has been difficult to analyze because of its large size and low abundance in tissues and cells. Boletta et al.[152] used novel methods to establish cell lines with stable overexpression of PKD1. They had selected the MDCK cell line for this study because it is a renal epithelial cell line commonly used to study the process of tubulogenesis. Under normal conditions, MDCK cells form cysts when cultured in collagen, but produce branching

tubules if treated with hepatocyte growth factor (HGF).[153] Boletta et al.[152] found that the expression of recombinant PKD1 in MDCK cells resulted in spontaneous tubulogenesis, a reduced rate of growth, and induced resistance to apoptotic stimuli. The effects of PKD1 expression in this model are remarkably similar to what had been predicted based on the study of human and murine cystic tissues, and suggest that it may serve as a useful tool for understanding pathways regulated by polycystin-1. This unique system offers three phenotypic assays that can be used to test for the functional consequences of altering any of polycystin-1's domains. Given that each of the distinct phenotypes could be affected in a different way, this strategy might allow one to dissect out the various signaling pathways regulated by this multifunctional protein. One might also use such a system to test for functional consequences of missense changes identified solely in affected individuals but of uncertain consequence.

Mouse Models

Although cell culture systems are relatively easy to manipulate, they may not always mimic faithfully what occurs in vivo. Overexpression of proteins could conceivably activate pathways that are not important in the whole organism and, therefore, the significance of any phenotypic readout may be difficult to interpret. Animal models that faithfully reproduce human disease are therefore invaluable tools. They can be used to help define the pathophysiology of a disorder, identify factors that modulate the severity of disease, and to test therapeutic interventions.

For diseases that result from gain-of-function or dominant negative mutations, transgenic mice with overexpression of the mutant gene can be useful.[154] In this method, the cDNA of interest is injected into the pronuclei of fertilized eggs and then reimplanted in the uterus of females for carriage to term. Advantages of this approach include its wide availability, relatively low cost, and rapid readout. Transgenic animals can be produced within less than a month of producing the recombinant construct. Its major limitation is that the spatial and temporal pattern of gene expression of the transgene often fails to recapitulate that of the endogenous gene in a faithful manner. A second problem is that this strategy generally cannot be used for diseases that are recessive in nature. Overexpression of a null or functionally null allele is unlikely to reproduce the human disease, because the endogenous murine forms of the genes will be normally expressed. Finally, in most cases, the construct used to generate the mice exclusively contains the exonic sequences of one's gene and lacks all but perhaps a single intron (the latter has been shown to improve transgene expression). One consequence is that the transgene does not undergo any of the splicing normally observed for the endogenous gene.

In light of these limitations, a second approach that avoids many of these pitfalls has now become the standard of the community. Its goal is to generate mice with targeted mutations of the endogenous murine gene.[155] By altering the gene in situ, one preserves most of its intrinsic structural features such as regulatory elements and introns. In most examples to date, the technique has been used to produce a "knockout" through the introduction of deletions that abrogate a gene's function. More recent refinements allow one to introduce more subtle changes such as nucleotide substitutions that encode missense changes ("knockin"). Such strategies allow one to generate models that carry the exact genetic defects observed in humans in a much more faithful manner.

The ability to do gene targeting depends on two principles. First, one must be able to perform an in vivo "swap" of the engineered gene segment for the endogenous sequence through the process of homologous recombination. Next, one must have a way of creating an animal from cells with the targeted mutation. Sperm and egg cells would seem like natural choices but unfortunately cannot be used for this purpose. Embryonic stem (ES) cells, removed from the earliest stages of development, do work well because they are easily cultured and totipotent—they can give rise to any organ system or cell type. This allows one to "pass" the mutation through the mouse germ line.

The first step in gene targeting is to design a targeting construct that contains a genomic segment of the target gene into which has been inserted a neomycin resistance gene. The latter step allows for the selection of ES cells that have "taken up" one's construct. For "knockout" constructs, the neomycin gene has replaced a portion of the coding sequence of the target gene so that its integration would interrupt the target's coding region. For "knockin" constructs, the neomycin gene is typically placed within an intron where it is less likely to disrupt the gene's function. To create the mutation, the targeting construct includes a segment of the normal gene that has been altered to include the desired variant. Properly targeted clones are identified by Southern blot analysis. The appropriate ES cells are then injected into a mouse blastocyst in a process that produces an adult chimeric mouse. Chimeric mice can be readily identified by their mixed black and agouti coat color, because the ES cells typically come from mice with agouti coat colors and the blastocysts are derived from animals whose coat color is black. The chimeric mice are bred and if their germ line is also chimeric, then the F1 generation should have some progeny that are heterozygous for the targeted disruption. In order to produce homozygous mice, the F1 generation can be interbred to one another.

"Knockout" models are useful in a number of ways. For example, they may confirm the genetic basis of a disease. As noted in a previous section, genetic studies of cystic tissue have indicated that ADPKD is likely to be recessive on a molecular level. This would suggest that mice heterozygous for Pkd mutations should survive to adulthood with few, if any, symptoms. In contrast to their human counterparts, they would be predicted to have relatively few cysts because of their far shorter lifespan and much smaller organ size. A smaller kidney or liver would have far fewer cells at risk of acquiring mutations over a period of 1 to 2 years of observation. These predictions were confirmed in studies

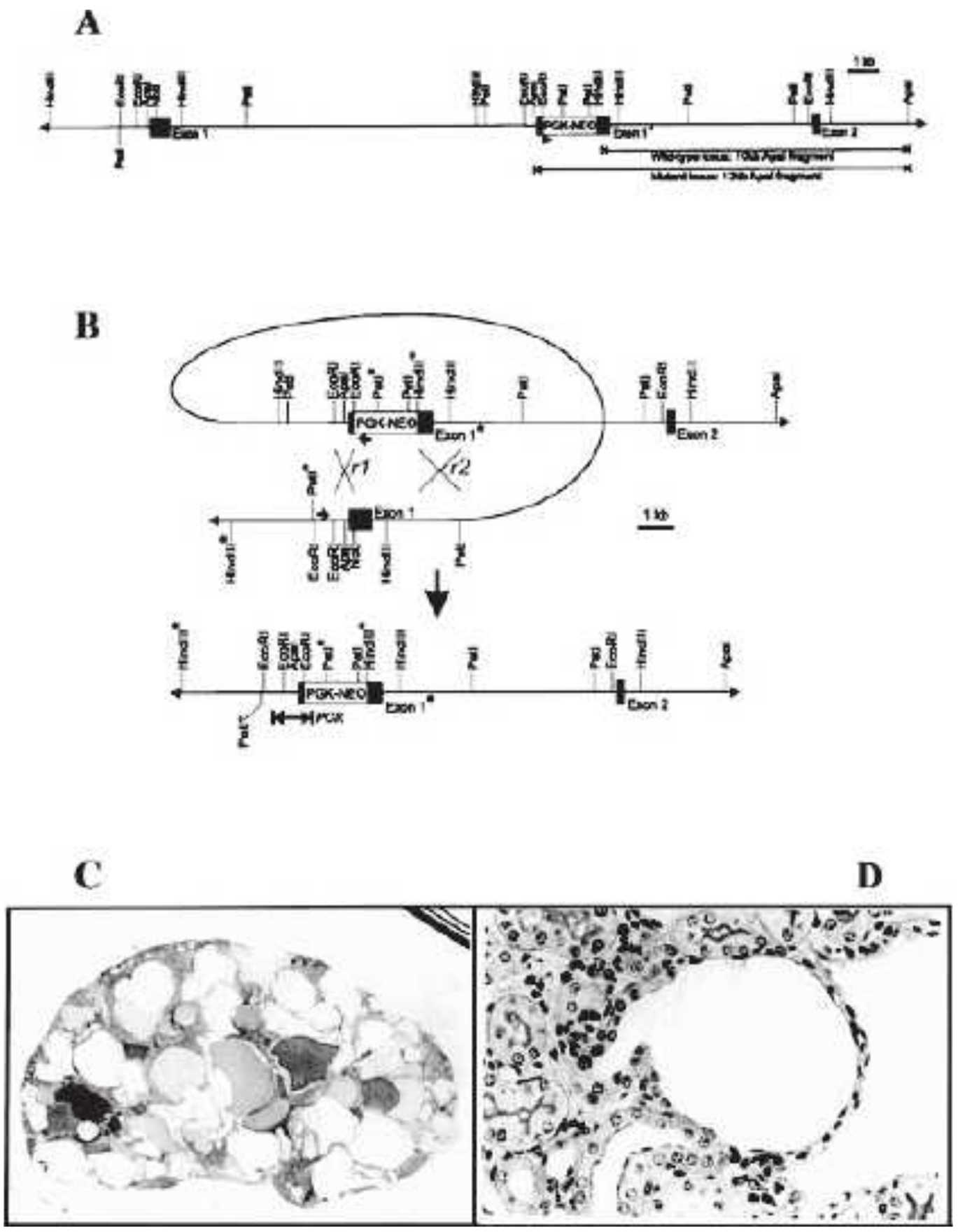

FIGURE 14.6 **A:** Restriction map of the *Pkd2*^{WS25} allele created by gene targeting. Instead of replacing the normal 5′ end of the *Pkd2* with the mutant sequence, the targeting construct integrated within the 5′ end to create a local duplication. Locally duplicated sequences are known to be prone to genomic rearrangement by unequal sister chromatid exchange. **B:** A schematic representation of intragenic homologous recombination that is observed for the unstable *Pkd2* allele, *Pkd2*^{WS25}. Two different recombination processes can occur. Recombination r1 produces a null allele, whereas recombination r2 results in a wild-type allele. Both the wild-type and the unrecombined *Pkd2*^{WS25} allele have normal functional activity. **C:** A cross-section of a murine kidney taken from a homozygous mouse carrying two unstable *Pkd2* alleles (Pkd2$^{WS25/WS25}$). Severe cystic disease is observed, mimicking the pattern of human ADPKD (2.5×). **D:** Cysts have a focal origin. A magnified view of a murine kidney with the Pkd2$^{WS25/WS25}$ genotype. An incipient cyst is shown forming as an outpouching from a normal tubule. Presumably, the cyst developed as the result of homozygous inactivation of the *Pkd2* gene in one tubular cell (320×). (From Wu G, D'Agati V, Cai Y, et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell.* 1998 Apr 17;93(2):177–188. Used with permission.)

of mice that were heterozygous for inactivating mutations of either Pkd1 or Pkd2.[156–159] Wu and associates[156] had developed a particularly elegant model. They had generated an unstable Pkd2 allele by inserting a second copy of the first exon of Pkd2 in tandem to the endogenous exon (Fig. 14.6). This allele was prone to acquiring somatic mutations via intragenic homologous recombination. Mice heterozygous for the null allele and the unstable allele consistently developed an ADPKD phenotype similar to that observed in humans. These mice did not die in utero but developed significant renal and hepatic cystic disease by 11 weeks of age. This model has proven to be an invaluable tool for studying the pathophysiology of the disease[160] and testing putative therapies.[161,162] One can easily imagine how it also might be used to identify factors that predispose to the acquisition of somatic mutations or influence the progression of disease.

A common problem limiting the use of standard gene knockout technology is that complete homozygous inactivation of a gene often results in a nonviable fetus that fails to complete gestation. This situation is usually recognized by a distortion in the Mendelian ratio of observed versus expected genotypes of living offspring. In the case of Pkd1, this property severely limited the use of the many models that had been generated by gene targeting. However, newer technologies have allowed investigators to circumvent this problem and have expanded the capability to manipulate the murine genome at will. Gene-targeting techniques can be used to create mice that have latent mutations, which can be activated upon demand (conditional mutations, otherwise known as "floxed alleles").[163–169] In this approach, the targeting construct is designed to include very short DNA sequences that serve as specific recognition sites for nonnative DNA recombinase enzymes in introns flanking key functional aspects of a gene of interest. If carefully positioned, the short DNA sequences (either loxP or frt sites) have no effect on the function of the gene until after recombination has been induced. Since the recombinase enzymes (Cre and Flp) are not normally present in mice, one can determine when and where one wishes to inactivate the gene of interest by controlling the spatial and temporal expression of the recombinase. This usually is

achieved by breeding mice with either the loxP- or frt-allele to animals transgenic for the corresponding recombinase (Cre and Flp, respectively). By altering the regulatory elements that control Cre or Flp expression, one can define the conditions under which one wishes to inactivate the gene.

This strategy has been successfully used to generate viable adult mice that otherwise would have died in utero of their homozygous mutations. For example, this approach has been used to create mouse models that allow for the conditional inactivation of Pkd1.[170–172] These models have proven to be invaluable in the study of ADPKD, particularly with respect to defining how developmental context influences renal cyst development. For example, Piontek et al.[173] showed that Pkd1 inactivation in mice before postnatal day 13 results in the rapid development of severe polycystic kidney disease, whereas inactivation on or after postnatal day 14 results in much slower cyst development. These experiments defined a key developmental switch influencing cyst development and provided important mechanistic insights into the acquisition of cysts in human ADPKD. In addition, mouse models with slower, adult onset cystic disease will be an extremely valuable resource for testing therapeutic interventions and may prove more predictive of human responses to therapy.

Finally, a conditional model of gene inactivation also allows for the study of organ-specific properties of a gene's function. In the case of ADPKD, germ line homozygous mutations of Pkd1 and Pkd2 in mice result in embryonic or perinatal lethality.[156,157,159,170,174] Mutants uniformly demonstrate cystic kidneys, polyhydramnios, edema, and hemorrhage, but the primary cause of fetal death remained unclear. Using a series of mouse models with conditional Pkd1 and Pkd2 mutations, Garcia-Gonzalez et al.[175] were able to show that placental abnormalities appear to be the primary cause of fetal demise, but that deletion of Pkd1 or Pkd2 in endothelial cells alone is not sufficient to cause the dramatic vascular phenotypes observed in null animals. These findings revealed a surprising complexity in the functional roles of the polycystins, and further studies with organ-specific Cre recombinases will allow for the further delineation of tissue-specific functions of the proteins.

The Use of Nonvertebrate Organisms as Models

The numerous genetic tools available for manipulation of the mouse genome along with the wide availability of genetically defined strains has fostered great interest in using mouse models to identify genetic loci that encode modifiers of disease severity or potential interacting partners. Unfortunately, there are currently no efficient techniques for using large-scale genetic screens to identify enhancing or suppressing genes. In some cases, crossing inbred mutant strains to mice of a different background has revealed the influence of genetic modifiers that have been mapped to large chromosomal regions by quantitative trait analysis. Despite great success in localizing many of these factors,[176–181] progress in identifying the precise loci has been very slow. For this reason, many groups have turned to the use of invertebrate organisms such as Caenorhabditis elegans (roundworm) and Drosophila melanogaster (fruit fly) to complement the human and mouse studies. As noted in an earlier section, greater than 60% of human disease genes are estimated to have orthologs in Drosophila. In addition, the signaling systems involved in a number of basic processes such as cell–cell signaling (i.e., JAK/STAT/cytokine, Wingless/WNT) appear to have conserved components in vertebrates and flies. We have already alluded to one of the most striking examples of how this approach has enhanced our understanding of a vertebrate gene linked to renal disease. Ito and Rubin[182] identified Drosophila homologues of Tsc2 and showed that its loss resulted in a dramatic change in cell size. Several groups linked Drosophila Tsc1 to the same pathway soon thereafter and, capitalizing on the model system's many advantages, linked both proteins to the insulin-signaling pathway.[183–185] Subsequent studies soon showed that the Tsc1/Tsc2 complex regulates the TOR kinase in both Drosophila and vertebrate systems and identified Rheb as the small GTPase that is the likely target of Tsc2 GAP (GTPase-activating protein) activity.[186–196] The most exciting consequence of these studies is that they led to a possible therapy (discussed in the following text). The subsequent development of methodologies that can create Drosophila mutants by homologous recombination have now allowed investigators to manipulate its genome at will to make simple models of human disease "on the fly."[197–202]

Insights are also likely to come from unanticipated sources. In the case of PKD, a strategy of randomly inactivating genes by insertional mutagenesis revealed that the mutation of the Tg737 gene resulted in a phenotype similar to that of human autosomal recessive polycystic kidney disease.[203] The gene sequence initially provided few clues as to the protein's function. In an unrelated project focused on identifying genes in algae (Chlamydomonas) that regulate formation and function of flagella, investigators determined that one of the genes that caused flagellar defects was a homologue of Tg737.[204] The gene encodes a protein, IFT88, that helps to carry "cargo" to the flagella in a process called intraflagellar transport. Subsequent studies showed that IFT88 homologues in other organisms have an essential function in the formation and function of a related structure, the cilium.[205] In vertebrates, cilia have been found on almost all cells and are either motile or nonmotile. The latter are thought to possibly function as sensory organelles for a variety of stimuli.[206] In the kidney, they may act as mechanosensors for flow.[207,208] There now is a very large body of data that links defects in cilia and related structures such as the basal body/centrosomal complex to renal cystic disease (Fig. 14.7),[206,209–212] and a comparative genomic approach capitalized on this observation to identify a locus for BBS.[35] In sum, appreciation of the central role that the primary cilium appears to play in normal development and in renal cystic disease was sparked by studies of simple algae.

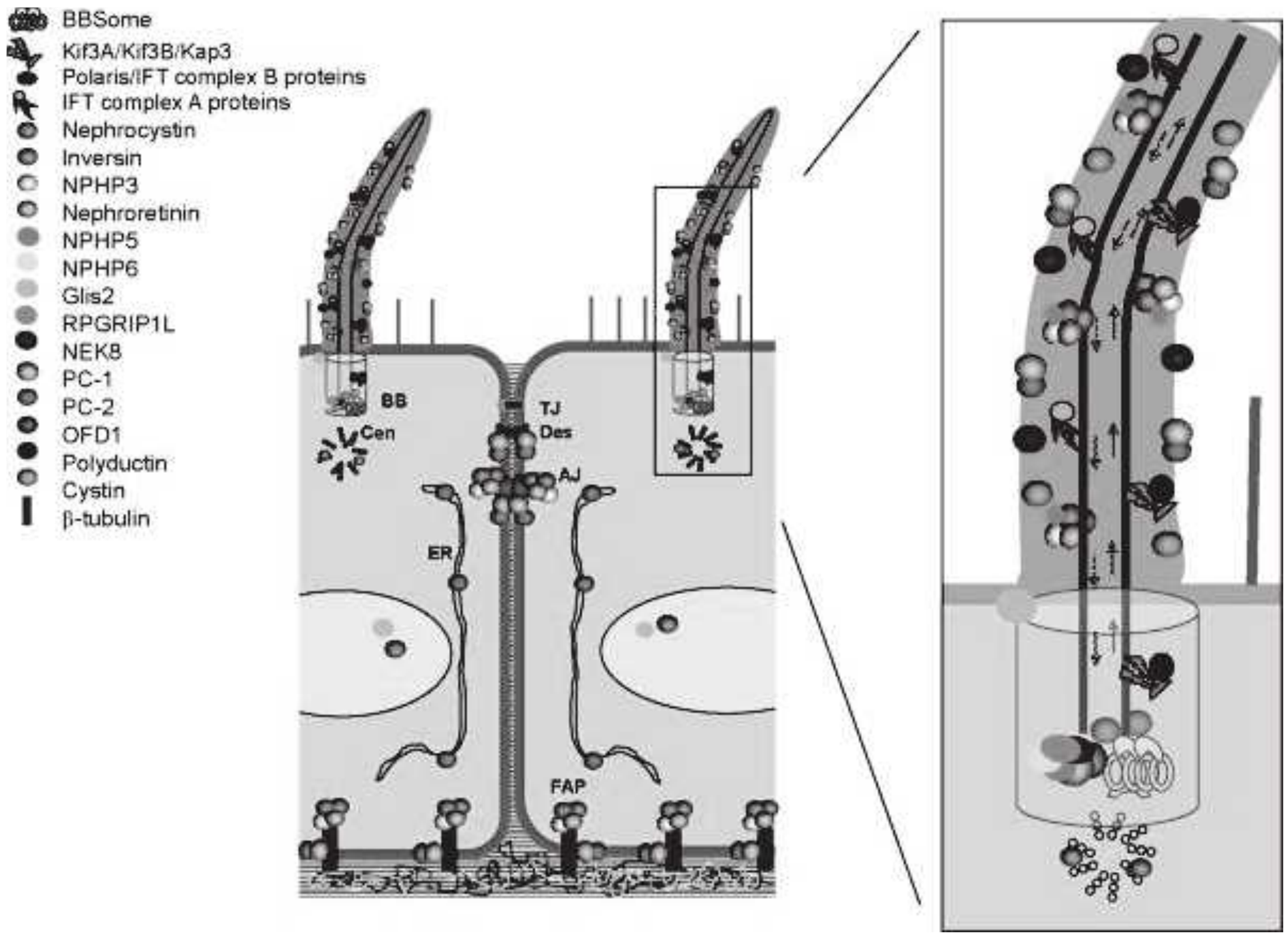

FIGURE 14.7 The subcellular localization of cystoproteins. Numerous cystoproteins have been localized to more than one intracellular domain. In this illustration, all known members of an interacting complex are shown colocalized to the same locations, even though this has not necessarily been shown for each unit. There are two exceptions: (1) polycystin-2 has been reported to have endoplasmic reticulum-specific functions as a calcium release channel that are independent of polycystin-1, and (2) at least one form of inversin has been localized to the centriole and to the nucleus. The *speckled arrow* in the primary cilium indicates the direction of anterograde transport along the microtubule system mediated by kinesin-II, a heterotrimeric protein composed of two motor units (Kif3a, Kif3b) and one nonmotor unit (KAP3). AJ, adherens junction; BB, basal body; Cen, centriole; ER, endoplasmic reticulum; FAP, focal adhesion plaque; TJ, tight junction; PC-1, polycystin-1; PC-2, polycystin-2. (Adapted from Watnick T, Germino G. From cilia to cyst. *Nat Genet*. 2003 Aug;34(4):35–56 and Menezes LF, Germino GG. Polycystic kidney disease, cilia, and planar polarity. *Methods Cell Biol*. 2009;94:273–297. Used with permission.) (See Color Plate.)

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

The advances described thus far may seem basic in their scope, but they also have far-reaching implications with respect to diagnosis and, ultimately, the treatment of inherited renal diseases. The most immediate clinical impact of gene identification is the development of molecular diagnostic tests. There are a number of circumstances in which DNA-based diagnoses may be helpful to clinicians managing the care of affected individuals and their families. In the case of X-linked nephrogenic diabetes insipidus, DNA testing can be used to identify at-risk male fetuses or newborns before they suffer repeated episodes of dehydration.[213] DNA testing is particularly useful in the management of families with VHL syndrome.[214–216] This information can provide reassurance to noncarriers and can focus close attention on those individuals most likely to develop complications that will need repeated computed tomography (CT) scans or magnetic resonance imaging (MRI). An additional benefit of DNA testing in this disorder is that it can identify the subgroup most likely to develop pheochromocytomas.

DNA tests are also used to evaluate presymptomatic individuals who seek genetic counseling regarding their prognosis or for the purpose of family planning. In general, presymptomatic DNA testing will probably be more widely used in the future if protective therapies for diseases such as ADPKD are developed that must be administered early in life before a clinical diagnosis can be made. A final use of DNA testing is in the evaluation of "at-risk," presymptomatic individuals as potential living-related transplant donors. This application is particularly important for diseases like ADPKD that have a late onset.

Historically, the primary methodology for DNA testing was by linkage analysis. This has been largely supplanted by newer methodologies (discussed in the following text). However, linkage analysis remains an important tool for diagnosis in families in whom the affected gene is unknown, or when a mutation cannot be detected in the gene of interest. Linkage analysis can also be helpful when results of direct DNA sequencing are indeterminate. For example, Zhao et al.[217] described a family in which a 28-year-old woman with a strong family history of ADPKD was being considered as a living kidney donor for her affected father. Because ultrasound screening in younger individuals cannot definitively rule out ADPKD, a genetic confirmation was pursued before clearing her as a kidney donor. Direct sequencing of the PKD1 and PKD2 genes in her father revealed two PKD1 variants of unknown clinical significance, so linkage analysis using markers at both PKD1 and PKD2 loci was performed on several affected and unaffected family members. This revealed that only a certain PKD1 haplotype cosegregated with all of the affected individuals, and the 28-year-old woman did not share this haplotype (Fig. 14.8). She was therefore cleared as a kidney donor.

Direct DNA sequencing is currently the most commonly used method of testing because it can often unequivocally determine whether the proband has inherited a disease-causing mutation without the participation of family members. There are certain limitations of direct mutation analysis, however, that need to be considered in interpreting a negative test. For example, one must consider whether genetic heterogeneity exists for the disease in question, and if so, whether all genes had been systematically evaluated. A second problem arises when a disease is not associated with a set of common mutations. In these cases, a laboratory will have to evaluate the gene's entire length for mutations. Depending on the size and complexity of the gene, the laboratory may elect to use screening techniques such as denaturing high performance liquid chromatography (dHPLC) rather than direct sequencing to search for suspected mutations, with DNA sequence analysis reserved only for suspicious changes.[218–221] None of the screening methods in common use have 100% sensitivity. A related problem is that the laboratory usually screens for mutations in a set of overlapping PCR products that includes only the coding region and the immediately adjacent splice sites. This strategy will miss pathogenic changes occurring in intronic sequences. Inevitably, a proportion of mutations will be missed, which may limit the conclusions that can be drawn from the data. Lastly, the implications of all changes may not be absolutely clear. Although some mutations (such as deletions or insertions) are clearly pathogenic, the status of amino acid substitutions may not be obvious. For example, it may be difficult to distinguish between a simple polymorphism and an amino acid change that disrupts a particular protein function.

The list of diseases for which DNA testing is available has grown considerably since the previous edition of this text and includes some forms of nephronophthisis, ADPKD, autosomal recessive polycystic kidney disease (ARPKD), medullary cystic disease, and hereditary nephrotic syndrome. In some cases, both linkage testing and direct mutation analysis is available. GENETests, an invaluable Web-based resource hosted at the University of Washington and funded by the NIH (http://www.genetests.org/) provides a comprehensive list of academic and commercial facilities that offer testing on either a clinical or research basis. The website also provides a library of contemporaneous reviews on numerous disorders as well as a variety of very informative educational resources.

The $1,000 genome, soon to become a reality, will significantly change the approach to DNA testing. The reduced cost and the increased throughput of "massively parallel" sequencing technologies will likely dramatically increase the number of diseases that can be evaluated. With these technologies, neither the number of genes that need to be considered nor the length of their respective sequences pose challenges. DNA testing services in the future may simply evaluate all nephrotic syndrome, NPHP, or PKD genes in a single test. Some investigators have suggested a future in which every individual has his or her DNA sequence determined as part of routine clinical practice and then given a risk assessment for both Mendelian and more common diseases. In addition to the obvious clinical challenge of how to interpret the numerous presymptomatic "risk variants" that will be identified by this approach, there are important technical, ethical, privacy, and intellectual property issues that must first be resolved. Without question, though, these technologies are rushing forward and will certainly impact clinical practice.

The impact of molecular studies on the treatment of disease has been, to date, somewhat limited. For diseases that result from inadequate amounts of functional protein (recessive diseases, haploinsufficiency states, tumor suppressor-type genes like PKD1 or VHL), strategies aimed at replacing the lost activity may treat the disorder. The most direct manner is by using gene therapy to express normal amounts of the correct protein in the target tissues. There remain huge obstacles to the widespread implementation of this approach for any disorder. Nonetheless, this strategy remains the "holy grail" of the field and may, in time, yield rich rewards.

Parallel efforts are underway to develop a detailed understanding of disease-associated proteins and the pathways in which they participate. The hope is that this knowledge will be used to target intermediate steps in disease pathogenesis for therapeutic intervention. This strategy may yield effective treatments before the barriers to gene therapy can be overcome. The cell culture and animal model systems described in earlier sections of this chapter are invaluable reagents for this type of research, and recent studies highlight the important contributions these resources offer. In the case of TSC, the studies first in Drosophila and then in vertebrate cells linked the TSC proteins to the regulation of mTOR kinase. Rapamycin, an agent widely used to prevent rejection in transplant patients because of its immunosuppressive properties, is known to inhibit this kinase.

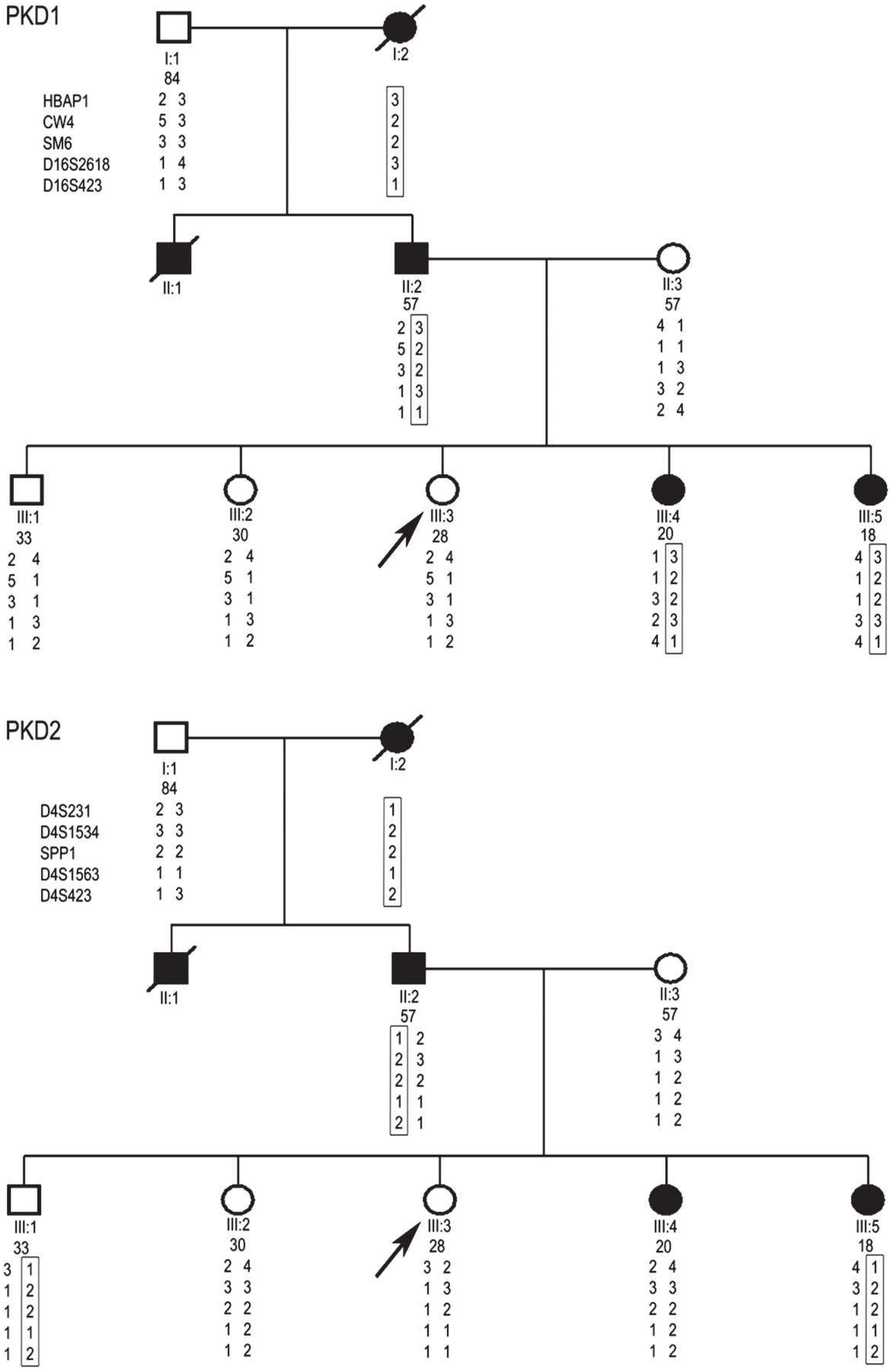

FIGURE 14.8 A haplotype analysis of an autosomal dominant polycystic kidney disease (ADPKD) family. The proband, a 28-year-old woman, is denoted by the *slanted arrows*. *Filled symbols* represent clinically affected individuals, and *unfilled symbols* represent unaffected individuals. Individual identification and age are indicated below each symbol. In this family, only the *PKD1* haplotype 3-2-2-3-1 (*top*) but not the single *PKD2* haplotype (*bottom*) cosegregated with the affected members. These data are consistent with this family's disease being *PKD1*-linked. The finding that the proband (III:3) did not carry the putative *PKD1* disease haplotype provided further reassurance that she was unaffected. (From Zhao X, Paterson AD, Zahirieh A, et al. Molecular diagnostics in autosomal dominant polycystic kidney disease: utility and limitations. *Clin J Am Soc Nephrol.* 2008 Jan;3(1):146–152. Used with permission of the Clinical Journal of the American Society of Nephrology.)

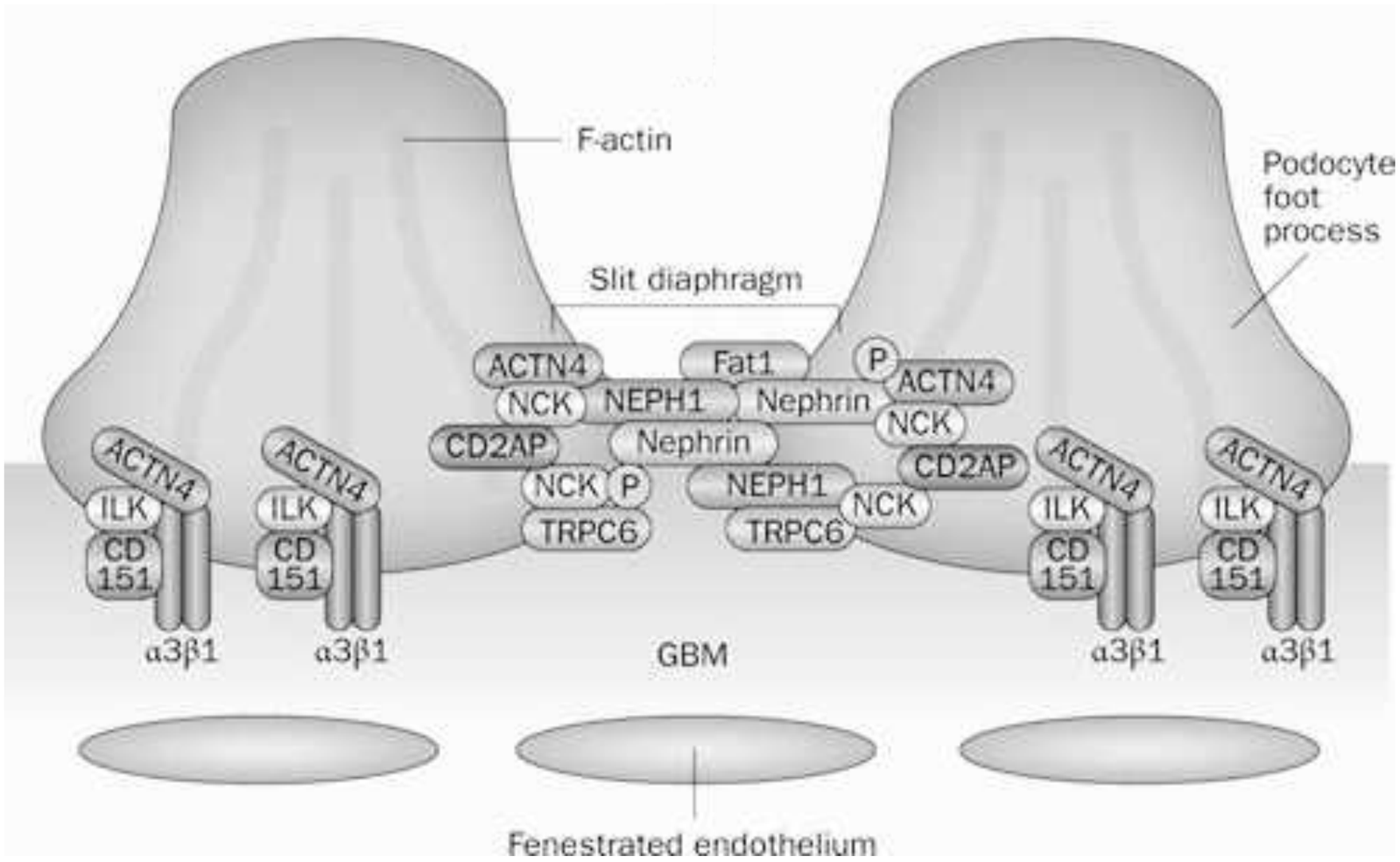

FIGURE 14.9 Podocyte foot processes, interconnected by slit diaphragms, form the final barrier for filtration. Normal function of the filtration barrier depends on proteins that anchor the foot processes to the GBM, including $\alpha 3\beta 1$ integrin, ACTN4, ILK, and the tetraspanin CD151, as well as those that are associated with the slit diaphragm, such as nephrin, NEPH1, podocin, Fat1, ACTN4, the adaptor protein NCK, CD2AP, and TRPC6. As outlined in Table 14.1, defects in many of these proteins have been found in various hereditary forms of nephrotic syndrome. ACTN4, α-actinin-4; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; ILK, integrin-linked kinase; P, podocin; TRPC6, transient receptor potential cation channel 6. (From Patrakka J, Tryggvason K. New insights into the role of podocytes in proteinuria. *Nat Rev Nephrol.* 2009 Aug;5(8):463–468. Reprinted by permission from Macmillan Publishers Ltd.)

Studies in mice and rats have shown that rapamycin or related compounds are effective at reducing the severity of TSC-related disease,[222–225] and clinical trials in humans are ongoing.[226–230]

In the case of ADPKD, insights gleaned from a variety of approaches culminated in an exciting new therapeutic initiative. In vitro studies of cultured cyst-lining cells from human specimens suggested that cystic epithelia responded to increased cyclic adenosine monophosphate (cAMP) activity with an increase in cell proliferation.[231–233] Reasoning that the vasopressin V2 receptor was the predominant regulator of cAMP activity in the collecting duct, the most prevalent site of cyst formation, Gattone and colleagues[234] tested a V2 receptor antagonist previously developed to treat hyponatremia in rodents with a syntenic form of ARPKD (PKHD1) and nephronophthisis (NPHP3). The investigators found a striking reduction of cyst volume and decreased interstitial fibrosis. In a follow-up report, they tested the same agent in a Pkd2 mouse model and found the same beneficial effects.[161] A clinical trial examining the efficacy of this class of drugs on the progression of human ADPKD is underway and should be completed in 2012.

Last, much of the focus of this chapter has been on advances in relatively rare monogenic disorders. Knowledge that has been gleaned from these studies, however, may also be applied to understanding the pathophysiology of more common disorders. For example, as a result of the identification of the genes involved in hereditary nephrotic syndromes, we have been able to synthesize a more coherent model of normal and abnormal podocyte structure and function (Fig. 14.9).[235] Similarly, mutations of the VHL gene have been implicated in the majority of clear cell renal carcinoma.[236,237] The result of studies of BBS have blurred the boundary between "traditional" Mendelian disorders and complex traits, and highlighted the likely continuum that exists between the two sets of conditions.[238] Significant advances in the ability to perform large-scale, population-based genome-wide association studies (GWAS), using databases such as the International HapMap Project (http://www.hapmap.org) and the 1000 Genomes Project (http://www.1000genomes.org) have allowed investigators to uncover associations between genetic variants and diseases with complex non-Mendelian inheritance such as hypertension, chronic kidney disease (CKD), and focal segmental glomerulosclerosis (FSGS). Notable discoveries using this approach include the association of variants in UMOD (uromodulin) with increased risk for CKD,[239–241] and of variants in APOL1 (apolipoprotein L1) with increased risk of FSGS in African Americans.[242,243] Continuing advances in population genetics research will likely provide further insights to decipher the genetic basis of these and other common polygenic disorders.

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CHAPTER

15 Nephronophthisis—Medullary Cystic Kidney Disease

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A group of hereditary renal diseases is summarized under the term NPHP-MCKD complex,[1,2] because the different disease entities share several features regarding (1) macroscopic pathology, (2) microscopic pathology, and (3) clinical symptoms (Table 15.1A). In this way the complex describes a distinct clinicopathologic entity.[3] The term nephronophthisis (NPHP) is used for the autosomal recessive variants, which lead to end-stage renal disease (ESRD) in the first 3 decades of life, whereas the term medullary cystic kidney disease (MCKD) refers to the autosomal dominant forms, in which ESRD develops in the third to seventh decade of life. Extrarenal manifestations such as ocular motor apraxia, retinitis pigmentosa, hepatic fibrosis, skeletal defects, and cerebellar vermis aplasia have exclusively been described in association with juvenile nephronophthisis. The only extrarenal associations in MCKD are hyperuricemia and gout. The identification of causative recessive genes in nephronophthisis has implicated the function of primary cilia and centrosomes in its pathogenesis.

FEATURES SHARED AMONG DISEASES OF THE NEPHRONOPHTHISIS-MEDULLARY CYSTIC KIDNEY DISEASE COMPLEX

Macroscopic Pathology

A major feature shared among the disease entities of the NPHP-MCKD complex (Table 15.1A[i]) is the appearance on macroscopic pathology as described in 27 patients with juvenile NPHP by Waldherr et al.[1] Kidney size is normal or moderately reduced. Cysts primarily appear at the corticomedullary border of the kidneys (Fig. 15.1). This is quite distinct from autosomal dominant and recessive polycystic kidney disease, where kidneys become grossly enlarged as a result of cystic dilatation throughout the organ. From the external surface, the kidney is indistinguishable from the kidney affected by glomerulonephritis or pyelonephritis. The surface usually has a finely granular appearance, most likely due to the protrusion of dilated cortical collecting ducts. Calices and pelvis appear completely normal. There are from 5 to over 50 cysts of 1 to 15 mm in diameter located preferentially at the corticomedullary border (Fig. 15.1). The cysts primarily arise from the distal convoluted and medullary collecting tubules as shown by microdissection,[4] but may also appear in the papilla. Cysts are not always present, but do occur in about 70% of autopsy cases. They apparently arise late in the course of the disease[5] and do not seem to be important for disease progression to renal failure.[6] Therefore, the presence of cysts is not a prerequisite for diagnosis.

Microscopic Pathology

The second shared feature among diseases of the NPHP-MCKD complex pertains to renal histology (Table 15.1A[ii]). The histologic changes are characteristic, but not pathognomonic, for the disease group. The characteristic histologic triad of NPHP-MCKD consists of (1) tubular basement membrane disintegration with irregular thickening as well as attenuation of the tubular basement membrane, (2) interstitial round cell infiltration with marked fibrosis and, (3) later in disease development, tubular atrophy with cyst development, which occurs predominantly at the corticomedullary junction (Fig. 15.2). Cysts seem to be the result rather than the cause of the atrophic process, although this time course could not be corroborated by statistical analysis.[1,7] Sometimes, a communication between a cyst and a tubule can be seen. The tubular basement membrane (TBM) is extremely thickened and multilayered. Fibroblasts are noted between the membrane layers. TBM changes and diverticulum formation are most prominent in the distal tubules, where cysts are lined with a single layer of cuboidal or flattened epithelium. In the advanced stage, the picture merges into a diffuse sclerosing tubulointerstitial nephropathy, the characteristic picture of end-stage NPHP-MCKD. The only significant glomerular change in early stages involves periglomerular fibrosis with a splitting and thickening of the Bowman capsule and glomerular obsolescence only in nephrons that

15.1 Shared and Distinguishing Features Among Diseases of the NPHP-MCKD Complex

A. Shared Features		
(i) Macroscopic pathology:	Corticomedullary cysts	
(ii) Microscopic pathology:	Tubuli: basement membrane disruption (thickening and attenuation), distal tubular atrophy and cysts Interstitium: round cell infiltration, fibrosis Glomeruli: periglomerular fibrosis only	
(iii) Symptoms:	Polyuria, polydipsia, anemia, growth retardation, ESRD	
B. Distinguishing Features		
	NPHP	**MCKD**
(i) Inheritance:	Autosomal recessive	Autosomal dominant
(ii) Median onset of ESRD:	Juvenile NPHP1: 13 yrs Infantile NPHP2: 1–3 yrs Adolescent NPHP3: 19 yrs NPHP4: 20 yrs NPHP5: 13 yrs	MCKD 1: 62 yrs MCKD 2: 32 yrs
(iii) Extrarenal associations:	Retinal degeneration, cerebellar vermis hypoplasia, hepatic fibrosis, cone-shaped epiphyses	Hyperuricemia, gout

ESRD, end stage renal disease; NPHP, nephronophthisis; MCKD, autosomal dominant medullary cystic kidney disease.

have been destroyed by the tubular alterations. An escape of Tamm-Horsfall (uromodulin) protein from damaged collecting tubules into the interstitium has been demonstrated in about 50% of patients with NPHP-MCKD as a periodic acid-Schiff (PAS)-positive material and by specific immunofluorescence staining with an anti-THP antibody.[8] Immunofluorescence does not otherwise contribute to the diagnosis of NPHP-MCKD.

Characteristic changes demonstrated by transmission electron microscopy include thickening, splitting, attenuation, and granular disintegration of the TBM (Fig. 15.3). The transition between these alterations is abrupt.[7] Fibroblasts are seen in direct contact with the TBM. At the base of the tubular epithelial cells, a marked increase of microfilaments is seen. The thickening is either homogeneous or has a lamellated, annular, and ringlike appearance. The glomerular basement membrane is normal. Multiple tubular diverticula are seen but the connections between cysts and distal tubular segments are patent.

Clinical Presentation

The third group of features shared among different diseases of the NPHP-MCKD complex involves clinical symptoms (Table 15.1A[iii]). Classical symptoms are polyuria, polydipsia, decreased urinary concentrating ability and, in children, anemia and growth retardation. The insignificance of the symptoms together with the lack of edema, hypertension, and urinary tract infections characteristically leads to a delayed diagnosis and therapy in NPHP-MCKD. In all variants of NPHP-MCKD, terminal renal failure insidiously ensues at characteristic age ranges, necessitating renal replacement therapy (Fig. 15.4). Disease recurrence has never been reported in kidneys transplanted to NPHP patients.[9]

FEATURES DISTINGUISHING DISEASE ENTITIES OF THE NPHP-MCKD COMPLEX

There are three features that clearly distinguish different disease entities of the NPHP-MCKD complex: (1) the mode of inheritance, (2) the age of onset for ESRD, and (3) the type of extrarenal organ involvement (Table 15.1B).

The Mode of Inheritance

In the NPHP-MCKD complex, the mode of inheritance can be either autosomal recessive or autosomal dominant.

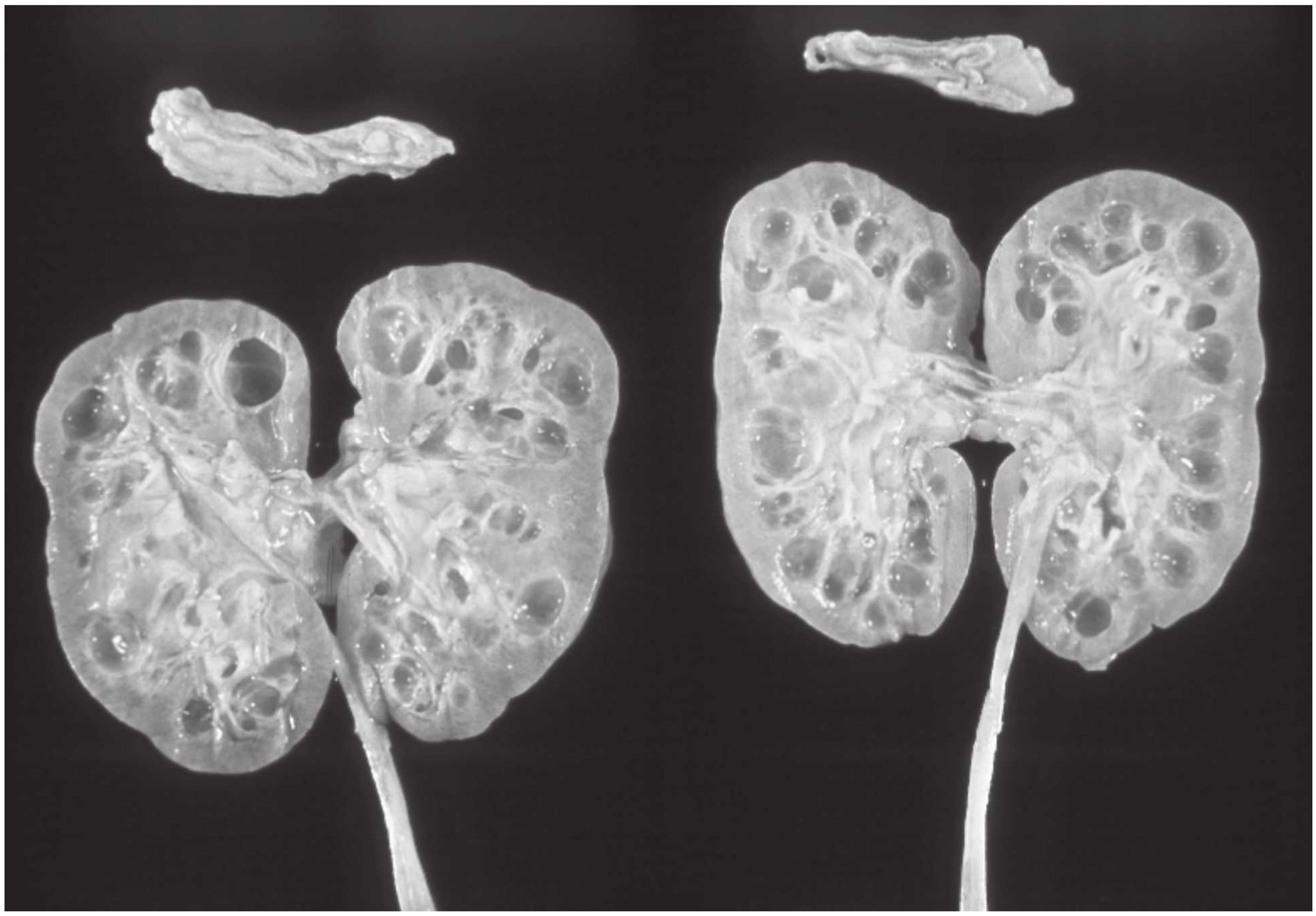


FIGURE 15.1 Juvenile nephronophthisis (autopsy case, 13-year-old girl). Note the numerous cysts of varying size in the medulla and at the corticomedullary junction. (Reproduced with permission from Hildebrandt F, Waldherr R, Kutt R, et al. The nephronophthisis complex: clinical and genetic aspects. *Clin Invest* 1992;70:802.)

For the recessive forms the term nephronophthisis (NPHP) is used, whereas the designation medullary cystic kidney disease (MCKD) denotes the dominant variants of the complex (Table 15.1B[i]).[10,11]

The Onset of End-Stage Renal Disease

The second distinction pertains to the age of onset of ESRD (Table 15.1B[ii]). In all variants of NPHP-MCKD, ESRD ensues at characteristic age ranges, necessitating renal replacement therapy (Fig. 15.4). In NPHP, chronic renal failure develops within the first 3 decades of life.[12–14] In a study conducted in 46 children with juvenile nephronophthisis (NPHP1), a serum creatinine value of 6 mg per deciliter was reached at a median age of 13 years (range: 4 to 20 years).[12,15] In a study by Waldherr et al.[1] ESRD was reached at a median age of 11.5 years. Gretz et al.[16] showed that the rate of deterioration of renal function was homogeneous in a study of 29 patients with NPHP1. The median time elapsing between a serum creatinine of 2 and 4 mg per deciliter was 32 months, between 4 and 6 mg per deciliter was 10 months, and between 6 and 8 mg per deciliter was 5 months.[16] A high concordance of the development of renal failure was noted in monozygotic twins.[17,18] Infantile nephronophthisis (NPHP2) is characterized by an early onset of ESRD between the neonatal period and 3 years of age.[14] In adolescent nephronophthisis (NPHP3), terminal renal failure develops at a median age of 19 years, which is 6 years later than in NPHP1.[13] The median age of ESRD in patients with NPHP4 and NPHP5 mutations is 20 years[19] and 13 years,[20] respectively. If renal failure has not developed by the age of 25 years, the diagnosis of recessive NPHP should be questioned and a pedigree analysis should be intensified to exclude dominant MCKD.

In MCKD, terminal renal failure occurs only in adult life. Two different variants are known, MCKD1 and MCKD2, with a median onset of ESRD of 62 years[21] and 32 years[22] respectively (Fig. 15.4).

Extrarenal Associations

The third distinguishing feature among variants of NPHP-MCKD is represented by the degree to which extrarenal associations occur (Table 15.1B[iii]). Extrarenal disease manifestations have only been described in recessive forms. One exception to this rule is the occurrence

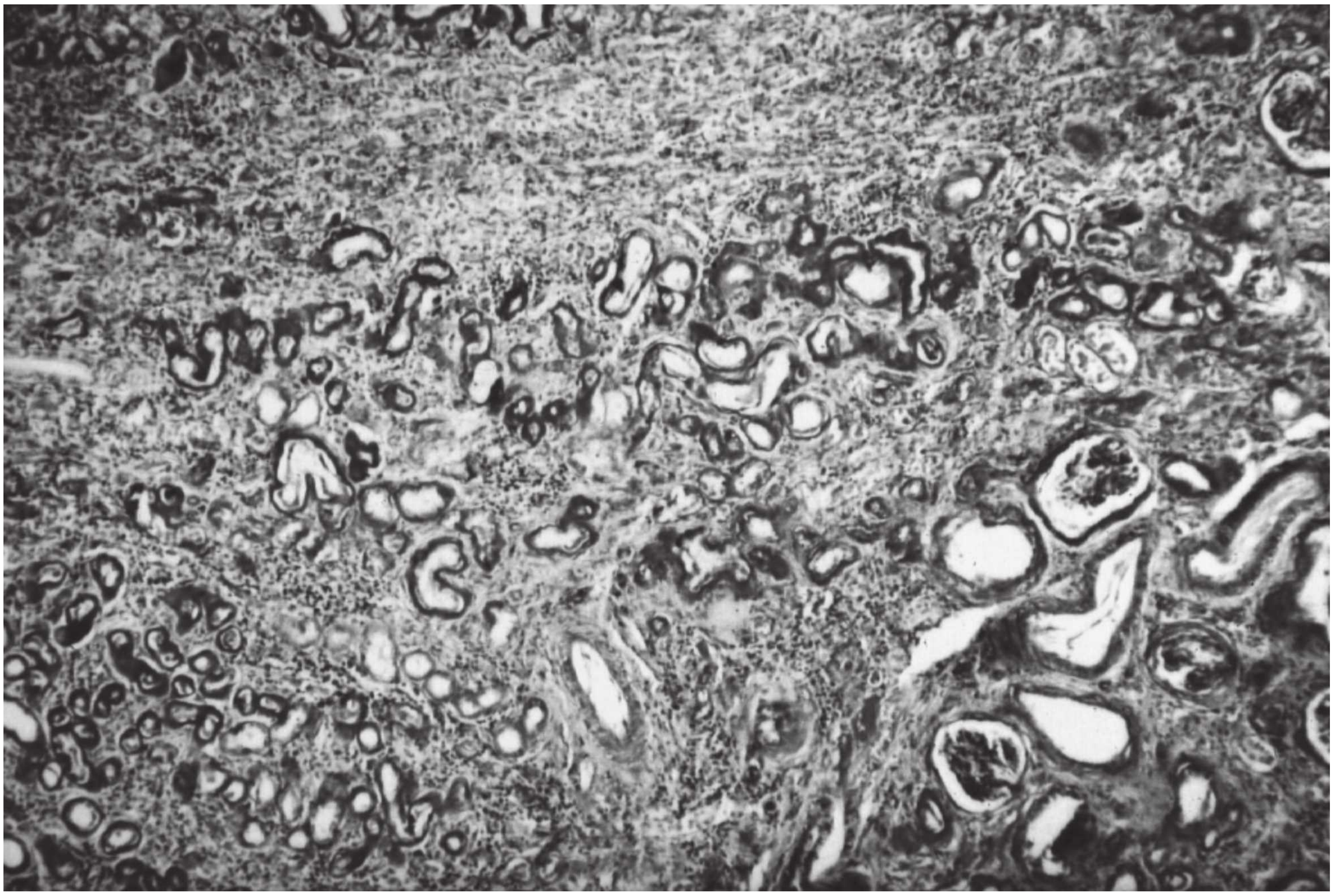


FIGURE 15.2 Renal histology in juvenile nephronophthisis (NPHP1). Note the characteristic triad, which consists of (1) tubular basement membrane disintegration with thickening as well as attenuation of the tubular basement membrane, (2) interstitial round cell infiltration with marked fibrosis, and later on (3) tubular atrophy and cyst development. (Courtesy of Prof. R. Waldherr, Heidelberg, Germany.)

of hyperuricemia and gout in MCKD1[23] and MCKD2.[22] MCKD2 patients with UMOD mutations also may exhibit defects in urine concentrating ability.[24] Recently, an extensive study on genotype–phenotype correlations in mutation of NPHP genes has been published.[25] NPHP1 can occur in combination with ocular motor apraxia Cogan type,[26,27] with retinitis pigmentosa in Senior-Løken syndrome (SLSN),[20] with liver fibrosis[28] with cone-shaped epiphyses in Mainzer-Saldino syndrome,[29] and with coloboma of the optic nerve and cerebellar vermis aplasia in Joubert syndrome type B (JBTSB) (Tables 15.1B[iii] and 15.2).[30] Infantile NPHP (type 2) can be associated with situs inversus[31] and one case report describes a patient with a nonsense inversin mutation with retinitis pigmentosa.[32] NPHP4 patients may have retinitis pigmentosa (SLSN) and Cogan syndrome.[33] NPHP5 patients display early onset retinitis pigmentosa (SLSN) in all known cases.[20] NPHP6 and NPHP8 patients have SLSN, Joubert syndrome, or Meckel-Gruber syndrome (MKS).[25,34,35] NPHP9 is associated with SLSN.[36] NPHP10 patients display SLSN and Bardet-Biedl syndrome (BBS)-like phenotypes,[37] whereas patients with NPHP11 show JBTS, MKS, and liver fibrosis.[38,39] NPHP12 patients exhibit Jeune asphyxiating thoracic dystrophy.[40]

Epidemiology

NPHP and dominant MCKD seem to be distributed evenly among males and females. NPHP has been reported from virtually all regions of the world.[41] Information on the incidence of the disease has been estimated at 9 patients per 8.3 million[42] in the United States or 1 in 50,000 live births in Canada.[1,43] The condition constitutes the most frequent genetic cause for ESRD in the first 3 decades of life and is a major cause of ESRD in children, accounting for 10% to 25% of these patients.[41,44,45] In contrast, in the North American pediatric ESRD population, pooled data indicate a prevalence of less than 5%.[46,47]

MCKD has initially been reported in the United States.[10] Its prevalence in Europe might have been underestimated because recently, kindred have been reported from Cyprus,[23] Italy,[22,48] France,[49] England, Finland,[50,51] Belgium, Czech Republic,[52] and Germany.[53,54] The diagnosis of MCKD may be frequently missed because clinical symptoms and signs are subtle.[55]

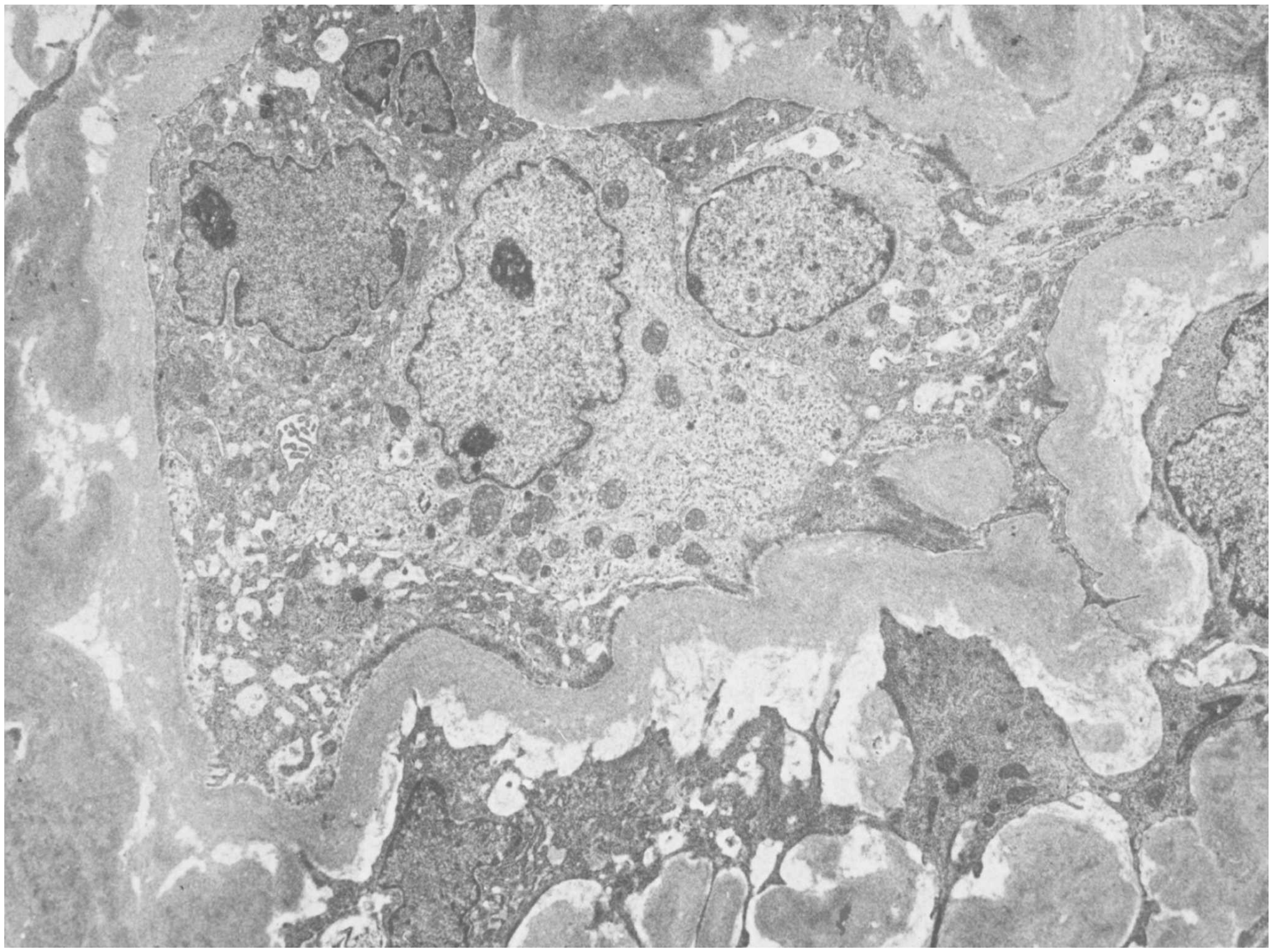


FIGURE 15.3 Thickening, wrinkling, and double layering of tubular basement membranes with intermembranous fibroblasts and dedifferentiation of tubular epithelial cells. An electron micrograph. (Reproduced with permission from Hildebrandt F, Waldherr R, Kutt R, et al. The nephronophthisis complex: clinical and genetic aspects. *Clin Invest* 1992;70:802.)

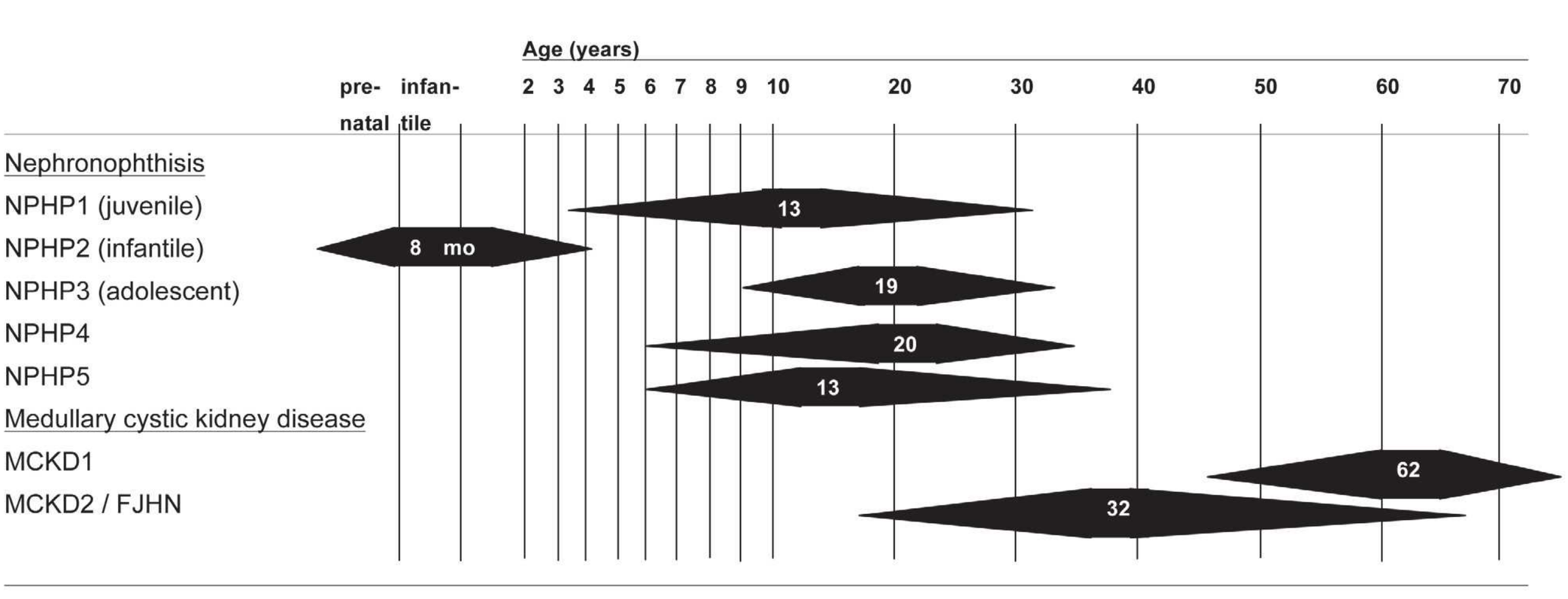


FIGURE 15.4 The time course of renal failure in NPHP-MCKD. Range for age of onset of end-stage renal disease is shown as solid triangles. Numbers indicates median age in years. *NPHP*, nephronophthisis; *MCKD*, medullary cystic kidney disease; *FJHN*, familial juvenile hyperuricemic nephropathy.

MOLECULAR GENETICS OF THE NEPHRONOPHTHISIS-MEDULLARY CYSTIC KIDNEY DISEASE COMPLEX

Classification of disease variants of the NPHP-MCKD complex has recently become more definite through the identification of distinct genes for the different variants. The disease complex is genetically very heterogeneous. Aspects of disease nomenclature, known genes, and extrarenal involvement within the NPHP-MCKD complex are summarized in Table 15.2.

Recessive Disease Variants: Nephronophthisis

Within recessive variants of the NPHP-MCKD complex, the different forms are distinguished on the basis of the mutated gene. To date, thirteen distinct genes (NPHP1–12, NPHPL1) have been identified by positional cloning and massively parallel sequencing.

Nephronophthisis Type 1 (Juvenile Nephronophthisis): Clinical Features

The first case of juvenile nephronophthisis was described by Smith and Graham[56] in 1945. This report of a sporadic case was followed by the publication of two large kindred with familial disease by Fanconi et al.,[57] who introduced the term familial juvenile nephronophthisis. This disease variant is now classified as nephronophthisis type 1 (NPHP1). Since the first description, over 300 cases of NPHP have been published in the literature.[2] NPHP1 is the most common variant within the NPHP-MCKD complex. Penetrance of recessive mutations is 100% by adolescence.

In juvenile NPHP, the symptoms of polyuria, polydipsia, decreased urinary concentrating ability, and secondary enuresis are the earliest presenting symptoms in over 80% of cases[41] and occur at around 4 to 6 years of age. Pallor, weakness, and generalized pruritus are also common. Anemia[51] and, in children, growth retardation occur later and are pronounced. In juvenile NPHP, children usually start to drink regularly at nighttime around age 6 years. This characteristic symptom should actively be sought when taking the patient's history. The mild nature of symptoms, together with a frequent lack of edema, hypertension, and urinary tract infections, characteristically leads to delayed diagnosis and therapy in NPHP-MCKD. Due to the late detection of symptoms, there is a small but definite risk of sudden death from fluid and electrolyte imbalance. For NPHP1, definite molecular genetic diagnosis is possible (see the text that follows). Disease recurrence has never been reported in kidneys transplanted to NPHP patients.[9]

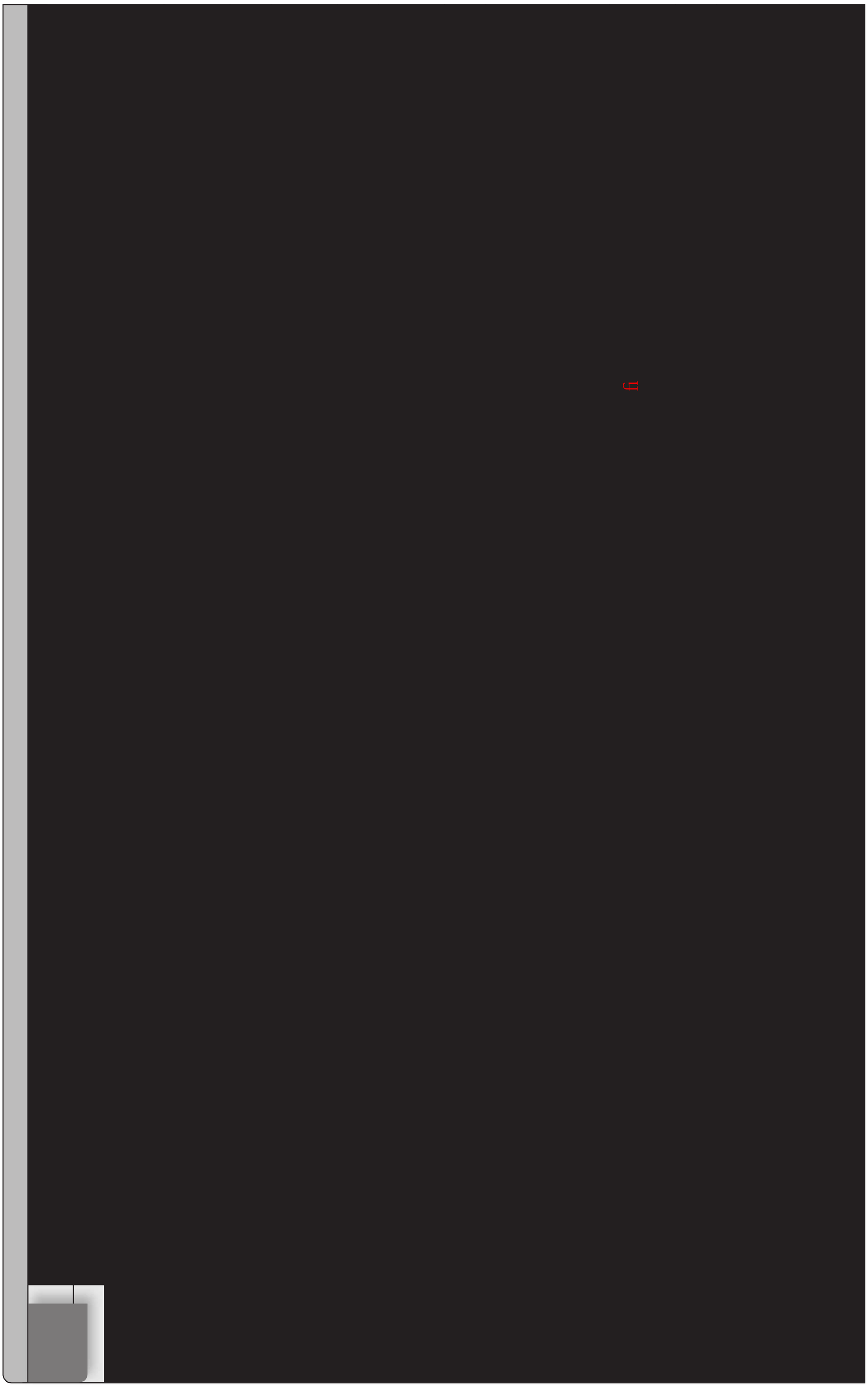
Nephronophthisis Type 1: Molecular Genetics

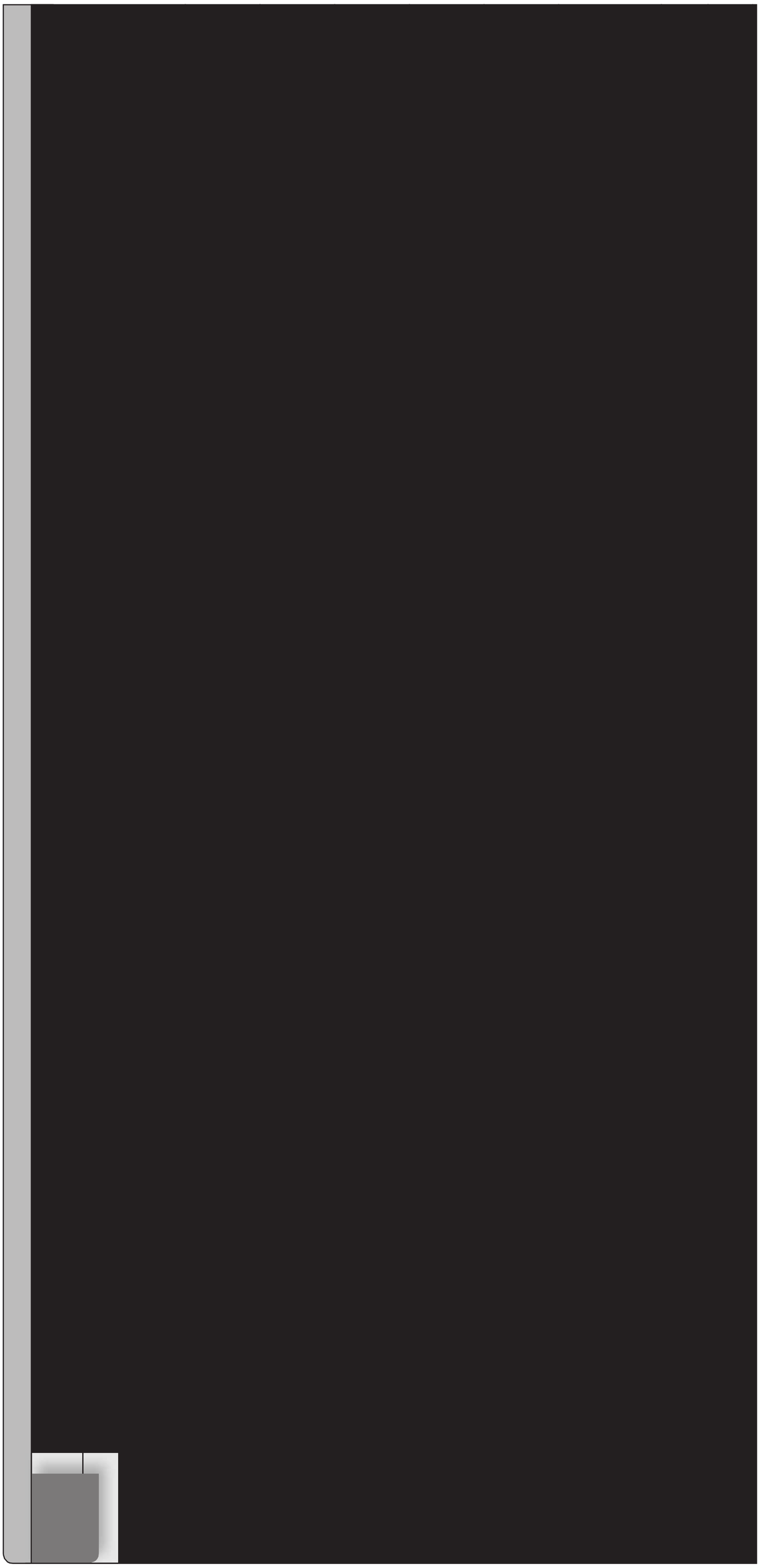
Because little was known about the pathogenesis of NPHP, a positional cloning approach was used for gene identification. By total genome search for linkage, a gene locus for juvenile NPHP1 was mapped to human chromosome 2q12-q13.[58,59] The critical genetic region was subsequently cloned in yeast artificial chromosome (YAC) and P1-derived artificial chromosome (PAC) contigs, which led to the identification of the NPHP1 gene, defects in which are responsible for NPHP1.[60,61] About 66% of children with juvenile NPHP harbor large (250 kb) homozygous deletions of the NPHP1 gene, whereas some carry point mutations in combination with heterozygous deletions.[62–64] Through gene identification, a molecular genetic diagnosis in NPHP1 has become possible (see the text that follows).[64–67]

The NPHP1 gene spans 83 kb, consists of 20 exons, and encodes an mRNA of 4.5 kb. It is flanked by two large (330 kb) inverted duplications. In addition, a second sequence of 45 kb, which is located between the centromeric inverted duplication and the NPHP1 gene, is repeated directly within the telomeric inverted duplication. In several NPHP1 families, the deletion break points have been localized to the 45 kb direct repeats using pulsed field gel electrophoresis.[63] Chromosomal misalignment followed by unequal crossing over or the formation of a loop structure on a single chromosome has been suggested as a potential cause for these deletions. In addition, there is a high degree of further rearrangements known to occur in this region of chromosome 2.[63] Furthermore, an unusual maternal deletion in a child with NPHP1 molecularly characterized, showing that the centromeric break point occurred within a long interspersed nuclear element-1 (LINE1).[68] The NPHP1 gene is a novel gene, which is not related to any known gene families. Expression studies in humans and mice revealed a broad tissue expression pattern. In addition, in situ hybridization studies of whole mount mouse embryos showed ubiquitous but weak Nphp1 expression at all embryonic stages between days 7.5 and 11.5 postconception.[69] In the adult mice, there was also a strong expression in testes.

Nephronophthisis Type 2

A second gene locus (NPHP2) for recessive NPHP has been localized to chromosome 9q31.1 in a large Bedouin pedigree by homozygosity mapping (Table 15.2).[14] This disease variant is termed infantile nephronophthisis (NPHP2) due to its prenatal, perinatal, or infantile onset. The clinical course and histology in this disease are quite different from other forms of NPHP.[70] The inv mouse model, in which a disruption of the protein inversin led to a consistent reversal of the left–right body axis,[71] was noted to have cystic kidney disease.[72,73] These observations led to the identification of inversin (INVS) as the gene mutated in NPHP2 with and without situs inversus.[31] INVS encodes a 1,062 amino acid protein containing 16 ankyrin repeats, a nuclear localization signal, and an IQ calmodulin domain.[74] Yeast two-hybrid and coimmunoprecipitation experiments have confirmed the interaction between inversin and calmodulin. By a knockdown of inversin expression in zebra fish, a polycystic kidney disease (PKD)-like phenotype in addition to a randomization of heart looping was observed.[31]





Inversin localizes to primary cilia, mitotic spindles, and centrosomes[74] and is intimately associated with the microtubule cytoskeleton.[75] INVS/NPHP2 mutations remain a rare cause of NPHP, accounting for <1% of cases.

Nephronophthisis Type 3

A third locus (NPHP3) for NPHP was mapped to chromosome 3q22.1 in a large Venezuelan kindred by a total genome search for linkage by applying the strategy of homozygosity mapping (Table 15.2).[13,76,77] This disease variant was termed adolescent nephronophthisis (NPHP3) because the onset of ESRD occurs 6 years later than in juvenile NPHP1, with a median onset of terminal renal failure occurring at age 19 years (Fig. 15.4).

Identification of the gene NPHP3, which causes adolescent NPHP, was carried out in the same Venezuelan kindred.[78] The novel NPHP3 protein interacts with nephrocystin-1. NPHP3 mutations were found in patients with isolated MPHP and in families with NPHP and hepatic fibrosis or tapetoretinal degeneration. Murine Nphp3 was shown to be expressed in the embryonic node, kidney tubules, retina, respiratory epithelium, liver, biliary tract, and neural tissues. A homozygous missense mutation in Nphp3 was identified as the underlying defect in the polycystic kidney disease (pcy) mouse phenotype.[78]

Nephronophthisis Type 4

By a total genome search for linkage, a fourth gene locus (NPHP4) was localized to chromosome 1p36,[79] including a family with SLSN. The respective gene (NPHP4) was subsequently identified as causing NPHP type 4 and SLSN type 4.[19,33] The gene and its gene product, nephroretinin/nephrocystin-4, are highly conserved in evolution. Nephrocystin-4 protein expression has been demonstrated in primary cilia, centrosomes, and near the cortical actin cytoskeleton, showing partial colocalization with β-catenin in polarized MDCK cells.[33] Co-immunoprecipitation experiments showed that nephrocystin, p130Cas, and Pyk2 are in a complex with nephrocystin-4.[33] More recently, it was demonstrated that NPHP4, in conjunction with NPHP1, interacts with the conserved polarity complex PALS1/PATJ/Crb3.[80] This data may reconcile the involvement of the nephrocystin complex of proteins in both, at the adherens junction and at the cilia/centrosomes (Fig. 15.5).

Nephronophthisis Type 5

NPHP5 (ICQB1) is a novel gene, which was identified by positional cloning as a cause of NPHP type 5.[20] All of the mutations found in NPHP5 and its protein product, nephrocystin-5, are the result of truncating mutations. Interestingly, all mutations were associated with the presence of early onset SLSN/retinitis pigmentosa where blindness occurred before the third year of life.[20] The nephrocystin-5 protein directly interacts with calmodulin and is in a protein complex with the retinitis pigmentosa GTPase regulator (RPGR), thus explaining the renal–retinal phenotype of the disease.

Nephronophthisis Type 6

By positional cloning, mutations in NPHP6 (CEP290) were identified as causing NPHP type 6.[81,82] Patients with truncating NPHP6 mutations displayed JBTS phenotype (JBTS6), whereas missense mutations caused SLSN. NPHP6 was shown to regulate the activity of the cAMP-regulated transcription factor CREB2/ATF4, suggesting that the loss of NPHP6 function leads to aberrant gene expression that may contribute to disease progression in NPHP6.[81] A direct interaction between NPHP5 and NPHP6 was demonstrated in zebra fish, where the depletion of either gene led to almost identical phenotypes.[83]

Nephronophthisis Type 7

Mutations in NPHP7 (GLIS2) have been identified in a single family as causing NPHP type 7.[84] GLIS2 is a transcription factor that negatively regulates the Sonic hedgehog pathway mediator GLI1 transcriptional activity by binding to GLI-binding sites.[85] NPHP7 activity is required in the adult kidney to suppress the Shh pathway activation. Loss of Nphp7 leads to the inactivation of the Snail and Wnt4 genes, which in turn initiates tubular dedifferentiation and epithelial-to-mesenchymal transition in the kidney, resulting in intestinal fibrosis.[86] NPHP7, together with NPHP6, is the second NPHP gene that is implicated in gene expression and regulation.

Nephronophthisis Type 8

Mutations in NPHP8 (RPGRIP-like 1, [RPGRIPL1]) were shown to cause NPHP type 8 in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel-Gruber syndrome.[35,87,88] Mutations in NPHP8 cause a wide range of phenotypes.[25]

Nephronophthisis Type 9

Homozygous mutations in NPHP9 (never in mitosis A-related kinase 8 [NEK8]) were shown to cause NPHP type 9.[36] NEK8 interacts and colocalizes with NPHP2/INV and NPHP3 to the proximal segment of the primary cilia.[89] The interaction of NEK8 with the autosomal dominant polycystic kidney disease (ADPKD) protein PKD2 suggests that NEK8 may regulate both the expression and posttranslational modification (phosphorylation) of both PKD1 and PKD2.[90]

Nephronophthisis Type 10

Mutations in the NPHP10 (SDCCAG8) gene were recently identified by candidate exome capture and massively parallel sequencing as causing NPHP type 10.[37] All patients carried two truncating mutations and developed juvenile NPHP with associated retinitis pigmentosa (SLSN). Interestingly, some patients presented Bardet-Biedl syndrome-like features, including obesity and hypogonadism.[37]

Nephronophthisis Type 11

Homozygous and compound heterozygous missense mutations in the NPHP11 (transmembrane protein 67 [TMEM67/MKS3]) gene were shown to cause NPHP type 11.[39] Similar

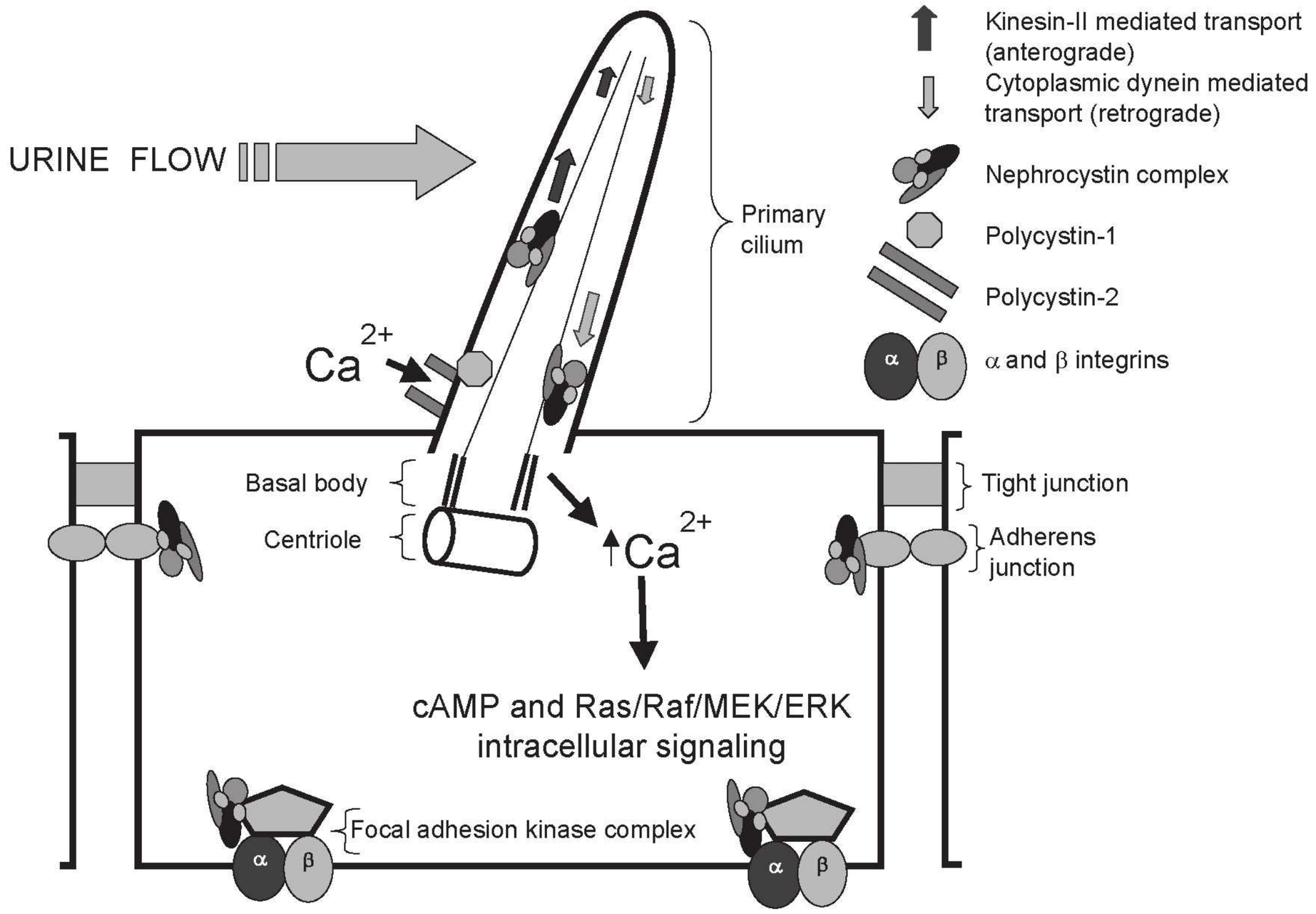


FIGURE 15.5 Localization of the nephrocystin complex to the renal epithelial cell primary cilium, the adherens junction, the focal adhesions, and the microtubule organizing centers (centrosomes). A primary renal cilium is shown bending as a result of urinary flow. Fluid shear forces lead to an increase in intracellular calcium, mediated by calcium permanent channels, such as polycystin-2, localized to the surface of the cilia. This initial calcium influx may lead to multiple downstream effects including calcium-induced calcium release, targeted fusion of cytoplasmic vesicles with the plasma membrane, protein kinase signaling cascades, and gene expression, which may modulate cellular proliferation, differentiation, and apoptosis. (Modified from Nauli SM, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 2003;33:129; and Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol* 2001;184:71.) Nephrocystin complexes are shown within the cilium, where they are moved up the cilium by intraflagellar transport protein complexes (kinesin-II) and down via the cytoplasmic dynein. The precise function of the nephrocystin complex within the cilium remains to be established. In addition to the cilium, inversin and nephrocystins have been localized to the adherens junctions, the centrosomes, and the nucleus (not shown). Nephrocystin complexes are also shown localized to the focal adhesion kinase complex, which includes proteins, such as pyk2 and p130Cas, and binding partners of nephrocystin-1 and nephrocystin-4. (Modified from Mollet G, et al. Characterization of the nephrocystin/nephrocystin-4 complex and subcellular localization of nephrocystin-4 to primary cilia and centrosomes. *Hum Mol Genet* 2005;14:645; and Benzing T, et al. Nephrocystin interacts with Pyk2, p130 (Cas), and tensin and triggers phosphorylation of Pyk2. *Proc Natl Acad Sci USA* 2001;98:9784.) Cell adhesion signaling events and polarity may be regulated by the nephrocystin complex at this location.

to NPHP3, NPHP6, and NPHP8, mutations in NPHP11 can cause a syndromic ciliopathy, with phenotypes ranging from SLSN and JBTS to MKS.[91]

Nephronophthisis Type 12

NPHP12 (tetratricopeptide repeat domain 21B [TTC21B]) is a novel gene that has very recently been identified as the cause of NPHP type 12.[40] Because mutations in NPHP12 were identified in 5% of other ciliopathy cases, it was suggested that NPHP12 mutations may have a role as genetic modifier mutations in ciliopathies.[40]

Nephronophthisis-like 1

Mutations in the XPNEPEP3 (X-prolyl aminopeptidase 3) gene were shown to cause an NPHP-like 1 (NPHPL-1) nephropathy.[92] Unlike other NPHP proteins, the NPHPL-1 gene product does not localize to the primary cilia or centrosomes. Instead, the enzyme XPNPEP3 harbors a mitochondrial leader sequence and localizes to mitochondria.[92] Possible involvement of NPHPL-1 in ciliary function is suggested by the presence of putative XPNPEP target sequences on several centrosomal proteins, including NPHP6/CEP290.[92]

Nephronophthisis with Extrarenal Associations

With the exception of the occurrence of hyperuricemia and gout in MCKD, extrarenal disease manifestations have only been described in recessive forms of nephronophthisis (Tables 15.1B and 15.2). Ocular motor apraxia Cogan type is a transient inability of horizontal eye movements occurring in the first few years of life. This has been described in patients with mutations in NPHP1[26,27] or NPHP4.[33]

SLSN, represented by a concomitant occurrence of NPHP with retinitis pigmentosa, was first described by Contreras et al.,[93] Senior et al.,[94] and Løken et al.[95] The designation SLSN seems more appropriate than the term retinal-renal dysplasia, because both renal and retinal changes are degenerative rather than dysplastic.[96] In NPHP types 1 through 4 retinitis pigmentosa occurs in about 10% of all affected families. In NPHP type 5, all patients exhibited early onset retinitis pigmentosa,[20] whereas in NPHP type 10, nearly all patients display late onset retinitis pigmentosa.[37] Retinitis pigmentosa is diagnosed by its specific findings on an ophthalmoscopy including increased pigment, attenuation of retinal vessels, and pallor of the optic disc, and is coupled with the results of an electroretinography and electrooculography. Retinal degeneration is characterized by a constant and complete extinction of the electroretinogram, which precedes the development of visual and funduscopic signs of retinitis pigmentosa.[97,98] The early onset and the late onset type of SLSN have been distinguished. The early onset type seems to represent a form of a Leber congenital amaurosis because children exhibit coarse nystagmus and/or blindness at birth or within the first 2 years of life.[99] Funduscopic alterations are present in all SLSN patients by the age of 10 years. The late onset form is characterized by the development of blindness during the school age years after a preceding night blindness. Other eye symptoms besides tapetoretinal degeneration include nystagmus, myopia, and coloboma of the choroidea.[57] The age of onset, symptoms, and a histology of renal disease is identical to what is known from patients with juvenile nephronophthisis without ocular involvement.

The association of NPHP with the degenerative phenotype of liver fibrosis was first noted by Boichis et al.,[28] and was later also reported by others.[100–102] All patients had hepatomegaly and moderate portal fibrosis with mild bile duct proliferation. This pattern differs from that of classical congenital hepatic fibrosis, where biliary dysgenesis is prominent. Cases with skeletal changes, predominantly in the form of cone-shaped epiphyses (type 28 and 28A) are known as Mainzer-Saldino syndrome, and were first published by Mainzer et al.[29] in combination with cases of retinal degeneration and cerebellar ataxia. Recessive mutations in the NPHP3, NPHP4, NPHP6, NPHP8, and NPHP11 genes have been described in patients with NPHP and liver degenerative phenotypes[25,78] where NPHP11 mutations seem to be the most frequently associated.[39]

In Joubert syndrome type B (JBTS), a developmental disorder with multiple organ involvement, NPHP occurs in association with coloboma of the eye or retinal degeneration, aplasia of the cerebellar vermis with ataxia, the facultative symptoms of psychomotor retardation, and neonatal tachy/dyspnea.[30,103,104] A diagnostic feature of Joubert syndrome on an axial magnetic resonance imaging (MRI) of the brain is prominent superior cerebellar peduncles, termed the molar tooth sign (MTS).[104] Thirteen genes have now been shown to cause Joubert syndrome. NPHP1 gene defects are a rare cause of Joubert syndrome in a subset of patients with NPHP.[105] Additionally, patients with mutations in NPHP3, NPHP6, NPHP8, NPHP11, and NPHP12 genes display JBTS.[25,35,39,40,81] A homozygous deletion of NPHP1 was found in two siblings and in a third patient with mild features of Joubert syndrome type B. The second gene defect was found in the Abelson Helper Integration Site (AHI1) gene, and its protein product has been termed Jouberin. The Jouberin protein has three known isoforms and possesses a coiled coil domain, at least six WD40 domains, and an SH3 domain. It is thus likely to be part of the nephrocystin complex of proteins. In the initial reports of AHI1 mutations, the phenotype included cerebellar abnormalities, but no renal phenotype was reported. Recently, Jouberin mutations were detected in patients with NPHP and JBTS.[106]

Additional phenotypes have been described in association with NPHP. These include Jeune syndrome (asphyxiating thoracic dysplasia)[107–109]; Meckel-Gruber syndrome[25,35,91]; Ellis van Creveld syndrome[110]; ulcerative colitis[111]; retinitis pigmentosa, hypopituitarism, nephronophthisis, and mild skeletal dysplasia (RHYNS) syndrome[112]; Alstrom syndrome[113]; Sensenbrenner syndrome[114–117]; and Arima syndrome.[118,119]

BBS[120,121] has been reported to exhibit renal histology findings reminiscent of NPHP. Recently, it was shown by using candidate exome capture and massively parallel sequencing that mutations in SDCCAG8/NPHP10 cause BBS-like phenotype (without polydactyly) in NPHP type 10 patients.[37] Gene identification of NPHP genes has revealed that the molecular relation between these diseases may lie in the expression of the respective gene products in primary cilia, basal bodies, or centrosomes of renal epithelial cells.[2]

Animal Models of NPHP

Genetic animal models resembling NPHP have been fruitful in the identification of underlying gene defects, and more recently in the experimental treatment of cystic kidney disease.

The Invs gene, when mutated, gives rise to renal cysts as well as left–right asymmetry, cardiovascular defects, hepatobiliary defects, and premature death in inv/inv knockout mice.[72,122] Collecting ducts of newborn inv/inv mice demonstrate diffuse cystic dilatation.[123] Mutations in INVS give rise to human NPHP type 2, with and without situs inversus. The pcy mouse model[123] demonstrates interstitial fibrosis and cystic kidneys. The underlying defect was shown to be a missense mutation in the Nphp3 gene.[78] Recently, mouse

models of Nphp1,[124] Nphp4,[125] Nphp7/Glis2,[86] and Nphp9/jck[126] have been reported. Unexpectedly, the phenotype of Nphp1$^{-/-}$ mice is relatively mild, affecting only spermatogenesis.[124] Another study, with a different Nphp1 knockout design, uncovered an epistatic relationship between Nphp1 and Ahi in regulating the severity of retinal degeneration phenotype first in mice and then in humans,[127] underlining the strength of the animal models in studying the pathomechanisms of NPHP. Similarly to Nphp1 knockout mice, homozygous mutant Nphp4 mice do not display renal phenotype, but present a severe retinal degeneration and male infertility phenotype.[125] The Nphp7 knockout mouse model has been instrumental in deciphering the underlying molecular defects causing NPHP7 by showing that the derepression of Sonic hedgehog (Shh) signaling in the adult kidney leads to epithelial to mesenchymal transition and, ultimately, interstitial fibrosis—the disease phenotype in NPHP7 patients.[84,86] The juvenile cystic kidney (jck) mouse model has been used extensively as a model for PKD, and its renal phenotype is caused by a recessive missense mutation in the Nphp9 gene.[126] This model has been fruitful for testing different modalities to ameliorate renal cystic disease progression. It was shown that cyst formation in jck mice can be suppressed by treatment with the CDK inhibitor roscovitine[128] or the glycosylceramide synthase inhibitor.[129]

The kd (kidney disease) mouse strain has also been reported as a genetic animal model of NPHP.[130,131] It shares several clinical and histologic[131] features with human NPHP. The mice are born healthy, but by 8 weeks of age, they develop severe interstitial nephritis that progresses to ESRD by 4 to 8 months of age. The defect is caused by a mutation of a gene that encodes a mitochondrial protein, namely prenyltransferaselike mitochondrial protein (PLMP).[132] Kd/kd mice were shown to have dysmorphic mitochondria within renal tubular epithelia.[132] Additional transgenic mouse models for NPHP, such as the tensin knockout mouse,[132] the bcl-2 knockout mouse,[133–135] and the Ace knockout mouse[136] will hopefully aid our understanding of the pathophysiology of NPHP.[137] A canine model of NPHP has also been reported.[31,138–140]

The Pathogenic Hypotheses of Nephronophthisis

The identification of mutations in the inversin gene, which cause NPHP type 2, established a link between the pathogenesis of NPHP to disease mechanisms of PKD.[31] The knockdown of invs in the zebra fish embryo causes a renal cystic phenotype. In addition, the positional cloning of the novel gene NPHP3, mutated in adolescent NPHP (type 3) and in the renal cystic mouse model pcy,[123] confirmed this paradigm. Nephrocystin interacts with both inversin and with b-tubulin, with colocalization of all three proteins in the primary renal cilia of epithelial cells.[74] Inversin was also shown to be localized to mitotic spindles and centrioles.[74] The IQ calmodulin-binding motif containing protein-1 (IQCB1), also known as nephrocystin-5, also reveals a ciliary and basal body colocalization.[20] All NPHP proteins identified so far share the localization to cilia, centrosomes, or the basal body, with the exception of mitochondrial NPHPL1. The finding that such nephrocystins colocalize to primary cilia, basal bodies, or centrioles together with other proteins that, if defective, cause renal cystic diseases, suggests a role within a functional module shared with other proteins (Fig. 15.5). Therefore, recently, a unifying hypothesis of renal cystogenesis has been established, thus characterizing renal cystic diseases as ciliopathies.[141] This hypothesis states that proteins, which, if mutated, cause renal cystic disease in humans, mice, or zebra fish, are part of a functional module, as defined by their subcellular localization to primary cilia, basal bodies, or centrioles.[2] This applies to polycystin-1 and -2, fibrocystin/polyductin, nephrocystin-1, -2 (inversin), -3, -4, -5, BBS-associated proteins, cystin, polaris, ALMS1, oral-facial-digital syndrome type 1 (OFD1), and others. The existence of such functional modules in ciliopathies was recently demonstrated by proteomic studies, showing that NPHP-JBTS-MKS proteins function in distinct modules that are mechanistically connected.[142]

A model of evolutionary conserved proteins involved in cilia has also recently added weight to the "cystogenes" hypothesis. Following identification the NPHP1, 2, and 4 genes, orthologs of these genes in the nematode Caenorhabditis elegans have been identified.[19,60,69] This strong evolutionary conservation of genes that, if defective, cause NPHP in humans, suggests that their products may be part of a functional module conserved in C. elegans. This assumption was also supported by the finding of cell-specific GFP expression under the nephrocystin-1 and -4 promoters in the same cell types of head and tail ciliated neurons, in which the C. elegans orthologs of other renal cyst-causing genes are expressed, such as pkd-1 (lov-1), pkd-2, and polaris (osm 5).[143]

In addition to the ciliary hypothesis, an adherens junction/focal adhesion hypothesis has also been suggested on the basis that nephrocystin-1 contains an SH3 domain.[60,144] This theory is based on the fact that most SH3 domains are found in adapter proteins, which have a function in focal adhesion signaling complexes of cell-matrix contacts.[145,146] Several findings support this hypothesis, such as:

1. Nephrocystin was shown to bind to the protein p130Cas ("crk-associated substrate"),[147–149] which is a major mediator of focal adhesion assembly[145] and to compete for binding with Src and Fyn.[147]
2. In children with NPHP, Rahilly and Fleming[150] described strong $\alpha 5\beta 1$ integrin expression in proximal tubules, from which $\alpha 5$ integrin is normally absent, which most likely results from defective $\alpha 6$ integrin expression. The $\alpha 5\beta 6$ complex is an important receptor for focal adhesion signaling in renal tubular cells.
3. The knockout mouse models for tensin[132] and for the Rho GDIa gene[151] both exhibit an NPHP-like phenotype, thereby implicating proteins of the focal adhesion signal transduction cascade in the pathogenesis of NPHP-like diseases.

Together, these findings may point to a pathogenesis of NPHP, which involves focal adhesion and/or adherens junction signaling processes. Data from Mollet et al.[152] demonstrated, in addition to ciliary and centrosomal localization, that NPHP4 was part of a subplasmalemmal protein complex which included NPHP1, p130Cas, and Pyk2. These data confirm the role of the nephrocystin proteins within both a cilial/centrosomal hypothesis and an adherens junction/focal adhesion hypothesis.

Medullary Cystic Kidney Disease

The first large kindreds of autosomal dominant medullary cystic kidney disease (MCKD) were reported by Goldman and by Gardner.[10,153,154] Dominant MCKD by a renal macroscopic pathology and histology is indistinguishable from recessive NPHP. In MCKD, terminal renal failure develops later than in NPHP, within the seventh decade of life (Fig. 15.4). The only extrarenal associations known to occur with MCKD are hyperuricemia and gouty arthritis, which have been described in the majority of the kindred reported.

Medullary Cystic Kidney Disease Type 1 (MCKD1)

A gene locus for MCKD1 was mapped to chromosome 1q21 in large pedigrees from Cyprus.[21] Further refinement of this locus has been possible using an observed recombinant from within a Belgian kindred,[155] reducing the critical genetic region to 2.1 Mb (Table 15.2). This disease form was associated with hyperuricemia and gout.[23] ESRD occurred at a median age of 62 years (Fig. 15.4).

Medullary Cystic Kidney Disease Type 2 (MCKD2)

A second locus (MCKD2) for medullary cystic kidney disease was localized to chromosome 16p12 (Table15.2).[22,156,157] In this variant, ESRD develops much earlier, at a median age of 32 years (Fig. 15.4). MCKD2 and an autosomal dominant disease formerly known as familial juvenile hyperuricemic nephropathy (FJHN)[158] have been shown to map to the same chromosomal region and suggest that they represent the same disease entity.[159,160] This was confirmed with the identification of mutations within the uromodulin (UMOD) gene in affected patients with phenotypes of FJHN and MCKD2.[161] UMOD encodes the Tamm-Horsfall protein, which is a GPI-anchored glycoprotein and is present abundantly in normal urine. It has been suggested that mutations within UMOD may disrupt the tertiary structure of UMOD.[161] A clustering of UMOD mutations was noted within the highly conserved exon 4 of the encoded sequence of UMOD.[162] An investigation of UMOD mutations in the urine of affected individuals and renal biopsies revealed an abnormal accumulation of uromodulin within tubular cells and reduced urinary excretion of wild-type uromodulin.[163] Glomerulocystic kidney disease (GCKD), characterized by dilatation of the Bowman space and the collapse of the glomerular tuft, is a renal disorder distinct from MCKD/FJHN, although some clinical features are shared. A clinical variant of GCKD demonstrates a reduced fractional excretion of uric acid, resulting in hyperuricemia. A mutation in UMOD was recently described in one family (three patients) with this condition, thus broadening the phenotype associated with UMOM mutations further.[164] Finally, there is evidence for at least one additional locus for MCKD.[53,54]

MOLECULAR GENETIC DIAGNOSIS, IMAGING, AND LABORATORY STUDIES

Molecular Genetic Diagnosis in Nephronophthisis

Nephronophthisis types 1 through 12 can now be unequivocally diagnosed, because direct molecular genetic diagnosis has become available through the identification of the responsible genes (www.renalgenes.org). Molecular genetic analysis is the only diagnostic procedure by which the diagnosis of NPHP can be made with certainty. It should be initiated to noninvasively prove or exclude NPHP before the invasive procedure of renal biopsy is performed. However, due to the presence of additional genes for NPHP, the lack of detection of mutations in NPHP1–12 and NPHPL-1 genes does not exclude the diagnosis of NPHP. In a similar manner, UMOD mutational analysis will allow a precise genetic diagnosis of MCKD2, but a lack of any mutations will not exclude MCKD. Molecular genetic testing should be performed only in the context of genetic counseling and within the guidelines of the National and International Societies for Human Genetics (www.ethics.ubc.ca). Prior to genetic counseling, a thorough pedigree analysis to distinguish recessive (early onset) from dominant (late onset) disease is mandatory, and extrarenal organ involvement should be sought.

Imaging Techniques

Renal ultrasound is a very useful imaging technique in the NPHP-MCKD complex. Kidneys are normal or moderately reduced in size and exhibit, typically, a loss of corticomedullary differentiation and an increased echogenicity. Later in the course of the disease, mostly when patients have reached ESRD, cysts can be detected at the corticomedullary junction (Fig. 15.6).[165–167] Garel and associates[168] have seen medullary cysts in 13 out of 15 children studied at the time of renal failure (mean age: 9.7 years).

Magnetic resonance tomography and computed tomography demonstrate the presence of cysts in MCKD.[169–173] The invasive procedure of renal arteriography is not indicated to demonstrate the presence of medullary cysts,[174] and caution must be exercised when performing contrast studies in patients with renal failure.

Laboratory Studies and Urinary Concentrating Ability

Besides a molecular genetic diagnosis of NPHP1-12, NPHPL-1, and UMOD, there are no chemical laboratory tests in

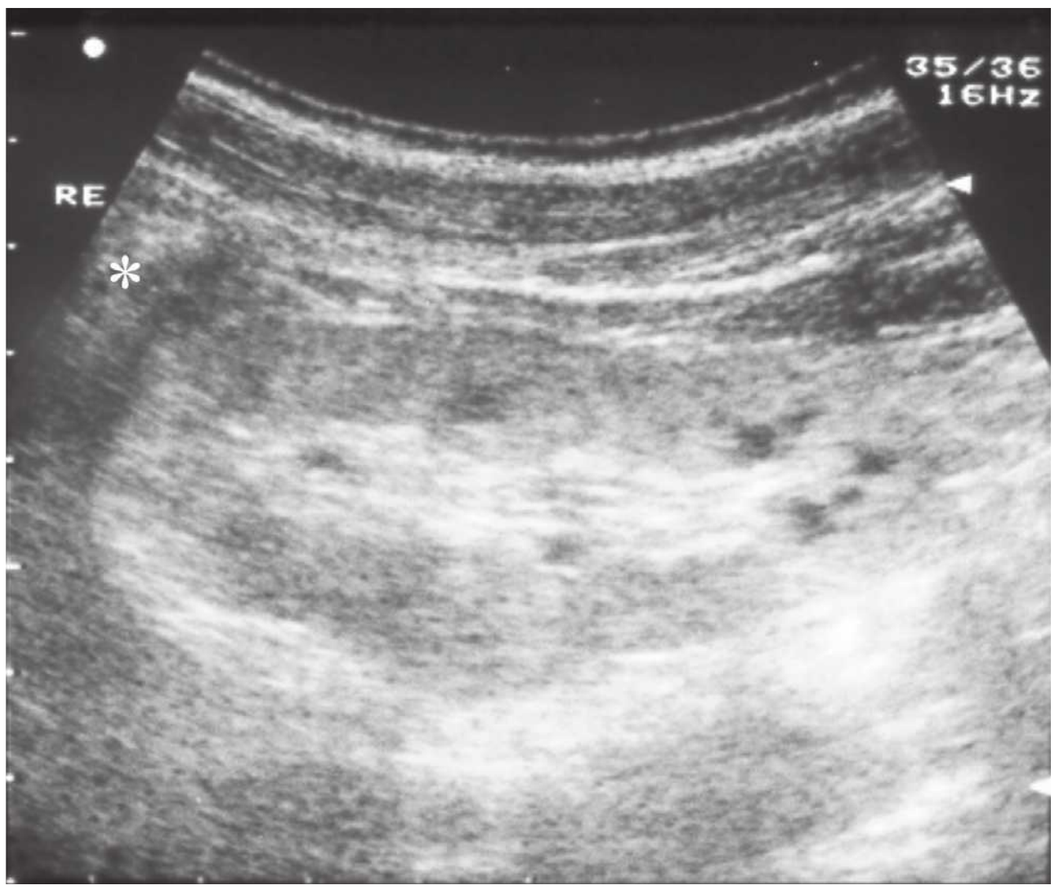


FIGURE 15.6 Characteristic renal ultrasound findings in juvenile nephronophthisis (NPHP1). Note the normal kidney size, the loss of corticomedullary differentiation, and the increased echogenicity, which renders the pattern of the kidney similar to that of the liver (*), together with the presence of cysts at the corticomedullary border of the kidney. (Courtesy of Prof. J. Dippel, Frankfurt, Germany.)

the NPHP-MCKD complex that specifically establish the diagnosis. Hematuria, proteinuria, and bacteriuria are typically absent in NPHP. In rare cases where proteinuria is present, it is usually mild and of the tubular type. Laboratory studies are needed to assess the severity of renal failure and generally demonstrate elevated serum creatinine, blood urea nitrogen (BUN), phosphorus, a metabolic acidosis, and anemia at the characteristic ages of the onset of ESRD for the different disease entities. Ophthalmoscopy should be performed in any patient to exclude SLSN. Liver function and hepatic ultrasonography should also be performed to facilitate the detection of patients with hepatic fibrosis.

A characteristic early finding in NPHP is the decreased ability to concentrate the urine following a water deprivation test.[41] An impairment of tubular function, with the constant finding of a renal concentration defect, usually precedes any documentable reduction in the glomerular filtration rate[10] and may be present with minimal histologic abnormalities.[175]

An intermediate defect of urinary concentration ability has been inconsistently demonstrated in the parents and some siblings of children with NPHP, and has been suggested to reflect the heterozygous state of the disease.[176] An 8-hour water deprivation test or vasopressin administration can be used to demonstrate a tubular concentration defect. Such tests should be performed with caution because dehydration may precipitate acute renal failure in patients with the disease or in unrecognized affected family members. In affected individuals, urine osmolality after 8 hours of water deprivation or vasopressin administration is $<$800 mOsm per kilogram of water. The diseases of the complex have also become known as salt losing nephritis. Poor renal uptake of 99^{m}-technetium-DMSA has been proposed as diagnostic of NPHP.[177]

Differential Diagnosis of Nephronophthisis-Medullary Cystic Kidney Disease

On histopathology, the NPHP-MCKD complex has to be differentiated from other forms of interstitial nephropathies like chronic pyelonephritis or drug injury. In oligomeganephronic dysplasia[178] kidney size is reduced and histology is distinct from NPHP. The paucity of urinary abnormalities, the frequent lack of hypertension, and the localization of renal cysts (if present) readily differentiate variants of the NPHP-MCKD complex from recessive or dominant polycystic kidney disease. Finally, a medullary sponge kidney[179] (see the subsequent text) does usually not lead to chronic renal failure and shows calcifications and calculi on renal ultrasound, and is, therefore, readily distinguishable from the complex.

PROGNOSIS, THERAPY, AND COUNSELING

Therapy of NPHP and MCKD is symptomatic and will pertain to the treatment of hypertension, if present, as well as the correction of disturbances of electrolyte, acid–base, and water balance. Hypokalemia may contribute to the polyuria, so that oral potassium supplementation may alleviate this symptom. Metabolic acidosis should be corrected, and osteodystrophy and secondary hyperparathyroidism should be treated with adequate calcium supplementation, phosphorus restriction or binders, and vitamin D therapy. Anemia can be treated with erythropoietin and growth retardation may require the administration of growth hormone if the diagnosis is made early enough for an intervention. Adequate nutrition (caloric and amino-acid supplementation) should be maintained with the help of a dietician. Salt wasting seems to be more frequent in the phase just preceding the development of end-stage renal disease. Patients are at risk for sudden water and electrolyte disturbances due to the high urinary output and salt loss. In some cases, an event of severe dehydration with acute renal failure can abruptly precipitate chronic renal failure. Sufficient salt and water supplementation is important at this stage, but may have to be restricted because hypertension develops late in the course of renal failure. Psychological counseling of the patients is an integral part of therapy because of the poor self-image associated with growth retardation and to alleviate pressures resulting from the need to comply with complicated medications and dietary prescriptions. All patients will require renal replacement therapy during childhood, adolescence, or in dominant disease, in adulthood.

At present, renal transplantation is the treatment of choice for ESRF associated with both NPHP and MCDK. Stavrou et al.[179] recently reported the outcomes of renal transplantation for 19 patients with MCKD type 1. Five-year graft survival was 90% with no evidence of recurrence of disease or specific complications.

Prior to genetic counseling, a thorough pedigree analysis to distinguish recessive (early onset) from dominant (late onset) disease is mandatory, and diseases other than renal organ involvement should be excluded. Siblings below 13 years of age should be reevaluated yearly by maximal urinary concentrating ability to allow for early detection and early prevention of complications. If a transplant recipient's renal histology suggests NPHP or MCKD and a living related donor is considered, an extensive search should be made to exclude or detect renal disease within the family.

Future therapeutic strategies targeted at renal cyst expansion may lead to the successful delay of ESRD. Vasopressin, a major adenyl cyclase agonist, acts via V2 receptors in the collecting duct. Recently, an antagonist of the V2 receptor (OPC31260) has been shown to inhibit renal cystogenesis in the pcy mouse, which is the murine equivalent of human NPHP3.[180] Clinical trials in patients with NPHP are eagerly awaited. Similarly, the antiproliferative agent rapamycin has been used in the Han:SPRD rat model of polycystic kidney disease, where a reduction in cyst volume density and a preservation of renal function was observed.[181] Thus, additional studies into therapeutic interventions in animal models are necessary to enable the development of therapeutic approaches to NPHP-MCKD.

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CHAPTER

16 Autosomal Dominant Polycystic Kidney Disease

Stefan Somlo • Arlene B. Chapman

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD; MIM 173990) is the most common hereditary renal disease occurring in 1:400 to 1:1000 individuals. It accounts for over 90% of all hereditary renal cystic diseases (Table 16.1). ADPKD is characterized by the presence of bilateral renal cysts that gradually grow and expand over time, resulting in significantly increased total kidney volume, progressive renal injury, and ultimately, end-stage renal disease (ESRD), usually in the sixth decade of life.

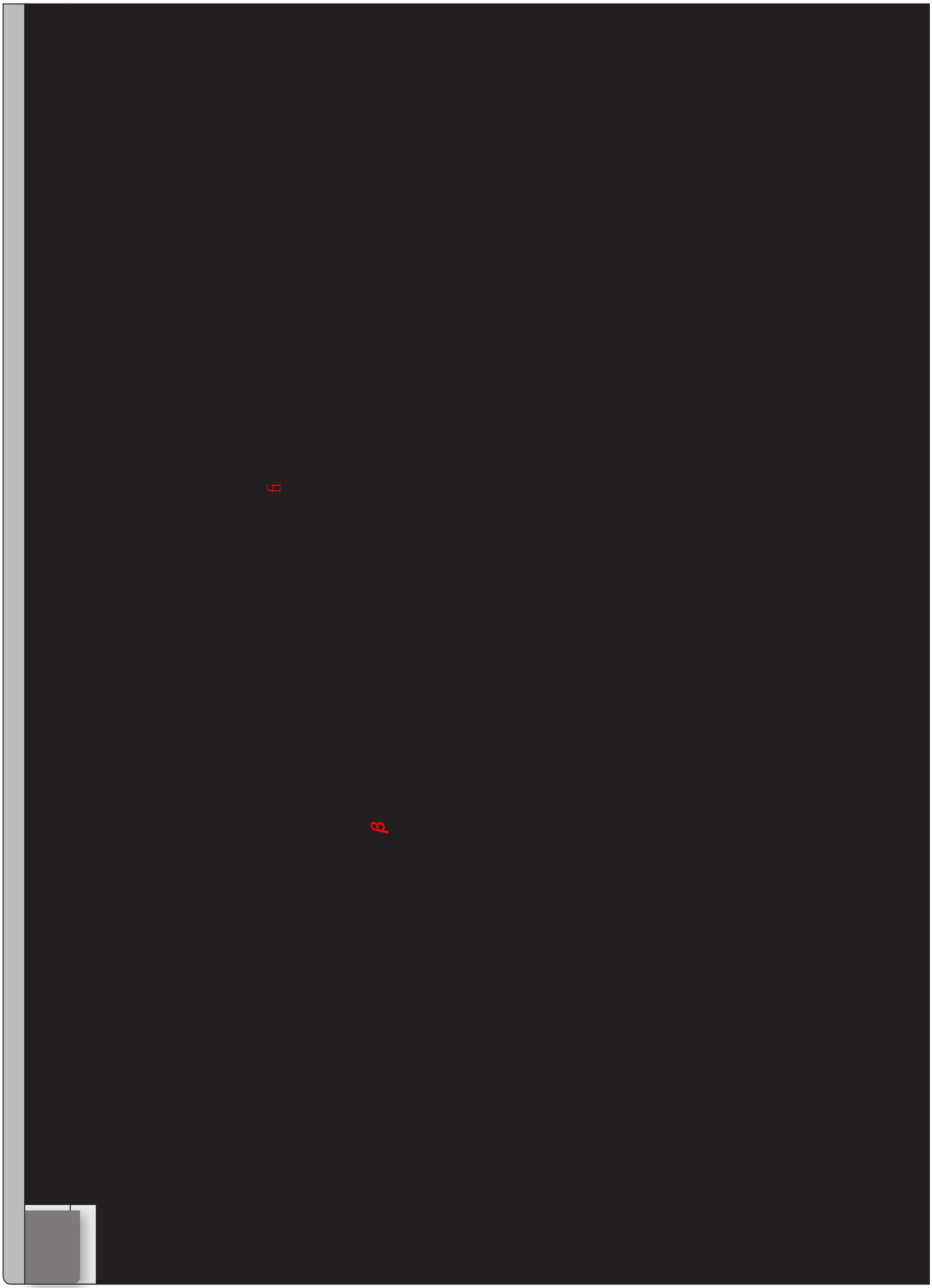
Other Mendelian diseases with varying degrees of fibrocystic involvement of the kidney are relatively rare. Autosomal recessive polycystic kidney disease (ARPKD; MIM 606702) occurs in 1:25,000; autosomal dominant tuberous sclerosis complex (TSC; MIM 191100 and 613254) occurs in 1:10,000; autosomal dominant medullary cystic disease or UMOD (MIM 174000) occurs in 1:35,000; and autosomal recessive familial juvenile nephronophthisis (NPHP; MIM 256100 and 602088) occurs in 1:40,000. In addition to the Mendelian diseases described previously, a variety of hereditary syndromes, such as Bardet-Biedl syndrome (BBS; MIM 209900) and Meckel Gruber syndrome (MKS; MIM 249000), result in a constellation of clinical manifestations that include renal cysts and are collectively termed ciliopathies (Table 16.1). In terms of clinical disease burden, however, all of the hereditary ciliopathies combined account for less than 1% of all renal cystic diseases.

This chapter will focus primarily on the single most common renal cystic disease, ADPKD. Current understanding of the epidemiology, clinical characteristics, and the pathology of ADPKD will be elucidated, and the appropriate approaches to the diagnosis and management of ADPKD will be provided. An overview of the genetics and the molecular pathways involving the polycystins is provided. Finally, a review of molecularly targeted therapeutic interventional trials will be presented to establish the potential future management for ADPKD individuals.

EPIDEMIOLOGY

Worldwide, ADPKD occurs between 1:400 and 1:1000 live births in all ethnicities when including ascertainment by autopsy.[1–3] Epidemiologic studies suggest that fewer than half of those with the disease are diagnosed during their life, with a majority of diagnoses occurring on autopsy after death from other causes.[1] Although not specifically evaluated in those studies, it is plausible that these individuals had a milder manifestation of disease. ADPKD has been estimated to be present in up to 600,000 individuals in the United States of America, and 12.5 million people worldwide (www.pkdcure.org). The disease accounts for about 6% of all patients on hemodialysis.[4] The incidence of ADPKD varies by genotype. PKD1 occurs in approximately 1:700 live births and PKD2 occurs in 1:15,000 live births. Reports from Japan, Denmark, the United States, Europe, India, Saudi Arabia, and Turkey show similar incidence rates, with no racial or ethnic predilection.[5–9] Patient and renal outcomes differ by PKD genotype. ADPKD resulting from mutations in PKD1 result in earlier mean age of onset of hypertension (29 versus 41 years of age), ESRD (55 versus 74 years of age), and death (68 versus 79 years of age) when compared to ADPKD due to PKD2 mutations.[10–12]

Current worldwide yearly incidence rates for ESRD due to ADPKD are 7.5 and 6.1 per million population for men and women, respectively. Gender differences with regard to disease progression and severity have been reported, with women having a more favorable renal outcome than men.[10,13] However, when gender differences and genotype are considered together, little or no differences in the age of onset of renal failure are found in PKD1 individuals. Importantly, significant differences in survival are found between men and women with PKD2 mutations (67.3 versus 71.0 years). In addition, previous age-adjusted sex ratios of the incidence of ESRD, which were greater in men than in women (1.4 to 1.6), are now beginning to reach parity in various countries, including Denmark and the United States.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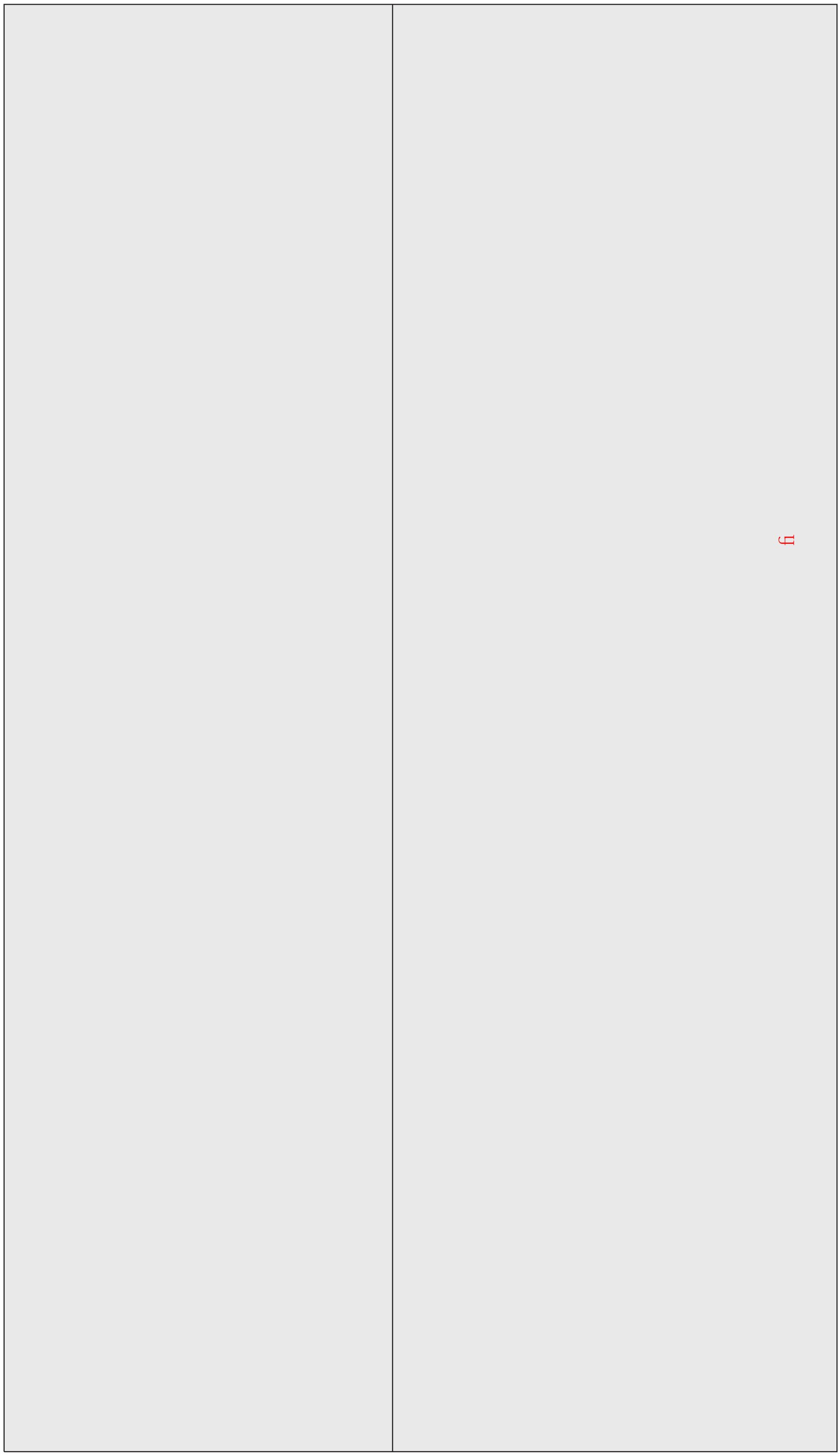
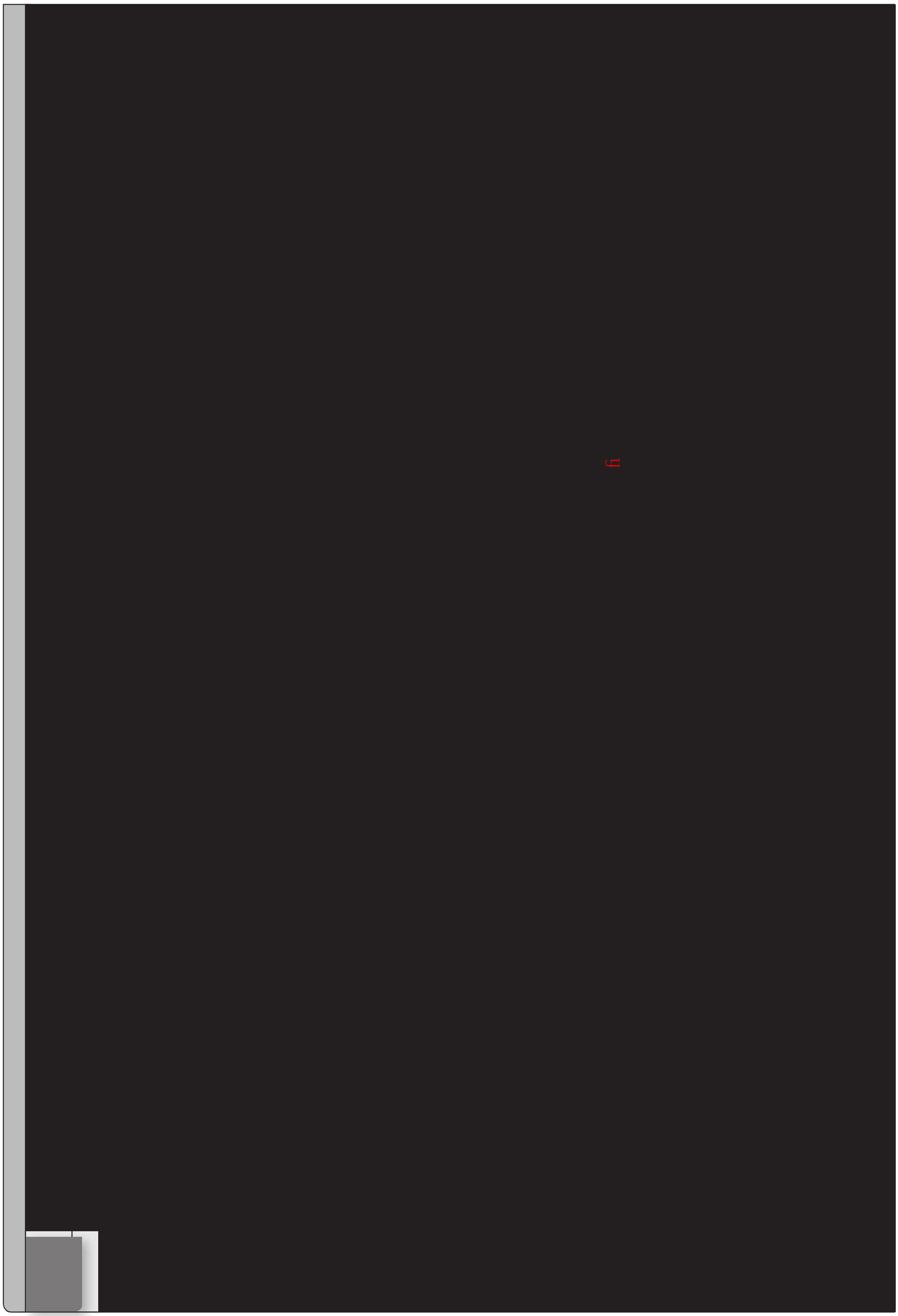




African American ADPKD patients have been reported to have a more aggressive renal course than their non-African American counterparts.[14–16] However, these reports are limited due to the small number of patients and the presence of other diseases affecting renal function, including the sickle cell disease or trait. Importantly, the relative incidence of ESRD due to ADPKD per million population in African Americans is lower than their non-African American counterparts.[4,17,18] Whether this relates to earlier mortality in African Americans or improved renal survival has not yet been determined.

During the past 3 decades, the average age of onset of ESRD, the incidence and prevalence rates of ADPKD in ESRD, and the survival rates of ADPKD in ESRD have increased. Incidence rates of ADPKD patients entering ESRD have increased significantly from 1990 to 2010 in the United States (35%), Japan (30%), and Denmark (33%).[5,17,19] The average age of onset of ESRD has increased in the United States (4.5 years), Denmark (5.1 years), and Japan (4.6 years). Importantly, the use and the number of antihypertensive agents, specifically inhibitors of the renin-angiotensin-aldosterone system (RAAS) are associated with decreased patient mortality and an increased age of onset of ESRD.[20,21] Taken together, these data suggest that patients are now more often surviving to start renal replacement therapy, and improved patient care has extended both renal and patient survival with a positive impact on patient mortality for those receiving renal replacement therapy.

DIAGNOSIS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Although symptoms in ADPKD individuals can present at a young age, a diagnosis of ADPKD is confirmed either with radiographic imaging or genetic testing. Before undertaking a diagnostic evaluation, counseling should be done to educate the family about the risk for inheritance. The mode of presentation for diagnostic screening has not changed over the last half century, with approximately 40% of individuals with a positive family history presenting asymptomatically.[13,22] The remaining patients present with clinical complications such as new onset hypertension, gross hematuria, acute flank pain, or fevers. The average age of presentation for diagnosis has also remained unchanged for the last 4 decades. With a number of potential beneficial therapies becoming available and evidence for improved mortality due to standard medical care, an earlier presentation for screening of at risk individuals will most likely occur.

The risk for discrimination in terms of insurability and employment has been reduced, but not eliminated, by the passage into law of the Genetic Information Nondiscrimination Act (GINA).[23,24] GINA prohibits insurers from canceling, denying, refusing to renew, or changing the terms or premiums of coverage based on genetic information. It also prohibits employers from making hiring, firing, promotion, and other employment-related decisions based on genetic factors. Genetic information is defined as information about an individual's genetic tests, the genetic tests of family members, or occurrence of a disease in family members of the individual. GINA, however, applies only to individuals who are asymptomatic, does not prohibit underwriting based on information about current health status, and does not apply to life insurance, disability insurance, or long-term care insurance.

Ultrasound imaging is the initial imaging modality of choice for a diagnosis of ADPKD (Fig. 16.1). Ultrasound is cost-efficient as compared to magnetic resonance imaging (MRI) or computed tomography (CT) and is not associated with the radiation exposure that occurs with CT imaging. The vast majority of affected ADPKD patients can be diagnosed by ultrasound imaging alone. Since the initial ultrasound criteria for a diagnosis of ADPKD were developed in 1994,[25] the imaging resolution for detection of small cysts with ultrasound, CT, and MRI have vastly improved.[26,27] The limit of detection of cysts using CT and MRI is now as low as 1 mm in diameter as compared to 0.5 to 1 cm with ultrasound.[28] Importantly, and relevant to the value of diagnostic imaging in ADPKD, simple renal cysts >1 cm in diameter remain relatively rare in childhood, occurring in <0.1% in the general population.

MR- and CT-based imaging studies of healthy young adults without a family history of ADPKD show that simple renal cysts of diameters as small as 1 mm are relatively common, even in young adults, occurring in 11 out of 35 or 28% in 18- to 29-year-olds and 97 out of 190 or 51% in 30- to 44-year-olds.[26] Therefore, if Ravine criteria using cyst number alone is used with CT or MRI in individuals between the ages of 18 and 45 years of age, more than one third would erroneously qualify for a diagnosis of ADPKD. However, if only those with cysts >1 cm in diameter are considered, the size of the cyst commonly seen in ADPKD individuals and detectable by ultrasonography, the number of incorrectly diagnosed individuals would remain <1%. Therefore, in at-risk individuals, sensitivity and specificity for a correct diagnosis of ADPKD using ultrasound remains intact and a single renal cyst in an at-risk child from a family with ADPKD is sufficient to make a diagnosis (Table 16.2).[29]

An age-based renal cyst number is required for a diagnosis of ADPKD, given that simple renal cysts are present with increasing frequency as age increases in the general population. Ultrasound still carries high sensitivity and specificity for the majority of PKD1 individuals over the age of 30 years.[30,31] At age 30, a negative ultrasound indicates a less than 5% likelihood of having ADPKD in individuals from PKD1 families. Negative ultrasound imaging is also informative at earlier ages in at-risk individuals from PKD1 families, with a negative ultrasound in an at-risk 20-year-old conferring a less than 10% chance of carrying the disease. Additional experience with screening PKD2 individuals provides a more accurate estimate of the relatively high false–negative rates when screening at-risk individuals under the age of 40.[32] Although the specificity and positive predictive value of sonographic criteria is very high in PKD1 individuals, their sensitivity and negative predictive value are low when applied to PKD2 in the 15 to 29 age group (69.5 and

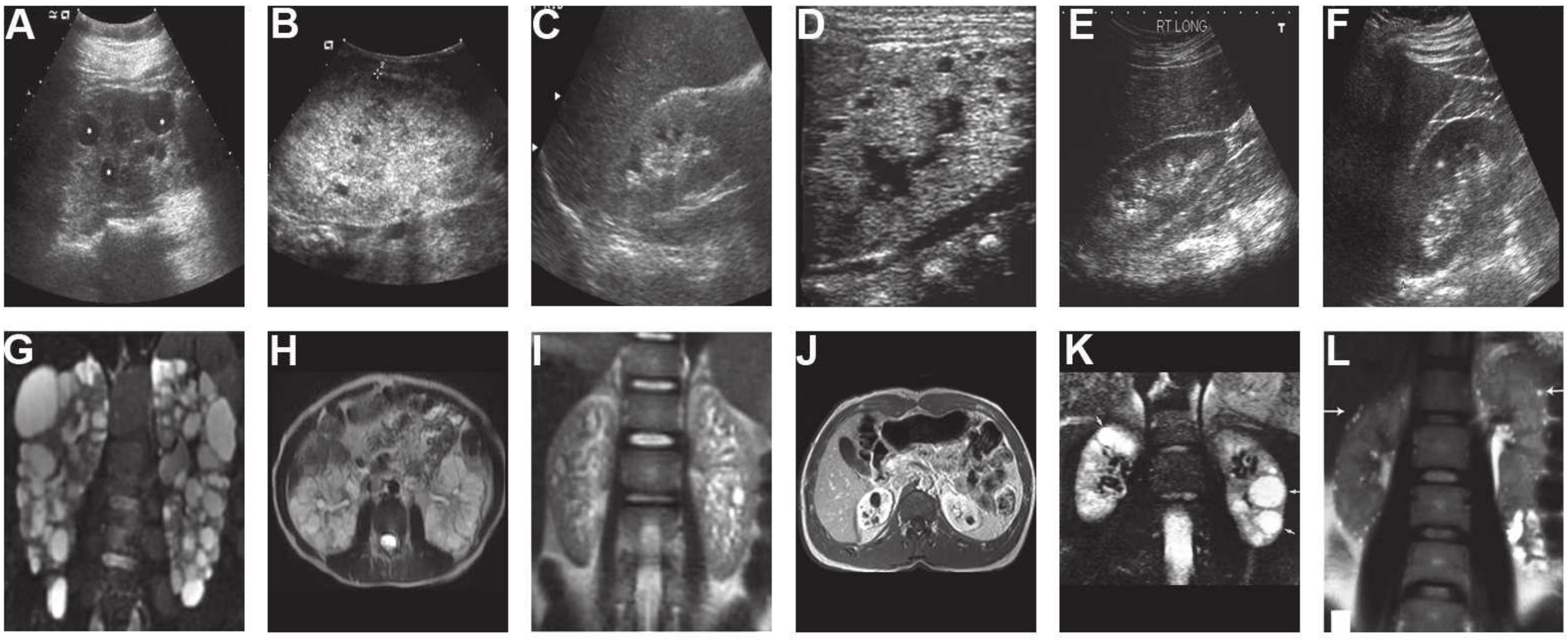


FIGURE 16.1 A radiographic appearance of hereditary renal cystic disorders. The top panels show ultrasonographic, longitudinal axis images of **(A)** autosomal dominant polycystic kidney disease (ADPKD), **(B)** autosomal recessive polycystic kidney disease (ARPKD), **(C)** familial juvenile nephronophthisis (NPHP), **(D)** glomerular cystic kidney disease (GCKD), **(E)** medullary cystic kidney disease (MCKD), and **(F)** tuberous sclerosis complex (TSC). The bottom panels show the same sequence of renal cystic disorders using either magnetic resonance imaging (MRI) or computed tomographic (CT) imaging: **(G)** a coronal MRI of early stage ADPKD, **(H)** an axial CT image of ARPKD, **(I)** a coronal MRI of NPHP, **(J)** an axial CT image of GCKD, **(K)** an axial CT image of MCKD, and **(L)** a coronal MRI of TSC.

16.2 Diagnostic Criteria for Autosomal Dominant Polycystic Kidney Disease

Age	PKD1	PKD2	Unknown ADPKD Gene Type
Diagnosis			
30–39 years	≥3 cysts[a] PPV = 100% SEN = 96.6%	PPV = 100% SEN = 94.9%	PPV = 100% SEN = 95.5%
40–59 years	≥2 cysts in each kidney PPV = 100% SEN = 92.6%	PPV = 100% SEN = 88.8%	PPV = 100% SEN = 90%
Exclusion			
30–39 years	≤1 cyst NPV = 100% SPEC = 96%	NPV = 96.8% SPEC = 93.8%	NPV = 98.3% SPEC = 94.8%
40–59 years	≤1 cyst NPV = 100% SPEC = 93.9%	NPV = 100% SPEC = 93.7%	NPV = 100% SPEC = 94.8%

[a]Unilateral or bilateral.
All values presented are mean estimates.
ADPKD, autosomal polycystic kidney disease; PPV, positive predictive value; SEN, sensitivity; SPEC, specificity; NPV, negative predictive value.
Derived from Pei Y, Obaji J, Dupuis A, et al. Unified criteria for ultrasonographic diagnosis of ADPKD. J Am Soc Nephrol. 2009;20(1):205–212.

78%, respectively). This is particularly a problem when evaluating potential young related kidney transplant donors, where the exclusion of ADPKD is important.

Based on this experience, Pei and colleagues[33] are now able to provide a unified age-based criteria for a diagnosis of ADPKD using ultrasound imaging for both PKD1 and PKD2 individuals (Table 16.2). This modification from the original Ravine criteria increases the age from 30 to 40 years for screening purposes. The presence of at least three (unilateral or bilateral) renal cysts and two cysts in each kidney are sufficient for a diagnosis of both at-risk individuals and those without a family history of ADPKD aged 15 to 39 years and 40 to 59 years, respectively. The requirement of three or more cysts (unilateral or bilateral) has a positive predictive value of 100% in the younger age group and minimizes false–positive diagnoses, because 2.1% and 0.7% of unaffected healthy individuals younger than 30 years have one and two renal cysts, respectively. In those 30 to 39 years old, both the original (two cysts in each kidney) and the revised (three cysts, unilateral or bilateral) criteria have a positive predictive value of 100%. Finally, for at-risk individuals aged greater than 60 years, four or more cysts in each kidney are required. Even with these criteria in place, there are still exceptions where ultrasound screening is not sufficient, usually when either a family history for ADPKD is absent and the clinical presentation is atypical, or when clinical suspicion for a positive diagnosis is high and evidence for renal cystic disease by ultrasound is lacking.

Although a minimum number of renal cysts are required for a diagnosis of ADPKD, other renal cystic diseases may also meet the requisite cyst number criteria to qualify for a diagnosis of ADPKD (Fig. 16.1). Additionally, given that approximately 15% of ADPKD individuals develop polycystic kidney disease (PKD) spontaneously and have unaffected biologic parents, other features of the clinical presentation are important to consider beyond simply the number of renal cysts identified. For example, the distribution and size of cysts can be informative. Cysts occurring predominantly in the medullary space with relatively small size are found in medullary cystic disease and familial juvenile nephronophthisis in the setting of normal or small kidney size and can be used to distinguish these from ADPKD. Angiomyolipomas are typically present in the kidneys of patients with tuberous sclerosis complex. Glomerular cystic disease is associated with relatively small widely distributed discrete cysts found predominantly in the cortex in the setting of normal kidney size.

In contrast to many other hereditary renal cystic diseases, ADPKD is characterized by significant renal enlargement in the setting of normal kidney function. Although renal enlargement is a feature of ARPKD, particularly in those diagnosed in utero or at birth, significant renal insufficiency usually accompanies this feature. The cysts in ARPKD tend to be diffuse, small, and relatively homogeneous and are more commonly described as fusiform and ectactic dilatations rather than discrete macrocysts. Significant renal enlargement is not present in other hereditary kidney diseases including von Hippel Lindau disease, nephronophthisis, and medullary cystic disease. Renal enlargement with significant renal cystic burden in the setting of normal kidney function, often with early onset, can be seen in specific individuals who have contiguous gene deletion mutations involving both the PKD1 and TSC2 genes.[34] This contiguous gene syndrome usually requires further genetic testing for accurate diagnosis. In addition to the diagnostic value of kidney enlargement in the setting of normal kidney function in ADPKD, the presence of radiographically visible liver cystic disease, when present, is also a unique and diagnostically useful feature of ADPKD. No other hereditary renal cystic disease is accompanied by polycystic liver disease with the exception of some instances of familial juvenile nephronophthisis. Importantly, familial instances of liver cystic disease indistinguishable from that seen in ADPKD but lacking kidney cysts is a genetically distinct disorder.[35]

Genetic testing may be required to confirm or exclude a diagnosis of ADPKD. Genetic testing is reserved for patients with renal cystic disease without a family history and with an uncertain presentation of ADPKD, or those with a negative ultrasound who are at risk for ADPKD but who need a confirmatory diagnosis for the purposes of living related kidney donation, family planning, or for occupational safety. Direct sequencing of the PKD1 and PKD2 genes is the most reliable approach to a genetic diagnosis.[36–38] A curated database of PKD1 and PKD2 mutations is available at http://pkdb.mayo.edu/. Mutation detection is successful in up to 85% of cases in research laboratories. Destructive mutations (i.e., those predicted to result in truncated proteins due to premature termination codons, aberrant splicing, or insertion-deletions resulting in frame shifting) are readily identifiable as pathogenic. The same is not true for mutations due to nonsynonymous amino acid substitutions, which may account for up to 30% of mutations in PKD1 and a significantly lower percentage of PKD2. The pathogenicity of missense sequence variations often need to be confirmed with segregation studies in other affected family members before a diagnosis can be confirmed. A genetic diagnosis is further complicated by the lack of commonly recurring mutations that have been identified in other diseases such as cystic fibrosis. As a result, most families have private mutations requiring the relatively expensive whole gene sequencing approach for detection that nonetheless yields a mutation detection rate (highest detection rate, 85%) lower than the rate of cyst detection by age-appropriate ultrasound (lowest detection rate, 99%). As a result, this approach is reserved in the clinical setting for a limited group of patients meeting the previous criteria.

PATHOLOGY OF POLYCYSTIC KIDNEY DISEASE

The kidneys of patients with polycystic disease gradually enlarge and attain an enormous size due to the growth of hundreds of cysts. Kidneys measuring 40 × 25 × 20 cm and weighing 7 to 8 kg have been reported. Usually, these greatly enlarged kidneys are seen in patients with ESRD undergoing

nephrectomy or at autopsy. These end-stage kidneys contain hundreds of fluid-filled cysts of widely differing sizes. The cyst walls can be thin and transparent, but calcification of cyst walls is also common.[39] The renal capsule may be thickened around infected cysts, and the kidney may be attached to adjacent abdominal organs such as the spleen and the adrenal glands by fibrous tissue.

Cut sections demonstrate cysts throughout the renal parenchyma. Islands of normal-appearing renal parenchyma can usually be found only in kidneys from young, nonazotemic patients. The cysts vary in size from 1 mm to 10 cm or more in diameter. Cysts in ADPKD arise from all segments of the nephron, and some cysts (~11%) retain the morphologic characteristics of proximal or distal tubules or collecting ducts. However, most (84%) are lined by a single layer of poorly differentiated columnar or cuboidal epithelium.[40,41] Approximately 5% of cysts are lined by a markedly hyperplastic epithelium, forming polyps and microadenomas.[40,42] This hyperproliferative epithelium typically has no signs of dysplasia or premalignant features. The cysts are surrounded by a fibrous stroma, which may contain bundles of smooth musclelike cells, likely transformed myofibroblasts. Inflammatory interstitial infiltrates are seen, and in advanced cases, the renal interstitium is replaced by fibrosis. Marked arteriosclerosis and arteriolosclerosis are found in nephrectomy specimens, evidence that ischemic injury and damage from hypertension contribute to tubular atrophy and glomerulosclerosis.[43]

Microdissection studies of human kidneys suggest that only 1% to 2% of nephrons are cystic.[40] These studies also have shown that cysts begin as focal dilatations of tubular segments.[44] When these dilatations exceed approximately 2 mm in diameter, they typically disconnect from the parent tubule; at least 73% of the cysts have no tubular openings when evaluated by scanning electron microscopy.[40] The cysts lining epithelial cells are joined together by junctional complexes like those seen in normal proximal tubules or by tight junctions typical of the distal renal epithelium.[41] Only a few microvilli are seen on the luminal surface and a few mitochondria in the cytoplasm. Some cells have prominent cilia, and different types of cells are found in some cysts. Occasional infoldings of the plasma membranes may be found on the basal surface. The basement membrane of most cysts is strikingly abnormal. There is pronounced splitting, duplication, thickening, and lamination of the basal lamina.

The osmolality of cyst fluids is similar to that of plasma, but sodium and nonsodium osmolyte concentrations vary significantly.[45–47] Sodium concentrations can vary between 3 and 207 mEq per liter, but often are either less than 60 mEq per liter or more than 75 mEq per liter.[46] Therefore, a distinction was made between low sodium and high sodium cysts. The high sodium cysts have sodium concentrations similar to plasma and therefore are also called nongradient cysts, whereas the low sodium cysts are gradient cysts because they are able to maintain steep concentration gradients not only for sodium but for protons, potassium, chloride, phosphates, and other ions.[48] Morphologically, the gradient cysts have long tight junctions (zonulae occludens depth >500 μm), making them impermeable to ions, whereas the nongradient cysts have short tight junctions (<500 μm), making them leaky for solutes and water.[46] These characteristics suggested that gradient cysts were derived from collecting ducts and nongradient cysts from proximal tubules. However, most nongradient cysts are lined by a poorly differentiated epithelium with few microvilli and few mitochondria, which does not resemble the proximal tubular epithelium.[46]

More recent studies have assessed the distribution of aquaporin-1 and -2 in ADPKD cysts. In normal kidneys, aquaporin-1 is expressed in proximal tubules and thin descending limbs of the Henle loop, whereas aquaporin-2 is expressed on the apical surfaces of the collecting duct epithelia. In ADPKD kidneys, approximately 30% of cysts stain positive for aquaporin-1, another 30% are positive for aquaporin-2, and the rest are negative for both aquaporin-1 and -2.[49,50] The aquaporin-1–positive cysts presumably are derived from proximal tubules or thin descending limbs, the aquaporin-2–positive cysts are from collecting ducts, and the negative cysts are from nephron segments that do not express these water channels (i.e., the ascending limb of the Henle loop and distal convoluted tubules). These results also imply that the expression of water channels is not a prerequisite for cyst expansion. Moreover, the aquaporins appear to retain their segment-specific expression in ADPKD even though the morphologic characteristics of proximal and distal tubules are lost.

In addition to electrolytes and water, cyst fluids also contain amino acids, glucose, urea; idiogenic osmoles such as sorbitol, betaine, and glycerophosphorylcholine; and proteins, such as β_2-microglobulin, erythropoietin, renin, and albumin.[47,48] Cytokines, specifically interleukin (IL)-1β, IL-2, and tumor necrosis factor (TNF)-α, growth factors (epidermal growth factor [EGF], hepatocyte growth factor, endothelins), and a nonpolar lipid cyst activating factor, are present in cyst fluids as well and likely play a significant role in the pathophysiology of ADPKD.[51–56]

Therefore, the cysts of polycystic kidneys are not simply impermeable cul-de-sacs that collect and store urine from more proximal nephron segments. They are complex structures that proliferate and undergo apoptosis; that synthesize or transport various proteins, hormones, and cytokines; and that actively secrete chloride and water. Under certain conditions, they also may be able to absorb solutes and water.[45] Most cysts are permeable to small solutes, but some are highly impermeable. Although most cysts are lined by morphologically undifferentiated epithelium, the differential expression of water channels is maintained.

CLINICAL CHARACTERISTICS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Although ADPKD derives its name from the kidney, it is a systemic disorder with extrarenal manifestations that are unique to ADPKD.[3,57,58] Renal manifestations of ADPKD are

the most common and include the presence of renal cysts, renal enlargement, a decreased renal concentrating ability, polyuria, nocturia, and increased thirst.[59,60] In addition, renal complications of ADPKD include flank or abdominal pain, gross hematuria, hypertension, urinary tract infections, and nephrolithiasis.[13,61,62] Extrarenal manifestations are common in ADPKD and involve the cardiovascular, gastrointestinal, male and female reproductive systems, and the thyroid, subarachnoid, pericardial, and bronchial spaces.[63–67]

Although ADPKD is a hereditary renal disease, patients are relatively oligosymptomatic until the second or third decade of life. Renal complications are the most common and occur with increasing frequency with age. Pain is the most common complication, followed by hypertension and gross hematuria. The age-dependent presentation of these manifestations relate closely to kidney size or total kidney volume.[62] By the third decade of life, less than 10% of all ADPKD patients are complication free, even though the mean age of diagnosis of ADPKD is 27 years.[13] Patient-centered outcome reporting demonstrates that thirst, pain, and urinary frequency are the most common patient concerns.[68] However, close attention to other renal complications is important, given their contribution to progressive renal insufficiency and ESRD. Not surprisingly, renal complications are associated with poorer renal and patient outcomes.

Pain is the most common clinical manifestation of ADPKD and is responsible for the majority of presentations for symptomatic diagnosis.[69,70] Pre-ESRD patients completing quality of life questionnaires demonstrate lower scores on the physical component summary suggesting that symptoms of discomfort significantly impact their quality of life. Focus groups determining the most common patient-reported outcomes find that pain along with thirst and polyuria are the most important. Pain can be managed effectively in most patients, but in a minority, chronic pain limits individuals' ability to function, resulting in sleep deprivation, fatigue, anxiety, and a decreased quality of life.[71] Acute and chronic pain in ADPKD is due to different causes. Acute pain syndromes in ADPKD are most often associated with cyst rupture, hemorrhage, renal infections, or nephrolithiasis. Chronic pain is a more complex and less defined problem. ADPKD patients report pain located in the back (71%), abdomen (61%), head (49%), chest (30%), and legs (27%).[72] Renal and nonrenal sources of pain not related to cystic disease should be considered including diverticulitis, ovarian cyst rupture, aortic or iliac aneurysms, or incarcerated hernias.

Chronic pain management related to polycystic kidneys requires a staged approach beginning with nonpharmacologic interventions including ice, heat, whirlpool, massage, and physical therapy as well as exercises to improve vertebral and abdominal wall support. When these approaches are not successful, other therapies including intermittent transcutaneous electrical nerve stimulation (TENS) unit and nonopioid analgesics, such as acetaminophen, can improve the level of pain in polycystic patients. There is less objective information regarding the benefits of other treatments including short- and long-term opioid medications, tramadol (Ultram), clonidine, gabapentin, or pregabalin. Nontraditional complementary medical approaches such as acupuncture may be helpful, although this may involve a placebo effect. Surgical approaches to pain in ADPKD patients are reserved for those who have systematically attempted all nonmedical and medical therapies over a reasonable period of time. The least invasive approach is percutaneous cyst aspiration with alcohol injection, typically done in patients with symptoms that can be matched locally to candidate cysts identified using CT or MRI. This can be done in interventional radiology suites as an outpatient procedure. Multiple cyst fenestrations or deroofing procedures (Rovsing procedures) can be done in more severe and complicated cases. Prospective studies report an immediate improvement in 85% to 90% of individuals with close to two thirds maintaining a benefit up to 2 years after treatment.[73–76] More recently, renal denervation procedures both abdominally and thoracoscopically with and without nephropexy have demonstrated early short-term pain relief.[77,78] Whether there are long-term benefits resulting from these interventions is not yet clear. Finally, partial or full nephrectomy or volume reducing procedures, including transcatheter arterial embolization, have been used with success in small numbers of patients with intractable pain.

Hypertension

Hypertension is common in ADPKD and, unlike in other tubulointerstitial diseases, it occurs in the majority of patients prior to the loss of kidney function.[79–81] The average age of onset is 29 years, and men are more often hypertensive than women early in the course of disease.[11,80] Evidence suggests that carotid and left and right ventricular structure are abnormal in asymptomatic ADPKD patients early in the course of disease prior to the development of hypertension. This is manifest by increases in carotid intimal wall thickness, reduced end-diastolic relaxation, decreased aortic relaxation, increased left ventricular mass, and left ventricular hypertrophy.[82–84] Hypertension is common in children with ADPKD, affecting 10% to 25% of individuals,[85–87] and it is associated with evidence of end-organ damage including increased left ventricular mass index and left ventricular hypertrophy. Whether primary cardiovascular abnormalities or increased systemic blood pressure are the primary hemodynamic abnormality to develop in ADPKD and how they relate to each other is unclear. A recent study suggested that an early intervention in ADPKD, particularly with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, may decrease the occurrence of left ventricular hypertrophy and has the potential to decrease cardiovascular mortality.[88]

Total kidney volume (TKV) is greater in hypertensive ADPKD adults and children with normal kidney function when compared to the respective normotensive ADPKD controls.[89–91] The increased cyst burden found in hypertensive ADPKD individuals is associated with evidence of systemic activation of the RAAS. Vascular imaging and MR-based quantification of renal blood flow demonstrate attenuated renal vasculature and reduced renal blood flow,

both of which occur early and are associated with hypertension and increased TKV.[92,93] Activation of the RAAS in ADPKD is most likely due to increased intrarenal ischemia secondary to cyst formation and expansion. Distortion of the renal vasculature due to increased cyst burden results in decreased renal nitric oxide production, increased reactive oxygen species formation, and further activation of the intrarenal RAAS.[94] Currently two large randomized clinical trials are under way to determine the impact of inhibition of the RAAS and the value of rigorous blood pressure control in disease progression in ADPKD.[95] Results of these trials will be available in 2014.

Gross Hematuria

Gross hematuria is a common initial clinical presentation in ADPKD, often presenting prior to the onset of hypertension. Although not prospectively established, gross hematuria tends to associate with rapid cyst expansion, increased physical activity, and cyst wall calcifications. Gross hematuria is significantly associated with increased kidney size and a poorer renal prognosis.[62,96,97] Gross hematuria may or may not be associated with renal pain. In patients with gross hematuria who are asymptomatic, cyst rupture into the urinary collecting system is the most likely cause. Renal imaging is encouraged to rule out other treatable causes of gross hematuria. In patients in whom symptoms develop, localized pain, fever, and dysuria are common. When these clinical signs and symptoms occur, it is important to rule out cyst infection, nephrolithiasis, pyelonephritis, or lower urinary tract infections.

Gross hematuria secondary to cyst rupture is typically self-limited and usually lasts 2 to 5 days. Increased hydration with oral fluid intake and bed rest with close monitoring of blood pressure is indicated given the increased risk for acute reversible kidney injury in the setting of antihypertensive medication intake, particularly angiotensin-receptor blocking agents and angiotensin-converting enzyme inhibitors.[98] Often, it is advised to temporarily discontinue these agents until the episode of gross hematuria resolves. It is important to monitor blood pressure closely with home blood pressure monitoring devices during this time.

Urinary Tract Infections

Urinary tract infections are common in ADPKD and occur more often in women as compared to men.[99] Unlike hypertension and gross hematuria, it is not well established whether urinary tract infections are associated with progressive renal injury. Given that lower urinary tract infections are relatively common in the general population, their occurrence in ADPKD may or may not be disease related. Differentiation between lower and upper urinary tract infections can be difficult, further complicating the discovery of any link between urinary tract infections and disease severity. Upper urinary tract infections in ADPKD may be due to cyst infections, nephrolithiasis, or pyelonephritis and require careful evaluation.[100,101] Therapy for cyst infections requires different antibiotic treatment than those recommended to treat pyelonephritis in the general population. Antibiotics that provide adequate cyst fluid concentrations are necessary.[102] In addition, a more prolonged course of therapy is needed to ensure successful eradication of the infection. Current recommendations for the treatment of cyst infections include a 2-week course of an oral quinolone or possibly trimethoprim-sulfamethoxazole.

The diagnosis of a cyst infection is often difficult. Clinical presentations vary ranging from local tenderness, fever, leukocytosis, and leukocyturia with positive urine cultures to diffuse abdominal discomfort or pain, absence of a fever, and negative urine cultures.[103] Importantly, blood cultures may more often provide evidence of the infecting organism than urine cultures given that many infected cysts do not directly communicate with the urinary collecting system. Occasionally, cyst infections do not respond to appropriate oral antibiotic therapy. Typically, this occurs in larger cysts (>5 cm in diameter) or when intracystic antibiotic levels are inadequate. It may be necessary to administer parenteral antibiotics, conduct imaging studies to rule out other causes of fever and pain (including nephrolithiasis), and to consider percutaneous cyst aspiration to obtain cultures or to decompress the large infected space.[104] In rare circumstances, frank pyonephritis may develop associated with sever malaise, sepsis, and shock and may necessitate partial or total nephrectomy.

Nephrolithiasis

Kidney stones are tenfold more common in ADPKD patients than the general population, occurring in approximately 25% of affected individuals.[105] Symptomatic nephrolithiasis typically occurs later than other renal complications in ADPKD.[96] Nephrolithiasis associates with increased TKV in ADPKD patients with normal kidney function. All types of kidney stones can occur in ADPKD; however, urate nephrolithiasis is more common than other types of kidney stones.[106] ADPKD patients develop hypocitraturia, even prior to a loss of renal function, and this may contribute to the increased frequency of urate nephrolithiasis. Whether the hypocitraturia associated with ADPKD is due to abnormalities in renal ammonia generation or other tubular defects is unknown. Of note, urinary biochemical parameters in ADPKD patients uniquely demonstrate normal urinary calcium excretion with increased oxaluria. Patients with nephrolithiasis typically present with unilateral flank pain, with or without radiation, and may have micro- (or rarely, macro-) hematuria. Those with nephrolithiasis diagnosed incidentally during renal imaging more commonly report lower unilateral back pain. Importantly, fevers and chills may occur in the setting of nephrolithiasis. This constellation of signs and symptoms overlap significantly with cyst infections and cyst hemorrhage. An evaluation of nephrolithiasis almost always requires renal imaging—most often, noncontrast CT imaging.[107] Ultrasound has a reduced sensitivity for the detection of kidney stones as compared to CT imaging

in ADPKD individuals. Excretory urography, abdominal flat plate X-rays, and ultrasound can all be used; however, the localization and detection of renal stones is achieved best with CT imaging. The differentiation of renal cyst wall calcification and nephrolithiasis is important and can be easily done when CT imaging is performed. Cyst wall calcifications are more common in ADPKD patients who demonstrate nephrolithiasis than those who do not.

The approach to the management of nephrolithiasis in ADPKD patients should involve a biochemical analysis of the urine and stone using crystallography if possible. Estimates of daily fluid intake and dietary intake of stone-forming elements should be established. Both of these evaluations can be determined from a single 24-hour urine collection. Urinary biochemical analysis should include calcium, urate, oxalate, citrate, and pH.[108] For the majority of stones formed in ADPKD, increases in fluid intake are the cornerstone of therapy. A minimum of 3 L per day of fluid intake should be established using home monitoring coupled with monthly 24-hour urine collections. The addition of bicarbonate or citrate is also helpful for those patients with urate nephrolithiasis to decrease urinary acidification and to increase potential stone dissolution. Dietary education is also helpful with regard to dietary intake of urate, calcium, and oxalate. For patients with calcium oxalate stones, the addition of thiazide diuretics will help to reduce urinary calcium excretion and, coupled with increased fluid intake to greater than 3 L per day, will reduce the concentration of urinary calcium and inhibit initial stone formation.

The most complicated stone in ADPKD patients is the struvite stone, or staghorn calculus. These stones are a constant nidus for infection, which can recur shortly after each treatment course and must be removed to avoid complications. These stones are a nidus for infection, which recurs after each treatment course, and must be removed to avoid complications. Collaboration with the urologic specialists is essential for the management of these patients. Depending of the size of the stone and its location, retrograde lithotripsy, extracorporeal shock wave lithotripsy (EWAL) therapy, or percutaneous stone removal may be necessary.

EXTRARENAL MANIFESTATIONS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Polycystic Liver Disease

Liver cyst formation is the most common extrarenal manifestation in ADPKD. Polycystic liver disease is due to the presence of multiple scattered cysts of biliary origin in the liver parenchyma. Among the hereditary renal cystic disorders, it is only found in ADPKD and, when present, is a useful adjunct in securing the diagnosis. In individuals with kidney cysts and no family history of ADPKD, the presence of liver cystic disease provides confirmation of the clinical diagnosis. Liver cystic disease occurs in over 85% of PKD1 patients by the age of 30.[109] An MRI analysis performed as part of the Consortium for the Radiographic Imaging Studies of Polycystic Kidney Disease (CRISP) study demonstrated that the prevalence of liver cysts increases with age, occurring in 58%, 85%, and 94% of affected individuals age 15 to 24, 25 to 34, and 35 to 46 years, respectively. The severity of cystic liver disease appears to parallel the severity of cystic kidney disease.[110,111] Liver cystic disease varies from a few cysts to massive cystic liver enlargement. Importantly, liver parenchyma and liver function are normal even in the setting of massive polycystic liver disease, and portal hypertension does not occur. Biochemically, the only liver function abnormalities found are mild elevations in the alkaline phosphatase and bilirubin. In contrast to renal cystic disease, polycystic liver disease occurs earlier and is more likely to be severe in women than in men and is influenced dramatically by estrogen exposure.[110] Liver cysts develop within specific segments of the liver, and there is no predictable segment sparing.

The signs and symptoms of polycystic liver disease include increased abdominal girth, increased clothing size, shortness of breath, early satiety, abdominal pain, and umbilical herniation. Morphologic studies of liver cysts demonstrate that they originate from biliary microhamartomas that arise from biliary ductules and peribiliary glands. As in the kidney, as liver cysts expand and enlarge, they become detached from their biliary tree of origin.[112] Similarly to polycystic kidney disease, cyst expansion is the result of multiple effects of proliferation of cyst-lining epithelia, fluid secretion, remodeling of the extracellular matrix, and neovascularization.[112] The genetic and molecular signaling mechanisms found in renal cystic epithelia have also been found in liver cystic disease in ADPKD, suggesting that the underlying disease biology in both organs are closely related.

Isolated Polycystic Liver Disease

Isolated autosomal dominant polycystic liver disease (ADPLD; MIM 177060, 608648) is an autosomal-dominant familial disease with significant genetic heterogeneity.[35,113] It is relatively rare, with an estimated incidence of $< 0.01\%$ of the population. This may be related to the low rate of clinical symptoms; autopsy studies suggest a rate of occurrence that is only slightly lower than that of ADPKD.[114] Although isolated ADPLD was reported as early as 1906,[115] it was not until 2003 that linkage analysis in eight Finnish families confirmed that isolated ADPLD is a disease genetically distinct from polycystic kidney disease. Subsequent gene discovery studies in patient families showed that ADPLD is genetically heterogeneous, with a subset of affected families having heterozygous mutations in PRKCSH or SEC63 in the setting of clinically indistinguishable clinical presentations.[116–118] Further genetic heterogeneity is suggested by the finding that only approximately 30% of ADPLD families have mutations in either of these two genes.[119] As a result, DNA testing in ADPLD patients has limited use because the mutated genes responsible for ADPLD have not been identified for the majority of families.

The natural history of ADPLD is relatively oligosymptomatic, characterized by associated symptoms in less than 30% of individuals. Those with symptomatic ADPLD complain of abdominal distention, fullness, discomfort, early satiety, and dyspnea and back pain. Individuals with ADPLD also demonstrate mild elevations in serum alkaline phosphatase as well as total bilirubin associated with lower total cholesterol and triglyceride levels. As in ADPKD, ADPLD women show a tendency toward significantly more liver cystic burden than men. As compared to unrelated and related unaffected individuals, mitral leaflet abnormalities may be more common, and other vascular malformations including intracranial aneurysms, carotid artery dissections, and ectatic cavernous arteries have been seen.[35] The differential diagnosis of ADPLD includes simple liver cysts and liver cysts resulting from other diseases, including ADPKD. Because simple liver cysts are common in the general population and occur with increasing frequency with increasing age, at least four liver cysts visible by ultrasonography are required for the diagnosis of ADPLD in individuals over the age of 40.

Intracranial Aneurysms and Other Vascular Abnormalities

The frequency of intracranial aneurysms (ICAs) is increased in ADPKD. A recent meta-analysis of 645 ADPKD patients with ICA demonstrated a 6.6-fold increased likelihood of an ICA developing compared to the general population.[120] This represents a frequency of 5.8% in selected ADPKD populations (as compared to 2.8% in the general population) and a 12% frequency in ADPKD patients with a positive family history of an ICA. ICAs tend to cluster in a small number of ADPKD families, and a positive family history of ICA is the only established risk factor associated with ICA in ADPKD. Gender, age, smoking exposures, hypertension, and race do not contribute to the risk of ICA formation in ADPKD. Mutations in the PKD1 gene tend to be closer to the 5′ end of the gene in families with intracranial aneurysms[121] and a common PKD1 mutation has been reported in individuals with a variety of vascular malformations including ICA,[122] but the genetic basis of ICA beyond the tendency to cluster in certain families is not well understood.

As compared to the general population, ICAs are more often found in the anterior circulation (84%) and rupture at an earlier age in ADPKD, but they do not differ in size at the time of rupture. As with all ICAs, the risk of rupture increases significantly once size reaches 10 mm in diameter. Given the serious consequences of an ICA rupture with permanent morbidity and mortality in excess of 40%, preventative screening and management are important aspects to patient care. Given that aneurysms are relatively rare, selective screening should be considered. Multiple studies of asymptomatic ADPKD patients demonstrate that only a positive family history associates with an ICA.[123,124] Repeat screening in individuals following an initial negative screen provided a very low yield of 2.4% in 76 individuals over 10 years.[20] Two longitudinal imaging studies of ADPKD individuals with documented small intact ICAs demonstrated a low frequency (8 out of 65) of increases in diameter over 243 patient years with the occurrence of six de novo aneurysms.[125,126] Taken together, these data suggest that the presence of ICAs in ADPKD patients is reflective of an initial expansion at the time of formation, perhaps with a higher risk of rupture. If the initial screening does not show vascular abnormalities, further imaging is not required unless individuals are symptomatic. Specific subgroups of ADPKD patients in addition to those with a positive family history of ICA, such as those considering organ donation or receipt, commercial pilots, or those with considerable personal burden due to concern about their status should undergo an initial screening for ICA.

Potential imaging modalities to assess the intracerebral vasculature include CT angiography, four vessel arteriography, or MRI. All modalities have excellent resolution, accuracy, and reliability. CTs and angiographies are associated with increased radiation exposure and potential complications. Therefore, MR angiography is the imaging modality of choice for screening. MRs with and without gadolinium can be used to clearly outline the cerebral vasculature. The management of ICAs in ADPKD patients relate primarily to the size of the ICA but also depend on location and whether they are asymptomatic. Typically, ICAs less than 5 mm in diameter are at low risk of rupture. In symptomatic individuals or those with ICAs greater than 7 mm, either surgical ablation or coil ablation or thrombosis can be used, depending on the size and location of the ICA. Complications related to surgical intervention are low (< 1%), but when they occur, they have significant morbidity, particularly with procedures performed in the posterior circulation of the circle of Willis. Therefore, patients with ICAs are advised to carefully review the risks of rupture and complications of treatment before moving forward with surgical or endovascular interventions.

Fertility in Autosomal Dominant Polycystic Kidney Disease

Women with ADPKD demonstrate fertility rates similar to the general population. However, an increased rate of ectopic pregnancy[127] has been reported that is potentially associated with abnormalities in fallopian tube function or ciliary motility. Importantly, seminal vesicle cysts are common in men and can be detected by transrectal ultrasonography in up to 40% of male ADPKD patients.[128] Abnormal sperm motility occurs in the majority of men with ADPKD, and although this has not been shown to directly relate to fertility, it may play a role in the increased occurrence of azoospermia reported in men with ADPKD.[129] Although women with ADPKD demonstrate a normal ability to become pregnant, the course of pregnancy is associated with increased maternal and fetal complications. In general, the likelihood of a successful pregnancy in ADPKD is similar to the general population,[127,130] but there are subgroups of patients, such as those with preexisting hypertension or with established renal insufficiency, who are at an increased risk for fetal loss.

Premature delivery, small for gestational age babies, and congenital abnormalities occur in a small percent of offspring

born to women with ADPKD, typically those older than 30 years or with preexisting hypertension.[127] In a large series of 605 pregnancies in 235 women with ADPKD, only 2 individuals had serum creatinine concentrations greater than 1.2 mg per deciliter prior to becoming pregnant. This is in large part due to the typically delayed occurrence of renal insufficiency in the fourth to sixth decades of life in ADPKD individuals. Given that renal function is usually intact in ADPKD individuals during their reproductive years, the typical complications of polyhydramnios and preterm labor that are associated with pregnancy in women with established renal insufficiency are not typical features of pregnancy management in ADPKD. Maternal complications occurred in 35% of women with ADPKD who become pregnant, including new-onset hypertension, worsening hypertension, preeclampsia, and acute kidney injury. These complications tend to be relatively mild and resulted in uncomplicated pregnancies in the majority of women with ADPKD.

In women with ADPKD who are planning a pregnancy, proactive management before and during pregnancy is critical. For women with hypertension planning to become pregnant, all inhibitors of the RAAS should be stopped prior to pregnancy given the untoward effects on the fetus even with first trimester exposure to this class of drugs. Once pregnant, women with ADPKD should be seen by a high-risk obstetrician and a nephrologist beginning in the middle of the second trimester, particularly if prepregnancy renal function is not normal. Monthly screening for the development of new or worsening hypertension should be conducted. Patients should also check their blood pressure in their home environment on a regular basis. Patients should have their urine reviewed for the presence of new or increased proteinuria, which is a potential sign for the development of preeclampsia. Blood pressure management during pregnancy should include using antihypertensive therapies approved for use in pregnancy including aldomet, hydralazine, clonidine, labetalol, or a dihydropyridine. Immediately postdelivery, women with ADPKD should be monitored closely for signs of worsening hypertension and should have their level of kidney function and blood pressure established approximately 6 weeks after delivery. Longitudinal studies of risk factors for the progression to renal failure have suggested that pregnancy number (particularly for those with more than three pregnancies) is an independent risk factor for the development of ESRD in ADPKD.[131] This association is weak compared to other risk factors such as the presence of hypertension or total kidney volume. The association with pregnancy number or use of estrogen/progesterone agents use is much stronger for liver cyst burden.[132]

THE PKD GENES AND THEIR PROTEIN PRODUCTS

The evolving understanding of the pathogenesis of polycystic kidney diseases has been punctuated by several critical discoveries. The most fundamental of these advances came with the identification of the genes and the respective protein products that are mutated in families with these diseases.[116–118,133–137] At the time of their discovery, the genes for ADPKD (and ARPKD) were completely novel and did not readily fit into in any known biologic pathways. The initial clues regarding their putative roles had to come from the predicted structure of the respective protein products and the knowledge that their functions are expected to intersect at the level of clinical human disease phenotype manifested as the dysregulated nephron tubule structure. This section will review the current state of knowledge regarding the protein products of the various genes associated with human polycystic kidney and liver disease.

PKD1 and Polycystin-1

PKD1 is located on chromosome 16p13.3.[133–135,138] The structure of the gene locus is complicated by the fact that the 5′ two-thirds of the gene is duplicated multiple times with very high sequence fidelity in pseudogenes located on more proximal regions of chromosome 16.[139–142] The need to resolve sequence variants occurring in the PKD1 gene itself as opposed to its homologs for purposes of mutation detection has complicated genetic testing in ADPKD, although current sequence–based technologies are able to address this complication. The protein encoded by PKD1 is called PC-1. PC-1 is a large, low abundance, polytopic integral membrane protein with complex domain structure suggestive of receptor function that undergoes multiple proteolytic cleavage processes (Fig. 16.2). This constellation of features coupled with the absence of direct biochemical and cell biologic assays for its function has made deciphering the mechanisms of ADPKD challenging despite the successful identification of the genes almost 2 decades ago.

Human PC1 is comprised of 4302 amino acids with a 3074 amino acid extracellular NH_2-terminus, 11 transmembrane domains, and a 198 amino acid cytosolic COOH-terminus (Fig. 16.2).[143] The extracellular NH_2-terminal domain contains a number of protein motifs including leucine-rich repeats, a WSC homology domain, C-type lectin domain, a low density lipoprotein (LDL)-A related domain, 16 immunoglobinlike PKD repeats,[134,144,145] a receptor egg jelly (REJ) module,[146] and a G-protein–coupled receptor (GPCR) proteolytic site (GPS).[147] The Ig-like polycystic kidney disease (PKD) domains occupy 40% of the extracellular portion of PC1 and contain a distinct β-sandwich fold structure[148] that is resistant to unfolding under mechanical force.[149–151] The stability of these PKD domains is altered by naturally occurring mutations in PKD patients.[152] The REJ module is comprised of four fibronectin type III β-sheet domains.[153] The first intracellular loop contains a highly conserved polycystin-1, lipoxygenase, alpha-toxin (PLAT) domain that may be involved in protein interactions.[154] The extracellular loop between the sixth and seventh transmembrane domains contains a highly conserved domain that is common to both PC-1 and PC-2 family members and is not found in any other protein families.[155] The region of the last six transmembrane domains of PC1 share sequence similarity with

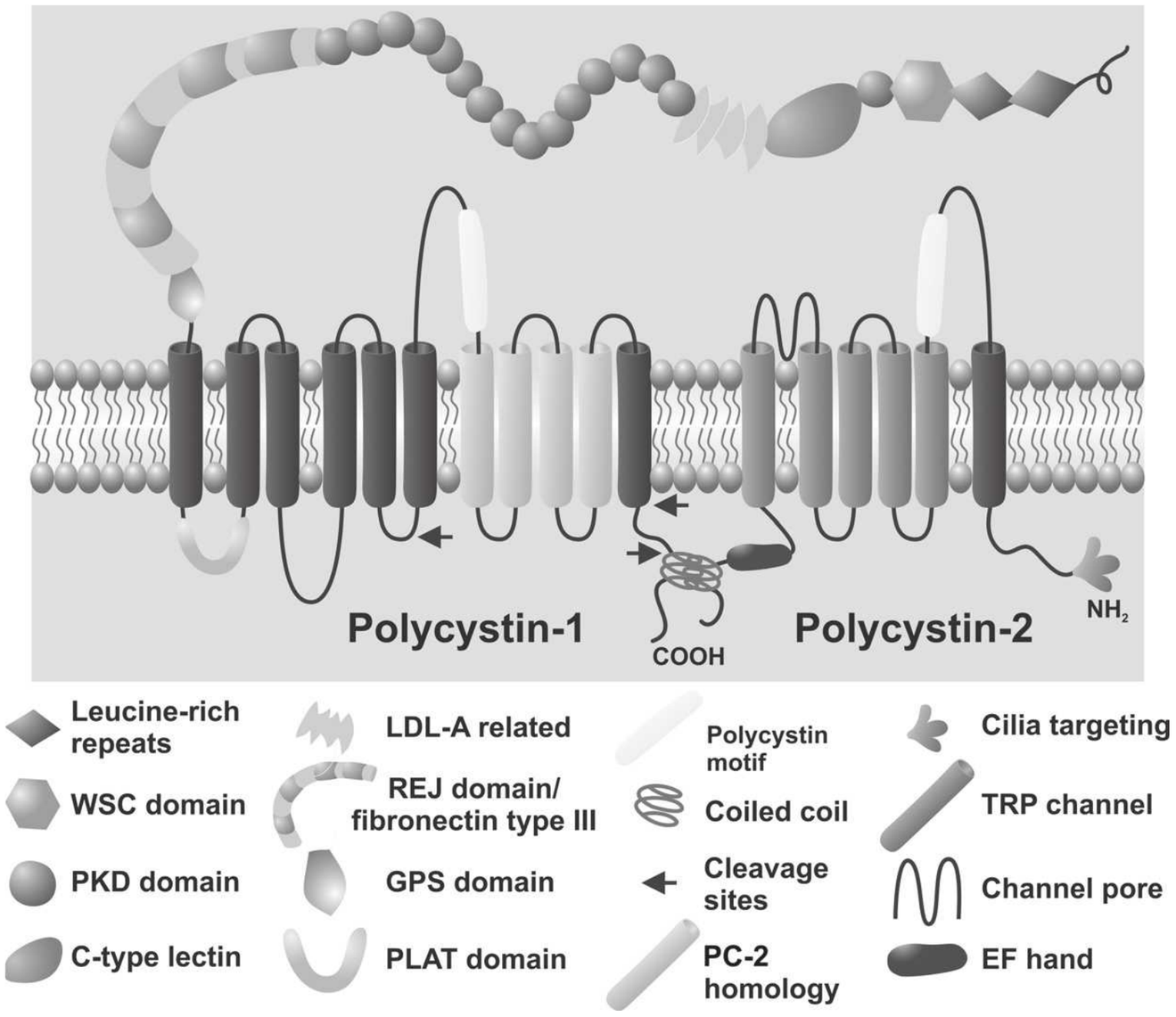


FIGURE 16.2 The protein products of the autosomal dominant polycystic kidney disease (ADPKD) genes *PKD1* and *PKD2*. The schematic drawing highlights the predicted domain structure of polycystin-1 (PC-1) and polycystin-2 (PC-2). PC-1 and PC-2 interact via coiled coil domains in their respective cytoplasmic carboxy (COOH)-termini to form a predicted receptor-channel complex; the most likely stoichiometry is three PC-2 molecules (only one is shown) and one PC-1 molecule. The extensive extracellular NH_2-terminal domain of PC-1 is cleaved at the G-protein–coupled receptor proteolytic site (GPS) in the endoplasmic reticulum (ER) but remains noncovalently associated with the intramembranous COOH-terminal PC-1 fragment. Additional putative cleavage sites are indicated by *arrows*. See the text for further discussion of the structural features of both proteins. PKD, polycystic kidney disease; LDL-A, low density lipoprotein-A; REJ, receptor egg jelly; PLAT, polycystin-1, lipoxygenase, alpha-toxin; TRP, transient receptor potential. (See Color Plate.)

PC-2 but lack critical residues suggesting that PC1 does not have the channel activity associated with PC-2 (see the following). The COOH terminus of PC1 contains a coiled coil domain that is necessary for interaction with PC-2.[156–158] In addition to the repeated partial PKD1 sequences on chromosome 16, there are four homologous protein products in the PKD1 gene family: PKD1L1, PKD1L2, PKD1L3, and PKDREJ. A complex of PKD1L3 protein with the PKD2 homolog, PKD2L1, has been assigned chemosensory function in sour taste and pH sensation,[159,160] although studies with Pkd1L3 knockout mice have called this into question.[161] PKD1L1, but not PKD1, has been implicated in left–right axis determination in mammals.[162]

Several functional cleavage processes have been defined for PC1. The best characterized of these is the autoproteolytic cleavage within the sequence HL↓T[3049] at the GPS in a process that requires an intact REJ module.[163,164] The GPS cleavage process occurs early in the secretory pathway, most likely in the endoplasmic reticulum (ER), and requires N-glycan attachment.[164] The resultant extracellular NH_2-terminal fragment and the intramembranous COOH-terminal fragment remain noncovalently associated with each other.[163] Although the GPS site is conserved in all PC1 homologs within and across species, at least two homologs, PKDREJ and the sea urchin protein SuREJ2, do not undergo GPS cleavage.[165,166] Experimental evidence for the functional importance of GPS cleavage in PC-1 comes from findings that pathogenic patient mutations in the REJ abrogate GPS cleavage, that cleavage deficient PC-1 does not support tubulogenesis and STAT1 activation in a cell culture assay, and that a GPS cleavage mutant knockin functions as a hypomorphic allele in mice.[163,167] GPS cleavage appears to promote cell surface expression of PC-1.[168] Cleaved PC-1 has been identified in urinary exosome–like vesicles, raising the possibility of a signaling function for shed polycystins.[169]

Additional cleavage products of PC-1 have been identified, although these are less well understood than the GPS cleavage. P100, a second intramembranous cleavage product of PC-1 encompassing the last six transmembrane domains, has been shown to diminish store-operated calcium entry by altering the translocation of the ER calcium sensor protein STIM1 to the cell periphery.[170] Two cleavages liberating different fragments of the cytoplasmic tail of PC-1 have also been identified. The first yields a 35 kDa fragment that translocates to the nucleus following cleavage step that is dependent on the presence of functional PC-2.[171,172] This fragment is released by gamma-secretase–mediated cleavage and regulates the Wnt and C/EBP homologous protein (CHOP) pathways by binding to respective transcription factors (TCF) and CHOP, thereby disrupting their interaction with a common transcriptional coactivator p300.[173,174] The second proposed COOH terminal cleavage product is a 15 kDa fragment that interacts with the transcriptional activator STAT6 and the coactivator p100 in a process that is enhanced by the cessation of flow-induced mechanical stimuli.[175] Inhibition of STAT6 in a Pkd1 mouse model results in the slowing of cyst growth.[176]

PKD2 and Polycystin-2

PKD2 encodes PC2, a 968 amino acid integral membrane protein with six transmembrane spans and intracellular NH_2 and COOH termini (Fig. 16.2).[135,177,178] It is a member of the transient receptor potential (TRP) family of cation channels and is also known as TRPP2.[179–182] Two mammalian homologs of PKD2, PKD2L1 (TRPP3) and PKD2L2 (TRPP5), have been identified. The TRP channel family is comprised of

28 different gene products, which function in diverse, mostly sensory, cellular processes including sensation of pain, temperature (hot and cold), taste, pressure, and vision.[183] The last five transmembrane spans of PC-2 have the greatest structural similarity with other TRP channels, with the region between the fifth and sixth transmembrane domains comprising the ion selectivity pore. An RVxP motif in the NH_2 terminus of PC-2 is necessary for its localization in cilia, and PC-2 can traffic to cilia independently of PC-1 (see the following).[184] Phosphorylation at serine 812 in the COOH terminus of PC-2 affects the channel properties[185] and trafficking[186,187] of the protein. The subcellular immunolocalization of PC-2 has been controversial. There is general consensus that PC-2 is abundantly expressed in the ER[177,179,188] and the primary cilium.[189,190] COOH truncated forms of PC-2 readily traffic to the plasma membrane,[177,184] and it has been suggested that coassembly with PC-1 is required for trafficking of full-length PC-2 to the generalized plasma membrane.[180] PC-2 has also been reported associated with centrosomes[191] and the mitotic spindles of dividing cells.[192]

The cellular mechanisms for trafficking PC-2 to the cilium and the somatic plasma membrane are divergent.[193] Trafficking begins with a coat protein complex II (COPII)-dependent process that delivers PC-2 from the ER to the cis side of the Golgi. From there, the bulk of PC-2 is returned to the ER in a process dependent on a 34 amino acid retrieval signal in the COOH terminus of PC-2. A minority of PC-2 enters a vesicular transport process directly from the cis part of the Golgi that delivers the protein to the cilium in a process that depends on Rab8a as well as the RVxP motif. If PC-2 is also to be delivered to the somatic plasma membrane (e.g., by truncation of the COOH terminus retrieval signal), this process traverses the Golgi in the conventional manner of other integral membrane and secreted proteins. PC-2, like PC-1 and the ARPKD (PKHD1) gene product fibrocystin, is a prominent component in urinary exosomeslike vesicles that are produced by the multivesicular body sorting pathway.[169] The PKD-related proteins and the exosomes that contain them may play a role in novel urinary signaling pathways in the kidney.

The COOH terminus of PC-2 contains EF hand[135,194,195] and coiled domains[156–158,195,196] and has been the subject of several biophysical and biochemical studies. The EF hand binds calcium[194] and may have a role in modulating channel activation and inhibition. The coiled coil domain is responsible for homo- and hetero-multimerization of PC-2 with itself and with PC-1, respectively. The structural data are most consistent with a complex consisting of three PC-2 molecules and one PC-1 molecule interacting through their respective coiled coil domains.[157,197] Critical residues in both proteins that weaken these interactions have been identified and should prove useful in modulating activity of the polycystins in experimental systems.[158,196] In addition to interacting with each other, PC-2 and to a lesser but still significant extent, PC-1, have acquired an extensive list of interacting proteins primarily associated with their respective COOH termini.[58] Although many of these interactions have shown functional effects in a variety of biologic systems, a direct role in the pathogenesis of ADPKD is lacking in most of them. This may in fact highlight the likelihood that PC-1 and PC-2 serve additional cellular and tissue functions that may not be directly related to their role in PKD. Identifying the roles specific to ADPKD remains a challenge especially in light of the fact that those roles might be subsumed by the minute fraction of each protein that appears in the primary cilium (see the following).

The Genes for Autosomal Dominant Polycystic Liver Disease

The two known genes for ADPLD, PRKCSH [116,117] and SEC63,[118] respectively encode the noncatalytic beta subunit of glucosidase II (GIIβ)[198,199] and SEC63p. These proteins work at the level of the ER to ensure the proper biogenesis of integral membrane and secreted proteins (Fig. 16.3). As such, their client proteins include up to one-third of the cellular proteome. GII is an ER luminal enzyme involved in glucose trimming of N-glycan moieties in the calnexin–calreticulin cycle. GII activity is necessary for proper folding and quality control of proteins passing through the ER translocon.[200] The ADPLD-associated GIIβ subunit contains an ER luminal retention signal and is required for the function of the GII holoenzyme.[201] SEC63p, an integral protein of the ER membrane, works upstream of GIIβ in concert with the SEC61 translocon pore and BiP, the major Hsp70 chaperone in the ER to effect the cotranslational targeting of precursor proteins with NH_2-terminal cleavable or noncleavable signal peptides.[202–205] SEC63p has a DnaJ-like domain located in the ER lumen, which recruits BiP to the translocon complex. BiP and SEC63p form a molecular ratchet that is responsible for the ATP-dependent vectorial movement of polypeptides into the ER lumen.[206] In contrast to the still incompletely understood functions of the polycystins, it is fairly clear that the two ADPLD gene products work together to facilitate cotranslational translocation across the ER membrane and the proper folding of nascent peptides destined to become either secreted or membrane-inserted proteins. Recent studies have elucidated the interrelationship of this function with polycystic kidney disease (see the following).

PKHD1 and Fibrocystin/Polyductin

A single gene, PKHD1, is known to be mutated in human ARPKD. Heterozygous carrier parents are typically asymptomatic, although a recent report has suggested that they have a predisposition to polycystic liver disease and increased renal medullary echogenicity by ultrasound,[207] whereas affected offspring show bile duct proliferation associated with fibrosis in the liver and relatively homogeneous fusiform dilation of collecting duct segments in the kidney. The protein product of PKHD1 is a 4074 amino acid type I integral membrane protein with a 3858 amino acid extracellular NH_2-terminus, a single transmembrane domain, and a 192 amino acid cytoplasmic tail called fibrocystin/polyductin

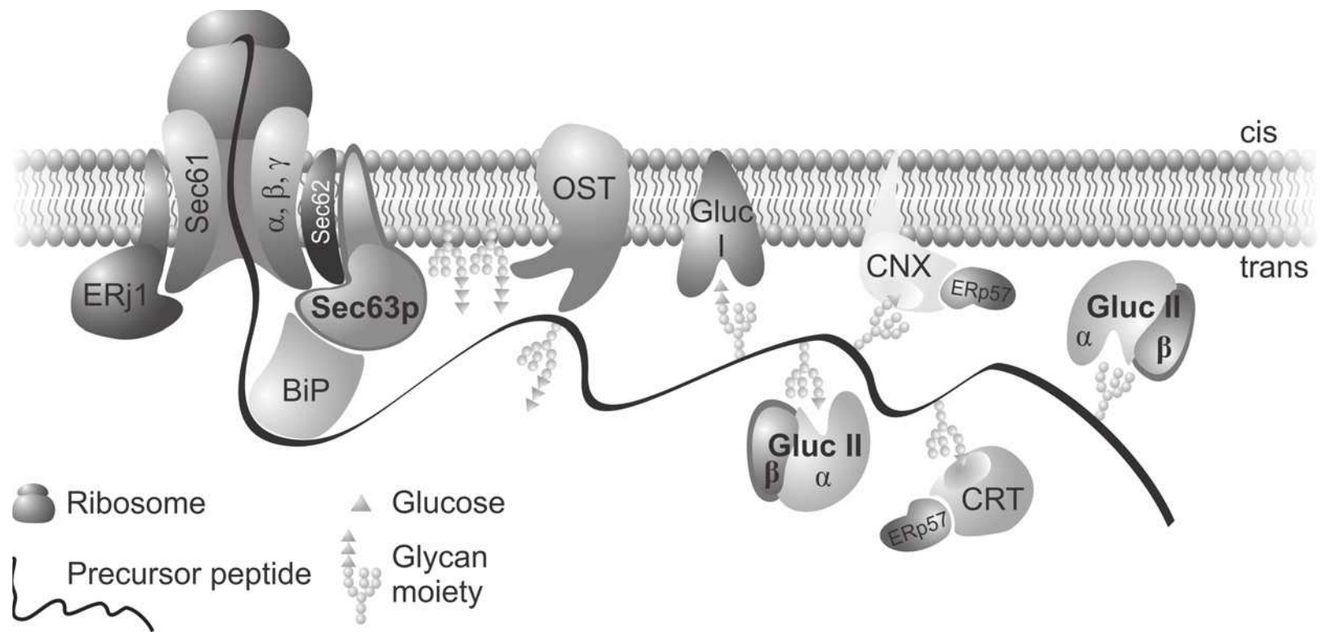


FIGURE 16.3 The functional pathway for isolated polycystic liver disease genes, *SEC63* and *PRKCSH*. The respective products Sec63p and glucosidase IIβ (Gluc IIβ) are resident endoplasmic reticulum (ER) proteins that function in the biogenetic pathway for integral membrane and secreted proteins. The ribosome and precursor polypeptide docks with its receptor comprised of the Sec61α,β,γ/Sec62 complex on the cytoplasmic (*cis*) aspect of the ER membrane. The ER luminal Hsp70-type chaperone protein BiP, acting in concert with the Hsp40 type cochaperones, Sec63p and ERj1, facilitates the cotranslational translocation of nascent peptides with cleavable or noncleavable NH_2-terminal signal peptides. The J domain of Sec63p recruits BiP to the translocation pore complex and activates it by converting its low substrate affinity ATP-bound form to its high affinity ADP-bound form. BiP binds the nascent peptide and acts as a molecular ratchet, facilitating translocation. Oligosaccharyl transferase (OST) catalyzes the attachment of the core glycan moiety to asparagine residues (*N*-glycosylation). Gluc I removes the terminal glucose from the glycan and Gluc II, comprised of α and β subunits, removes the second glucose. This allows the nascent peptide to complex with the chaperone proteins calnexin (CNX) and calreticulin (CRT), both of which associate with thiol-disulfide oxidoreductase ERp57 and promote proper folding and quality control of the precursor polypeptides. Gluc II removes the third glucose from the *N*-linked glycan once the peptide is properly folded. Proteins failing to fold properly are targeted for ER-associated degradation (ERAD) by retro-translocation through the translocon complex followed by proteasomal degradation in the cytoplasmic compartment (not shown).

(FPC).[136,137,208] FPC undergoes "notchlike" proteolytic processing that leads to the shedding of the extracellular domain and the release of the intracellular COOH-terminal fragment through regulated intramembranous proteolysis.[209] FPC, like PC-1 and PC-2, has been found in urine and bile in exosomelike vesicles (ELV),[169] with a recent study suggesting that the majority of mature FPCs are targeted to ELVs.[210] ELV components may have a role in noncell autonomous signaling along the nephron.

MOLECULAR GENETICS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

The discovery of the genes for ADPKD, ADPLD, and ARPKD has enabled an emerging understanding of the increasingly complex molecular bases for cyst formation. Elucidation of these mechanisms is essential for the future development and validation of therapies for ADPKD. The discovery of the genes for ADPKD demonstrated that the preponderance of human disease mutations in both PKD1 and PKD2 were heterozygous loss-of-function variants. This made it unlikely that ADPKD occurred by a true dominant or gain-of-function mechanism, leaving the paradox of an apparent disconnect between germline heterozygous mutations that affect all cells in the body and the focal nature of cyst formation affecting discrete points in a small subset of nephrons. Studies using human material and mouse models of ADPKD and related diseases have implicated a complex interplay between multiplicity of factors, all related to germline mutations in PKD1 or PKD2, giving rise to cyst formation. Over the past decade, advances in the field have defined the occurrence and timing of somatic second hit mutations, the dosage of PKD genes, and most specifically, of PKD1. The effects of noncell autonomous factors including kidney injury, inflammation, and as yet undefined local signals all play a central role in determining the severity and progression of polycystic kidney disease.

The "Two-Hit" Mechanism

A preponderance of evidence supports the notion that homozygous inactivation of either PKD gene during kidney development or adult life is sufficient to result in cysts. Such a cellular recessive mechanism proposes that the critical cyst-initiating event in an affected heterozygous individual occurs when a somatic mutation inactivates the remaining normal copy a PKD gene in an epithelial cell(s) along the nephron. Such a cell undergoes a change in phenotype manifest through increased proliferation coupled with other processes such as a change to a secretory phenotype that, over time, give rise to the focally derived cysts that predominate in ADPKD (Fig. 16.4A). Direct experimental evidence of such a mechanism was first described using human polycystic kidneys from which cells lining individual cysts were

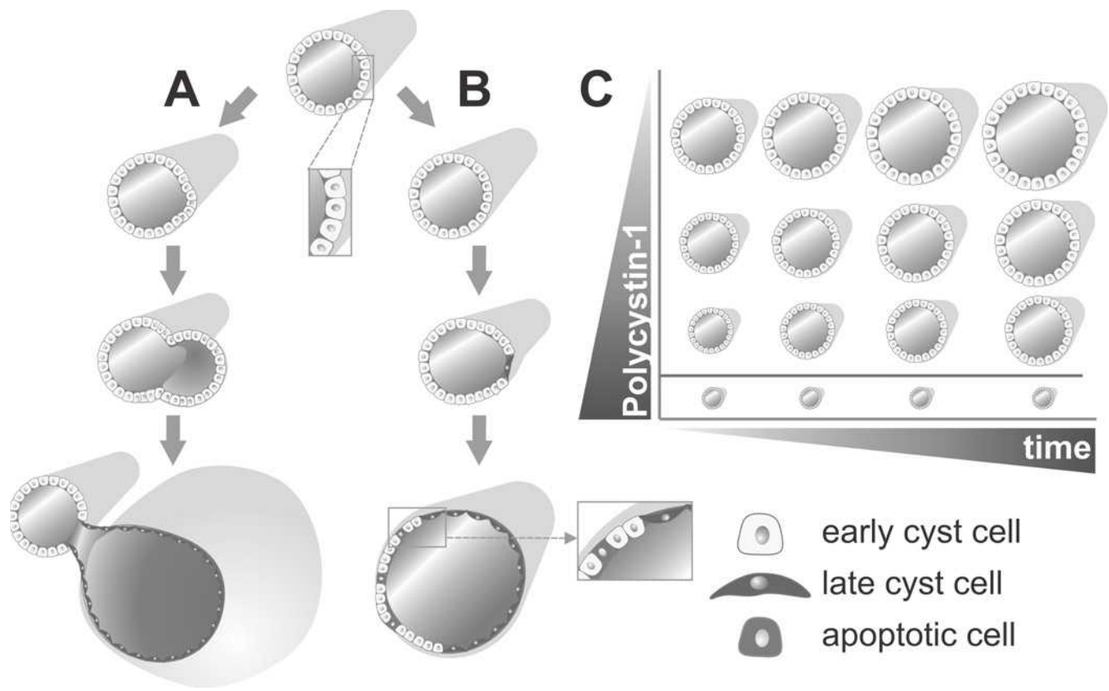


FIGURE 16.4 Mechanisms of cyst formation. **A**. Individual tubular cells undergo a somatic second hit mutation of the normal allele (early cyst cell) and these cells proliferate, forming a focal out-pouching from the tubule that progressively enlarges and eventually loses its connection with the tubule of origin. **B**. Additionally, as the early cyst cell mass expands following the second hit, non–cell autonomous processes can contribute to apoptosis in surrounding cells and to expansion of the tubule to form mosaic cysts comprised of normal tubule cells as well as early and late cyst cells. **C**. Cyst formation can occur as a function of reduced polycystin (PC)-1 dosage and time. When there is sufficient PC-1 (below the *line*), tubules maintain normal structure. Once PC-1 levels fall below the critical threshold (above the *line*), progressive cyst formation ensues. The *vertical axis* illustrates the concept that the lower the dose of residual PC-1, the more quickly cyst growth occurs. The *horizontal axis* illustrates that at each level of reduced PC-1 dosage, cyst growth is progressive over time.

isolated and examined for a loss of heterozygosity (LOH) as a marker for the loss of the normal PKD1 allele.[211] Several subsequent studies confirmed the occurrence of LOH in a subset of cysts in both the kidney and liver due to mutations in either PKD1 or PKD2.[212–214] Together, these studies demonstrate an association between LOH and cysts and support the hypothesis that individual cysts represented clonal expansions of cells that had undergone somatic second hits.

Dynamic evidence of the two-hit mechanism of cyst formation was initially provided by a mouse model of Pkd2 in which the gene-targeting event resulted in a serendipitous allele, $Pkd2^{WS25}$, which undergoes spontaneous inter- or intragenic recombination resulting in conversion to a null allele or reversion to the wild-type allele.[215] This model spontaneously develops cysts over time that result from stochastic gene inactivation events that most closely models the human disease. Subsequently, the principle that second hit mutations are sufficient for cyst formation has been demonstrated in a multitude of knockout mouse models in which conditional alleles produced by flanking loxP sites in the mouse orthologs of Pkd1 or Pkd2 are inactivated by transgenically expressed Cre recombinase enzyme.[211,216–223] The expression of the Cre recombinase can be controlled spatially by tissue and nephron segment–specific promoters and temporally by using inducible forms of the Cre recombinase. The past decade has seen extensive use of these animal models to define additional mechanisms of cyst formation and to evaluate candidate target pathways in preclinical evaluations of therapy.[224–226]

Studies comparing the number and rate of growth of cysts in human PKD1 and PKD2 show that the relatively slower progression of disease in PKD2 patients is associated with a reduced number of cysts, but not with differences in the rate of growth of PKD1 and PKD2 cysts.[227] PKD2 patients also have the same spectrum of extrarenal manifestations as PKD1 patients. These data are most consistent with the interpretation that the rate of somatic second step mutations is lower for the much smaller protein coding region in the PKD2 gene, resulting in a reduced rate of cyst initiation and, therefore, a smaller number of cysts. Biologically, once either PKD gene is lost, they appear to be functionally equivalent as indicated by similar rates of PKD1 and PKD2 cyst growth in humans, similar cystic phenotypes in knockout mouse kidneys,[217,228] and the interchangeability of a mating defect observed in male Caenorhabditis elegans nematodes with inactivation of either gene's ortholog.[229]

Conditional and inducible gene inactivation models in the mouse have also played a critical role in defining the importance of cilia to the pathogenesis of PKD. The intraflagellar transport proteins and their associated motor proteins are necessary for the formation and maintenance of intact primary cilia in renal tubular epithelial cells. Inactivation of these genes in the mouse kidney results in cyst formation that is reminiscent of the cysts formed following the inactivation of Pkd1 and Pkd2.[230–232] These and related findings have solidified the link between the primary cilium in the pathogenesis of PKD (see the following). In addition, kidney-specific inactivation in mice has defined an in vivo system by which candidate genes whose function is thought to be central to maintaining normal tubular structure can be evaluated to determine whether or not they are associated with cyst formation. Because work with mouse models is both time

consuming and costly, the discovery that the inactivation of cilia and PKD-related genes in the vertebrate genetic model organism zebrafish can model cystic diseases[233] has permitted more rapid discovery and validation of important genetic components in the cyst formation process.

The Timing of Gene Inactivation

Taking advantage of the ability to temporally control activity of the Cre recombinase, and therefore of the timing of the inactivation of the mouse orthologs of Pkd1 or Pkd2, investigators set out to determine the differential effects of inactivation of the respective genes during and after kidney development. Inactivation of Pkd1 during kidney growth and development resulted in explosive cyst growth,[220,223] whereas inactivation in the postdevelopmental adult kidney resulted in a much slower, indolent progression of cystic disease.[220] These differences based on timing of gene inactivation were also seen with inactivation of Ift88,[231] an intraflagellar transport protein, and Kif3a,[234] a component of the heterotrimeric kinesin II motor protein complex required for integrity of cilia. These findings have raised questions regarding the timing of second step mutations in human ADPKD. Computational models based on the measured rate of cyst growth observed by volumetric MRI in human subjects suggest a disconnect between cyst size and the simple two-hit model beginning with single cells that can be reconciled if the bulk of the second hits take place in utero and are associated with extraordinary prenatal cyst growth.[235] An alternative explanation may be that cyst formation entails additional factors beyond the simple two-hit model beginning with a single cell.

The Role of Noncell Autonomous Factors in Cyst Progression

Studies in both heterozygous and knockout animal models of PKD orthologous genes or genes related to cilia structures have shown that acute injury promotes accelerated cyst growth in mice. Injury models using ischemia reperfusion,[234,236–238] nephrotoxins,[239] and compensatory hypertrophy following unilateral nephrectomy[240] resulted in marked acceleration of cyst progression in Pkd1 and Pkd2 heterozygotes, and Pkd1, Kif3a, or Ift88 adult knockout models. These findings have led to the proposal that environmental "third hits" are an essential part of disease progression in human ADPKD.[241] This effect is presumably related to the induction of a proliferative response following kidney injury because cells with reduced or absent polycystin expression or cells lacking cilia have a greater proliferative potential. It is likely that instances of acute kidney injury or perhaps subclinical injury in patients with ADPKD have deleterious effects on the progression of polycystic disease. Nonetheless, it should be kept in mind that cyst formation occurs, albeit at a slower pace, following adult second hit mutations without the requirement for third hits.[220,242,243] Therefore, the clinical significance of the contributions from kidney injury to cyst progression in the majority of ADPKD patients remains uncertain.

An intriguing study using chimeric mice produced by aggregation of $Pkd1^{-/-}$ pluripotent embryonic stem cells and wild-type morulae showed that cysts can be mosaic (i.e., comprised of both $Pkd1^{-/-}$ and $Pkd1^{+/+}$ cells).[244] The wild-type $Pkd1^{+/+}$ morulae were derived from mice that express lacZ ubiquitously so that cells from the wild-type lineage could be stained blue by adding substrate for β-galactosidase. The $Pkd1^{-/-}$ cells did not express lacZ and so could be distinguished from cells of wild-type origin throughout the life of the animals. The degree of cyst formation correlated with the extent of chimerism in this model; the greater the contribution from $Pkd1^{-/-}$ cells, the greater the cyst burden. Individual cysts were mosaic, comprised of both wild-type cells that could be stained blue and that retained their cuboidal epithelial shape as well as $Pkd1^{-/-}$ cells that did not stain blue and that had a squamoid shape typical of Pkd1 knockout cyst cells. Over time, the $Pkd1^{-/-}$ cells induced apoptosis in surrounding wild-type cells by a c-Jun N-terminal kinase (JNK)–dependent pathway.[244] This constellation of findings raises the possibility that noncell autonomous processes are active in cyst formation and that cystic disease progression and tissue remodeling in ADPKD involves proliferative expansion of the $Pkd1^{-/-}$ cells' mass coupled with either inclusion or programmed cell death of surrounding wild-type cells (Fig. 16.4B).

Inflammatory and fibrotic processes have long been identified in human ADPKD tissues. In most cases, this has involved late stage cystic kidneys that have undergone a lifetime of cyst infection and hemorrhage as well as systemic damage from hypertension and other factors. Proinflammatory cytokines such as IL-1β, TNF-α, and IL-2 have been identified in cyst fluid samples from human kidneys.[56] Monocyte chemoattractant protein-1 (MCP1) has been found in the urine of ADPKD patients.[245] Expression of MCP1 and macrophage infiltration was found in the nonorthologous Han:SPRD polycystic rat model,[246] and a transcriptome analysis revealed a strong innate immune response signature in another nonorthologous model, the cpk mouse.[247] Most recently, studies using early onset orthologous gene models based on Pkd1 and Pkd2 showed infiltration by an abnormally large number of alternatively activated macrophages in cystic mouse kidneys.[248] Therapeutic depletion of these macrophages resulted in decreased cyst formation, decreased proliferation of cyst cells, and improved preservation of renal function, suggesting that macrophage infiltration and differentiation may be another factor promoting progression of polycystic kidney disease.

Gene Dosage Effects

There is growing evidence to support the role of threshold dosage variation in both the initiation of cysts and the progression of PKD. The absence of pervasive cyst formation along the entire nephron in human ADPKD and in

heterozygous animal models indicates that a 50% reduction of PKD1 or PKD2 gene dosage resulting from the heterozygous germline state in ADPKD is not sufficient for clinical cyst growth. An analysis of individual human cysts identified some with transheterozygous mutations (i.e., mutations affecting one copy of PKD1 and one copy of PKD2 in the same cyst).[249] Subsequent studies in a unique family with a bilineal inheritance of PKD1 and PKD2[250] as well as transheterozygous mouse lines[218] showed that compound transheterozygous mutations in PKD1 in PKD2 are also not sufficient for cyst initiation. The two individuals in the family with bilineal inheritance who had mutations in both genes manifested severe but not explosive PKD with the onset of ESRD in their late 40s.[250] Transheterozygous mice developed more severe cystic disease than would be expected from a simple additive effect of second hits on both Pkd1 and Pkd2, but nonetheless showed that the transheterozygous state is not sufficient for cyst formation.[218] The extra additive effect on cyst formation in the transheterozygous mice suggested a possible threshold effect whereby second hit mutations producing hypomorphic alleles that normally would result in very slow or absent cyst formation can result in more active proliferation when there is haploinsufficiency of both PKD genes.[218]

More severe reductions in gene dosage, particularly of PKD1, have subsequently been associated with graded increases in the severity of cystic response, with a complete loss of polycystins leading to the most severe disease. The importance of dosage is apparent from animal models with mutant Pkd1 alleles that express reduced rather than absent functional PC-1. Mice homozygous for two such hypomorphic alleles develop polycystic kidneys.[251,252] Another example of cyst formation resulting from a reduced dosage of polycystin is seen following inactivation of the RNA-binding protein bicaudal C.[253] Bicaudal C normally antagonizes the inhibitory effect of the microRNA miR–17 on expression of PC-2, and the markedly reduced expression of PC-2 in the absence of bicaudal C is thought underlie cyst formation in this model.

The most compelling experimental data for the centrality of gene dosage come from a study that combined orthologous gene models of isolated ADPLD (Prkcsh, Sec63) with models of ADPKD (Pkd1, Pkd2) and ARPKD (Pkhd1) showing that PC-1 dosage is the central determinant of kidney cyst progression in all of these diseases.[254] The study showed that conditional inactivation of either Prkcsh or Sec63 in the kidney resulted in PKD. This finding extended the phenotypic interrelationship between ADPLD and ADPKD to include the kidney and showed that ADPLD likely occurs via a cellular recessive second hit mechanism similar to ADPKD.[255] The study went on to show that reducing the dosage of PC-1 or PC-2 further exacerbated the kidney cysts in the ADPLD knockout mice. Conversely, increasing the expression of PC-1 (but not PC-2) by transgenic expression ameliorated the kidney cystic disease. The ADPLD genes affected cyst formation by significantly reducing the steady-state levels of PC-1 due to defective biogenesis (Fig. 16.3). The ability to worsen and improve kidney cysts following ADPLD gene inactivation by either reducing or increasing the PC-1 dosage, respectively, indicates that PC-1 is the rate limiting component in this process. The rescue of the ADPLD cysts by overexpression of PC-1 was durable but not permanent, indicating that the time required for cyst formation is inversely related to the degree of reduction of PC-1 dosage. Taken together, these findings indicate that a combination of PC-1 dosage and time are key determinants of cystic progression (Fig. 16.4C). Furthermore, reduced PC-1 dosage resulted in worsened cyst formation following homozygous inactivation of the ARPKD gene, Pkhd1, suggesting that at least in mouse models the expressivity of the ARPKD phenotype in the kidney is modulated by functional PC-1 dosage.[254,256] In aggregate, this study defines dosage- and time-dependent interrelationships between ADPKD, ARPKD, and ADPLD in orthologous mouse models and establishes PC-1 activity as the central determinant of the phenotypes in all three disease models.

Genotype–Phenotype Correlations and Genetic Modifiers

Genotype–phenotype correlations are variations in disease progression (the phenotype) based on different mutations (the genotype) in the primary gene underlying a human Mendelian disease. The major genetic determinant of severity in ADPKD is the underlying disease gene locus effect—PKD1 mutations result in ADPKD with mean age of ESRD almost 2 decades earlier than PKD2.[10,227] In clinical studies, 85% of ADPKD families have PKD1 mutations and only 15% have PKD2 mutations. The milder phenotype has likely caused underascertainment of PKD2 families in these studies, and population-based ascertainment suggests that 29% of ADPKD families have PKD2.[13] In the group of individuals entering ESRD after age 65, 50% are PKD2 families.[257] Within the PKD1 population, a small subset of patients with mutations resulting in a contiguous gene deletion syndrome affecting the tuberous sclerosis complex 2 gene, TSC2, have severe PKD, often presenting in early childhood.[258,259]

Human ADPKD families with recessive inheritance of nonsynonymous amino acid substitution mutations in PKD1 have recently been described[260] and have been found to mimic ARPKD.[261] Although fetuses homozygous for complete loss-of-function PKD gene alleles are presumed to be nonviable, these recessively inherited hypomorphic PKD1 alleles have reduced function, allowing for live-born progeny. They develop early onset cystic disease with cysts that are homogeneous in size and distribution. This is in contrast to the usual heterogeneous and focal cyst population seen in typical ADPKD and are more reminiscent of ARPKD. Similar findings have been described in an individual homozygous for a PKD2 missense mutation due to uniparental disomy (two copies of part of a chromosome from one parent and none from the other).[262] A weak modifier effect resulting in

greater severity of ADPKD from PKD1 mutations in the 5′ end of the gene compared to the 3′ end has been reported.[263] Recently, a dominantly inherited heterozygous missense variant in PKD1 was identified in a family segregating a mild form of ADPKD that was clinically similar to PKD2-based disease.[264] This finding suggests that hypomorphic alleles in the heterozygous state in PKD1 can also result in disease that is clinically milder.

Genetic variations at loci other than the primary disease gene can modify disease severity. Because all affected individuals in a given family have the same germline mutation in either PKD1 or PKD2, the high degree of intrafamilial variability observed in the severity of ADPKD has been taken to suggest that genetic modifiers and environmental factors influence the progression of ADPKD. This conclusion should be tempered in light of the fact that the timing and extent of somatic second hit mutations will also contribute to intrafamilial variability. Although no strong genomewide genetic modifier effects have been identified in ADPKD, several lines of evidence support the hypothesis that such modifiers exist and should be discoverable. The observation that a variation in age at ESRD among siblings is significantly greater than the variation in genetically identical twin pairs is likely attributable to the existence of genetic modifier effects.[265] Similarly, the occurrence of severe neonatal PKD in the progeny of parents with very mild ADPKD due to PKD2 mutations suggests that genetic modifiers at non-PKD gene loci may impact disease progression.[266] Two studies[267,268] used variance component analysis to determine heritability in ADPKD (i.e., the proportion of phenotypic variance explained by modifier genes). They found that between 32% and 42% of the variance in creatinine clearance and 43% to 78% of the variance in age at ESRD were heritable.

The existence of genetic modifiers has been directly demonstrated in selected cases of ADPKD. A report identified severely affected ADPKD patients in whom heterozygous mutations in PKD1 were accompanied by heterozygous mutations in the transcription factor hepatocyte nuclear factor-1β (HNF-1β).[269] Recessively inherited mutations in HNF-1β result in glomerulocystic disease in maturity onset diabetes of the young type 5 (MODY5) and HNF-1β regulates transcription of several PKD genes, including PKD1.[270–272] This suggests that the severity of ADPKD may be modified by mutations that indirectly affect expression of PKD genes. A directed genetic association study analyzing 173 biologic candidate genes in 794 ADPKD patients from 227 families identified Dickkopf 3 (DKK3) as a possible genetic modifier for disease severity in ADPKD.[273] DKK3 antagonizes Wnt/β-catenin signaling, a pathway that has been implicated in cyst growth (see the following).

CILIA AND CYSTIC KIDNEY DISEASE

Following the discovery of the genes responsible for ADPKD, the next major quantum of advancement in the field arose from the understanding that the structure and function of the primary cilium is central to the pathogenesis of polycystic diseases.[274,275] All cells in the kidney tubule with the possible exception of intercalated cells have a single primary cilium extending from the apical surface into the tubule lumen. Primary cilia differ from motile cilia in that they have nine pairs of radially arrayed microtubule bundles but lack the central pair, hence they are called 9+0 cilia (Fig. 16.5). Cilia comprise a unique cellular compartment that is devoid of intracellular components such as membrane-bound vesicles and ribosomes.[276] As a consequence, all the protein components of cilia must be synthesized in the cell body

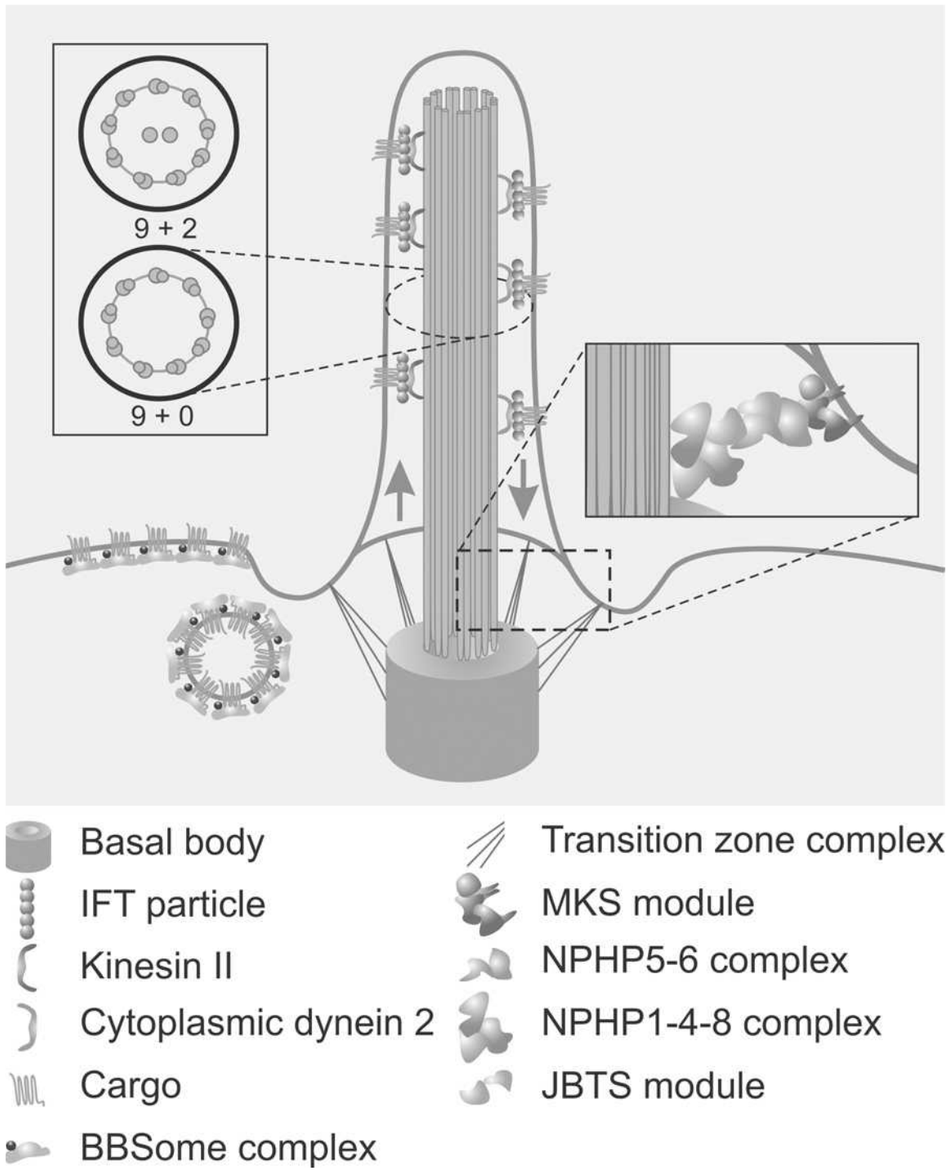


FIGURE 16.5 Cilia structure and function. Primary cilia are structured on nine pairs of radially arrayed microtubules without the central pair found in motile cilia. The ciliary axoneme emanates from the basal body, and the cilia compartment is separated from the body of the cell by the transition zone complex. The delivery of integral membrane proteins likely occurs by targeted exocytosis near the base of the cilia. The sorting of specific proteins destined for cilia may be mediated by the BBSome coat complex, which contains several of the gene products associated with Bardet-Biedl syndrome (BBS). The protein-sorting process is regulated by the transition zone complex, which has associated with it the protein products of the ciliopathy genes. The membrane proximal component of this complex is primarily composed of Meckel Gruber syndrome (MKS) proteins. Two nephronophthisis (NPHP) complexes, respectively based on NPHP5/6 and NPHP1/4/8 and in association with Joubert syndrome (JBTS) gene products, have also been identified. Within the cilium, cargo proteins are transported on IFT particles with kinesin II motor proteins responsible for anterograde transport away from the cell body and cytoplasmic dynein motor proteins responsible for retrograde transport back toward the cell body. (See Color Plate.)

and transported to cilia; similarly, the turnover of cilia components requires retrograde transport to the cell body. These transport processes are collectively referred to as intraflagellar transport (IFT). IFT particles are protein complexes necessary for bidirectional transport of cilia components that are moved along the microtubular ciliary axoneme by molecular motors.[276,277] The cilium is nucleated on the apical surface of epithelial cells by the basal body complex comprised of the centrioles in nondividing cells. The base of the cilium has a transition zone that serves as a sorting complex to regulate the trafficking of proteins into and out of the cilia.[278–280] Primary cilia subsume a broad array of sensory functions in different tissues and cell types.[281,282] As sensory organelles, cilia make use of their specialized structural features and the restricted access of cellular components to uniquely integrate signals from the cell's surroundings.

Several convergent lines of evidence support the connection between cilia function and the group of renal cystic disorders that include ADPKD and ARPKD as well as for a broader group of recessively inherited "ciliopathy diseases" such as NPHP, BBS, Joubert syndrome (JBTS), and MKS (Table 16.1). The early evidence for a connection between cilia and PDK came indirectly from findings in model organisms. The first gene identified for a recessive polycystic kidney phenotype in the mouse, Tg737,[283,284] was subsequently shown to be Ift88, an IFT complex B component.[285] Independently, the C. elegans homolog of PKD1, called lov–1, was found expressed in sensory neuronal cilia in male nematodes.[286] More direct evidence for the central role of cilia in ADPKD was obtained when PC-2 and PC-1 were localized to cilia in cells and tissues.[189,190] These findings led to a prospective study showing that kidney-selective inactivation of Kif3a, a component of the heterotrimeric kinesin-2 motor complex, resulted in the loss of cilia along the nephron, which recapitulated a polycystic kidney phenotype.[230] An unbiased phenotype-driven forward genetic screen for recessive loss-of-function alleles resulting in pronephric cysts in zebrafish identified 10 genes promoting cyst formation.[233] These included Pkd2 and Hnf-1β, IFT-related genes, and novel cilia-related genes, thus solidifying the connection between cilia structure and function and kidney cyst formation in vertebrates. Finally, human gene cloning for an extensive series of diseases that shared fibrocystic kidney phenotypes (e.g., NPHP, JBTS, MKS, and BBS) found that the respective protein products of these disease genes were mostly expressed in the cilia-basal body complex (Table 16.1).[275,287]

Polycystins have been localized to cellular compartments other than cilia.[288] Although it is widely believed that the function of polycystins in cilia is central to the pathogenesis of ADPKD, the contributions of polycystins expressed in other subcellular locations is uncertain. PC-1 has been localized to the basolateral membrane,[289] at sites of cell matrix adhesion[290] and intercellular adhesion,[291] and at desmosomes.[292] There is universal agreement that PC-2 shows its most abundant expression in the ER in cells[177] and in kidney tissues.[179] The localization of PC-2 to the generalized plasma membrane outside of cilia has been controversial.[177,185] The surface expression of PC-2 has been proposed as requiring coassembly with PC-1,[180] although PC-2 is able to traffic to cilia independently of PC-1.[184] The expression of PC-1 has shown developmental regulation with higher levels early in development and a reduced level of expression in adult tissues.[293,294] Similar changes in the expression of PC-2 with developmental stage have not been reported.

Ciliopathies

The recessive ciliopathy disorders (e.g., NPHP, JBTS, MKS, and BBS) have both clinical and genetic overlap amongst each other. The clinical overlap involves the range of tissues in which cilia function plays a central role.[275,282,295] For example, the outer segment of the retina represents a modified cilium, and retinal degeneration is seen in the Senior Loken variant of NPHP, as well as in JBTS, MKS, and BBS.[296] Cilia are also essential to Hedgehog signaling, which is important for patterning of the digits, and MKS, BBS, and another ciliopathy, such as orofacial digital syndrome, show digital defects.[282] Other manifestations associated with recessive cilia mutations include left–right asymmetry defects and associated cardiac heterotaxy syndromes,[297–299] central nervous system (CNS) malformations with developmental delay,[296] obesity,[231] anosmia,[300] and skeletal defects.[301] Genetic overlap among the ciliopathies occurs because mutations in the same gene can give rise to different diseases along this phenotypic spectrum (e.g., mutations in MKS3 can cause both MKS and JBTS; mutations in CEP290 can cause NPHP, JBTS, MKS, and BBS; and mutations in TMEM216 cause MKS and JBTS).[302,303]

The ciliopathy disease gene products have been segregated into distinct functional complexes within cilia (Fig. 16.5). Several of the BBS gene products comprise the functional BBSome that is central to the selective delivery of integral membrane proteins into cilia.[304–306] The gene products associated with NPHP, JBTS, and MKS comprise part of the transition zone gatekeeper complex at the base of cilia.[276,278,307] Within this transition zone complex, there are at least three functional subcomplexes.[307] NPHP gene products are active in apical organization and centriole/cilia integrity, MKS gene products are associated with Hh signaling,[307] and JBTS-MKS complex proteins regulate ciliary membrane composition.[278] Although functionally interrelated, the IFT, BBSome, and NPHP-JBTS-MKS complexes do not appear to interact physically, and only components of the latter two are associated with human diseases. It is possible that the absence of human disease resulting from recessive IFT mutants is due to lethality.

Relationship of Polycystic Diseases to Ciliopathies

Although it is tempting to categorize ADPKD as part of the ciliopathy spectrum of disorders, there are some important distinctions that support keeping ADPKD and diseases with close functional relation to it (ADPLD and ARPKD) in a conceptually and biologically distinct compartment. From a genetic

standpoint, the ciliopathies are all recessively inherited. It is likely that somatic second hits are affecting the normal copies of the ciliopathy genes in heterozygous carrier parents, yet these individuals do not develop clinical cystic phenotypes. This is in stark contrast to ADPLD, in which heterozygous patients still form liver cysts following second hit mutations in genes whose products only indirectly affect PC-1 and PC-2 activity.[254,255] The genetic evidence suggests that ciliopathy genes really have limited or absent functional interaction with the polycystins. These findings are also consistent with the view that the ciliopathy disorders are primarily developmental diseases with less of a role in structural homeostasis of the adult kidney than the ADPKD gene products. From a biochemical standpoint, PC-1, PC-2, and FPC are all integral membrane proteins and are notably absent from any of the complexes described for the other ciliopathy genes. Conceptually, the ciliopathy gene products function in establishing and maintaining the structural integrity and the unique molecular composition of cilia. PC-1 and PC-2 (and FPC) are part of that unique composition and form a receptor-channel signaling complex that subsumes a specific sensory signaling function within the primary cilium. The genetic and functional dichotomy between ciliopathies and polycystic diseases should raise a cautionary note in extrapolating molecular pathways defined in terms of defects in the cilia structure and function based on IFT, the transitional zone, and the BBSome mutations to disease pathogenesis in ADPKD, ADPLD, and ARPKD.

PATHOGENESIS: CELLULAR PATHWAYS AFFECTED BY THE POLYCYSTINS

Cellular Sensory Reception

The polycystins are integral membrane proteins expressed on a sensory organelle, the primary cilium. PC-1 has a structure suggestive of receptor function, and PC-2 is a member of the TRP nonselective cation on the channel family that largely serves as receptor-gated sensory signaling channels.[308] These features have been taken to indicate that PC-1 and PC-2 function as a sensory receptor-channel signaling complex, but the specific nature of the signal that the polycystins "sense" has remained elusive. The predominant hypothesis is that the polycystins comprise a mechanosensory complex within the cilia that detect luminal fluid flow or shear stress. The data to support this role come from laminar shear stress experiments performed on ciliated monolayers of cells in culture, which showed a global cytosolic calcium response that was dependent on the bending of intact cilia[309,310] and the presence of active PC-1 and PC-2.[311] Indirect in vivo evidence supporting this function came from a study examining defects in lateralized, flow-dependent calcium signaling in the embryonic node in mice.[299] The formation of the vertebrate left–right body axis requires leftward vectorial fluid flow generated by motile cilia in a transitory embryonic structure referred to as the embryonic node. Pkd2 knockout animals, which have a randomization of the left–right axis,[297] show a failure to initiate normal calcium transients along the left side of the node presumably due to the failure to sense the flow signal.[299] This flow sensor hypothesis suggests that renal tubular luminal flow per se is required for the normal homeostatic structural maintenance of the kidney, something that has not yet been directly demonstrated.

The alternative hypothesis that unknown ligands are involved in polycystin signaling remains plausible because the effects of flow or shear stress may be difficult to separate from the effects of signaling ligands that are delivered by flow. Recent evidence has shown that polycystins and FPCs are shed into the luminal space, and the ELVs in which they appear adhere to the surface of cilia.[169,210] These ELVs may be the vehicles carrying signals important in PKD. Earlier biochemical evidence supports homotypic interactions involving the extracellular domains of PC-1,[312] further posing the possible involvement of ligand binding in polycystin signaling. In the instance of flow-dependent embryonic node signaling in left–right asymmetry, shed vesicles carrying signaling molecules such as Sonic Hedgehog and retinoic acid have been shown to be essential in generating lateralized calcium signals, mentioned previously.[313] The PC-2–dependent cellular calcium response to flow stimulus was subsequently shown to require a heteromeric complex between PC-2 and transient receptor potential cation channel subfamily V member 4 (TRPV4).[314] Loss of TRPV4 abolishes the cellular calcium in response to flow in vitro yet does not result in cyst formation in the zebrafish model in vivo. This puts into question whether calcium transients resulting from ciliary flow sensing in vitro are a fundamental mechanism of cyst formation in vivo.[314] It is noteworthy that, to date, it has not been possible to document changes in local calcium within cilia in response to flow. A recent study linking the cilia-mediated flow response to regulation of the mammalian target of rapamycin (mTOR) signaling and cell size regulation showed that this was a function of the master kinase Lkb1 in cilia and specifically excluded PC-2 as the cilia sensor.[315] Finally, evidence that polycystin complex signaling is dynamically regulated by PC-1 dosage and not a binary on/off process[254] can be extrapolated to suggest that the signal sensed by PC-1 is also dynamically regulated. This should give pause to the notion that flow alone serves as the primary signal for determining kidney tubule lumen diameter. The increasing application of proteomic technologies to the analysis of signaling molecules in the urine may uncover novel signaling molecules and lead to improved understanding of the interrelationship between flow, ligands, and polycystin signaling.

Effector Pathways

A multitude of effector pathways have been proposed for the renal tubule cell response to a loss of polycystins.[58,316,317] Nonetheless, the molecular mechanism linking PC-1/PC-2 function in cilia to the extensive array of candidate cellular effector pathways has been elusive beyond a hypothesized role for calcium ions (Fig. 16.6).[58] The potential complexity of what is unknown in cilia-based signaling downstream of polycystins is best illustrated by what is known of Hedgehog

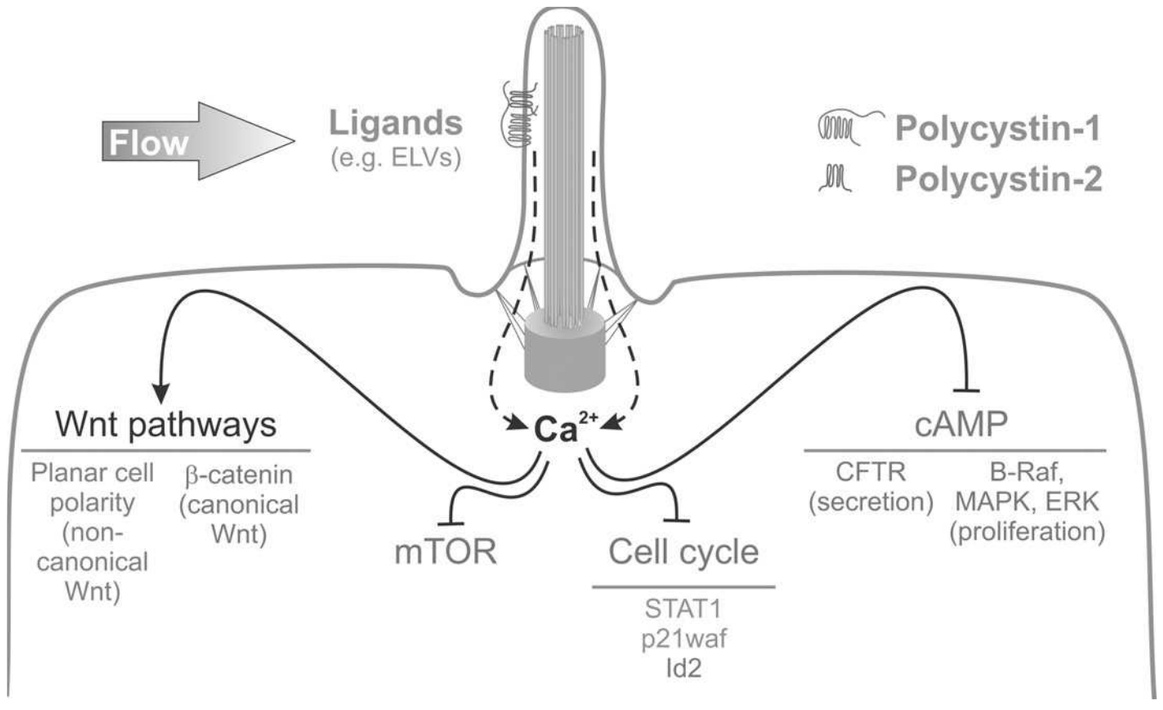


FIGURE 16.6 Polycystin (PC) signaling effector pathways. The PC-1/PC-2 receptor channel sensory complex on apical cilia on the renal tubular epithelium is activated by flow and potential ligands carried by the flow (exosome-like vesicles [ELVs]). This is thought to result in a rise in local and cellular calcium, which mediates signals to a number of effector pathways. Pathways or components shown in *blue* are activated by normal polycystin signaling, whereas those shown in *red* are inhibited by intact polycystin signals. A loss of PCs results in increased cyclic adenosine monophosphate (cAMP) production, which may increase apical secretion and the proliferation in cyst cells. The loss of PCs also increases the progression of the cell cycle, and may activate mammalian target of rapamycin (mTOR) signaling and may favor β-catenin-dependent Wnt signaling. STAT1, signal transducers and activators of transcription 1; CFTR, cystic fibrosis transmembrane conductance regulator; MAPK/ERK, mitogen-activated protein kinase/extracellular regulated kinase. (See Color Plate.)

(Hh) signaling in cilia.[281] In the absence of Hh ligand, the receptor Patched (Ptch) resides in cilia and inhibits the entry of the seven transmembrane receptor protein Smoothened (Smo) into cilia. In this state, the transcriptional repressor Gli3R inhibits Hh-responsive gene transcription, whereas the transcriptional activator Gli2 is sequestered in cilia by binding of Suppressor of Fused (SuFu). Upon binding of the Hh ligand, Ptch translocates out of cilia, thereby allowing Smo to enter the cilium. This in turn alleviates Gli3R repressor activity and promotes translocation of transcription activators Gli1 and Gli2 to the nucleus. All of the aforementioned components of the Hh signaling pathway have been found in cilia.[318,319] The lack of a comparable mechanistic understanding of polycystin signaling leaves open the possibility that many of the effector pathways identified to date represent secondary processes removed from the immediate molecular events of polycystin signaling. These secondary processes may contribute substantially to disease progression and therefore merit investigation and evaluation as potential targets for clinical therapy. In parallel, continued basic studies to identify the less understood, more proximate polycystin signaling events is essential to achieve the goal of identifying novel and specific therapeutic targets for ADPKD.

Planar Cell Polarity and Wnt Signaling

Cyst formation has been attributed to defects in a tissue organization process defined by planar cell polarity (PCP). In the case of the kidney, the developing nephron grows in the direction oriented in parallel with the lumen while not growing in the dimension perpendicular to the lumen. This asymmetric orientation of growth within the plane of the tubule epithelium is reflective of PCP (Fig. 16.7). "Convergent

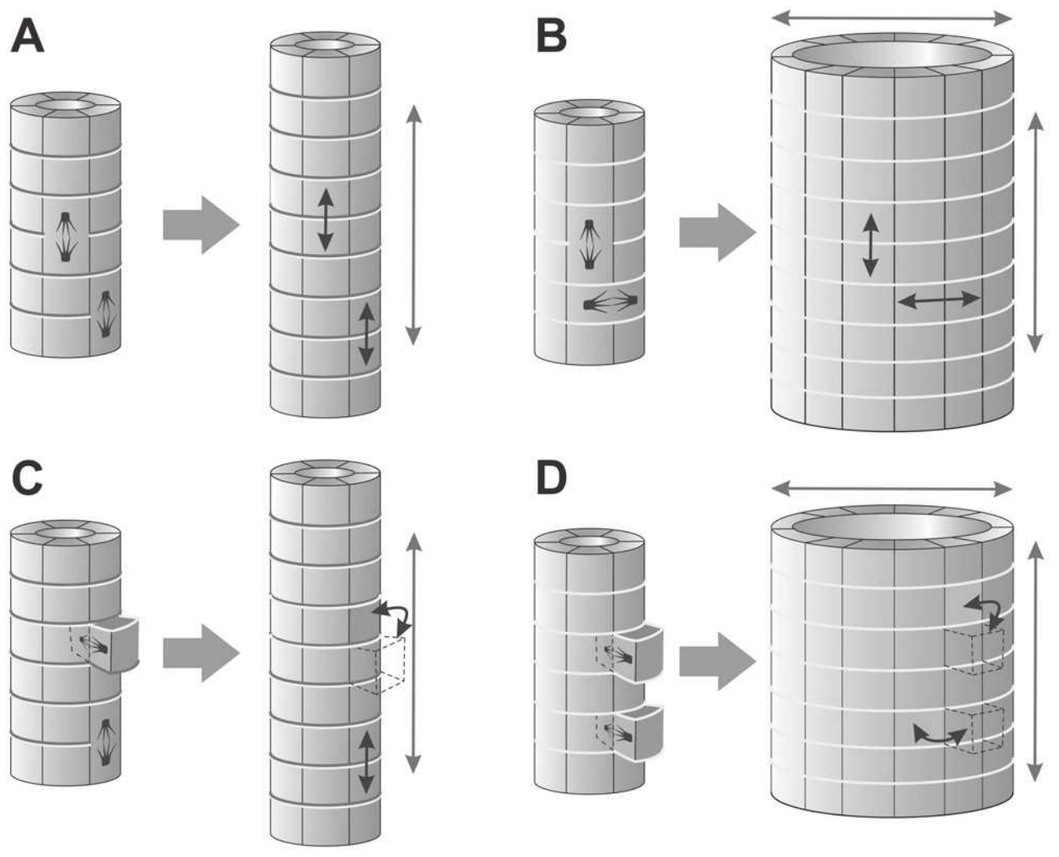


FIGURE 16.7 Planar cell polarity in the kidney. **A**: The postnatal elongating nephron shows oriented cell division (OCD) with mitotic spindle poles aligned with the long axis of the tubule within the plane of the epithelium. **B**: Randomization of the mitotic spindle axis, as may occur in recessive ciliopathies, can interfere with normal tubular elongation and give rise to dilated or cystic tubules. **C**: Planar cell polarity is also mediated by convergent extension movements whereby cells move toward a midline axis and along its length. In this schematic, a cell dividing out of the plane of the epithelium is shown entering that plane along the longitudinal axis. **D**: A loss of convergent extension movements can also result in impaired tissue polarity.

extension" movements, in which cells move toward a midline in one dimension and extend along that midline in a perpendicular direction, are one component of PCP. Another PCP component, oriented cell division (OCD), occurs when the axis of the mitotic spindle poles in anaphase and telophase align in parallel to the tubule lumen leading to cell division that is parallel to the axis of elongation and in the plane of the epithelium. Evidence linking cilia functions to PCP[320,321] has been provided by the association of Ift88 mutations with defective planar polarization of actin-based stereocilia in the inner ear[322] and by the roles in PCP processes of recessive ciliopathy genes for nephronophthisis (inversin)[323] and BBS.[324]

It has been proposed that a loss of OCD in elongating kidney tubules results in randomized orientation of mitoses, which replaces the directional tubule elongation process with cyst formation (Fig. 16.7).[325] Experimental studies have confirmed that elongating tubules in the postnatal mouse or rat kidney show OCD with mitotic spindles oriented parallel to the long axis of the tubule.[326,327] Consistent with the hypothesized pathogenic role of PCP defects in cyst formation, mice with mutations in Hnf-1β and rats with mutation in Pkhd1 show a loss of OCD in advance of the cyst formation.[326] Similarly, postnatal Ift20 mutant kidneys and injured adult Kif3a mutant kidneys, both of which have structural defects in cilia formation, also show a loss of OCD followed by cyst formation.[234,328] Finally, a loss of the PCP-related protocadherin Fat4 can result in cystic kidneys, a phenotype worsened by a reduced dosage of the core PCP protein Vangl2.[329] Although OCD is the predominant PCP mechanism in the postnatal elongating nephron in mice, convergent extension movements predominate during the embryonic phase of kidney development.[327] BBS genes (e.g., Bbs1, Bbs4, Bbs6) are required for PCP-associated convergent extension movements in zebrafish,[330] but a role for the human PKD genes (e.g., Pkhd1, Pkd1, Pkd2) in convergent extension movements has not yet been reported.

The previous data suggest that PCP-related processes are linked to cilia structure and function and are important in kidney development. The disruption of PCP processes during development can result in cyst formation. Despite this connection, the relationship of disruption of PCP to the pathogenesis of human ADPKD and ARPKD is less clear. Two mouse models of ARPKD that do not show a cystic phenotype in the collecting duct nonetheless show a loss of OCD in that segment in the postnatal kidney.[331] The same study also found that precystic tubules that had lost either PC-1 or PC-2 expression did not show a loss of OCD yet went on to form cysts.[331] The finding that mutations in Pkhd1 disrupt OCD during tubule elongation but do not lead to cysts calls into question whether a loss of OCD is sufficient for kidney cyst formation. The finding that a loss of PC-1/PC-2 can initiate cysts without the loss of OCD suggests that PCP defects are not necessary for cyst formation in ADPKD. One interpretation of these apparently discrepant results is that the mechanisms of kidney cyst formation in human ADPKD and ARPKD differ from those associated with the recessive ciliopathy diseases.

PCP is mediated by components of the noncanonical Wnt signaling pathway. Modulation of canonical Wnt signaling, which regulates cellular proliferation and differentiation, has also been associated with cystic diseases (Fig. 16.6).[332] Wnts are secreted glycoproteins that bind to Frizzled receptors, which signal through Dishevelled inside the cell. Downstream of Dishevelled, the canonical and noncanonical pathways diverge with β-catenin–dependent gene expression acting as the major effector of the canonical Wnt pathway. The nephronophthisis gene inversin modulates Dishevelled levels to determine the balance between canonical and noncanonical Wnt signaling.[323] Similarly, BBS genes are required for PCP-associated convergent extension movements in zebrafish, but suppression of these transcripts results in the activation of canonical Wnt signaling through stabilization of β-catenin.[330] These findings have led to the emergence of the concept that several ciliopathy genes function to regulate the balance between canonical and noncanonical Wnt signals. Implicit in such a hypothesis is the idea that cyst formation following the disruption of PCP pathways is fostered by the associated increase in canonical Wnt signaling.

The role of canonical Wnt signaling in cyst formation has been explored directly. The product of the JBTS gene Ahi1 supports canonical Wnt signaling by facilitating β-catenin accumulation and consequent transcriptional activation in the nucleus.[333] Loss of Ahi1 impairs the canonical Wnt response to kidney injury and results in cyst formation. Although cilia-related proteins may function in Wnt pathways, a direct role for cilia in Wnt signaling remains controversial given conflicting evidence that cilia inhibit canonical Wnt signaling[330,334] or have no role in it.[335,336] The data on the role of Wnt/β-catenin signaling in cyst formation in ADPKD is also confounded. Initial support for a role for canonical Wnt signaling in cyst formation came from studies showing that transgenic overexpression of a constitutively active β-catenin or kidney specific inactivation of the APC gene (which normally inhibits β-catenin signaling) results in cyst formation.[337,338] On the other hand, the study of Wnt9b-deficient mice that supported the role of noncanonical Wnt signaling/PCP in cyst formation did not find any evidence for β-catenin–dependent canonical Wnt signaling in the process.[339] The activity of COOH-terminal fragments of PC-1 in canonical Wnt signaling have been implicated in both activation[340–342] and repression[173,343] of the canonical Wnt pathway; the latter was also reported for PC-2.[344] Finally, a recent study using embryonic Pkd1 and adult Pkd2 mouse models of polycystic kidney disease in combination with a canonical Wnt activity reporter transgene failed to show evidence of β-catenin transcriptional activation in kidney cysts in vivo.[345] Although the absence of transgenic reporter activation in these mice does not entirely exclude a role for canonical Wnt signaling in cyst formation, it does pose a significant challenge to the model. In aggregate, the data suggest involvement of both canonical and noncanonical Wnt signaling in cystic processes, but this relationship is not universal and the direct relevance to human ADPKD remains uncertain.

Mitogen Activated Protein Kinase/ Extracellular Regulated Kinase

The mitogen activated protein kinase (MAPK)/extracellular regulated kinase (ERK) pathway is a kinase phosphorylation cascade that integrates extracellular signals received through receptor tyrosine kinases, G-protein-coupled receptors, and integrins. MAPK/ERK signaling is modulated by cAMP, protein kinase A (PKA), protein kinase C (PKC), and regulates a spectrum of cellular activities including cell cycle, gene transcription, protein translation, and epithelial morphogenesis (Fig. 16.6). Activation of the MAPK/ERK pathway is seen in cell culture models based on human ADPKD cyst lining cells that lack PC-1.[346,347] PC-2 has been implicated in providing tonic suppression of MAPK/ERK signaling.[348] MAPK/ERK activation occurs in vivo in mouse models based on nonorthologous[346,349,350] and orthologous ADPKD genes.[223] In human ADPKD cell culture models, the activation of the MAPK/ERK cascade is dependent on activation of B-Raf by cAMP—a paradoxical effect unique to PKD cells or in conditions of calcium deprivation.[346,347] Evidence for a similar mechanism of activation in vivo is lacking.[223] The presence of dysregulated MAPK/ERK activation associated with proliferation in both in vitro and in vivo models of ADPKD have identified this pathway as a potential target for therapy. To date, preclinical evaluations of inhibitors of this pathway have given mixed results. The MAPK/ERK blockade in a mouse model of NPHP reduced cyst formation,[350] but it had no effect in an early onset model of ADPKD.[223] It remains possible that an evaluation of additional agents targeting the ERK pathway in refined orthologous gene mouse models of ADPKD will identify agents suitable for human studies.

Mammalian Target of Rapamycin

The mTOR pathway integrates signaling input and nutrient availability to regulate a diverse set of processes including cell size, proliferation, metabolism, and survival. It has been implicated as an effector pathway in PKD (Fig. 16.6),[351,352] and mTOR inhibitors were evaluated in the first major randomized clinical trials for ADPKD (see the following). The earliest indication of a potential role for this pathway in PKD came from the observation that severe juvenile ADPKD was often the result of a contiguous gene deletion syndrome involving PKD1 and TSC2.[258,259] Mutations in TSC2 and TSC1 result in tuberous sclerosis complex. Together, their respective gene products have GTPase activity that inhibits Rheb, which is a master activator of the mTORC1 pathway. Experimental evidence for mTOR activation in PKD came from immunohistochemical studies showing phosphorylation of the mTORC1 target P70 ribosomal S6 kinase (S6K) in cells lining some, but not all, cysts; S6K phosphorylation was also observed in normal tubular epithelial cells in polycystic mouse models.[225,353,354]

Although there is evidence of activation of the mTOR pathway in PKD, as with the other putative effector pathways, the mechanisms of PC-1 or PC-2 to this mTOR activity remains incompletely understood. There has been a suggestion that the C-terminal tail of PC-1 interacts directly with TSC2 but the specificity of this putative interaction is uncertain given that no studies using the full-length PC-1 have been reported.[225] A functional interaction between PC-1 and mTORC1 has been suggested in cell-based studies where there was overexpression of PC-1–inhibited mTORC1 signaling, whereas cells lacking Pkd1 showed increased mTORC1 activation.[355] PC-1 inhibition of the mTORC1 cascade was dependent on its inhibition of ERK1/2 phosphorylation acting through TSC2. The mechanism of PC-1/mTOR interdependence remains unsettled because another study using just the COOH–terminus of PC-1 suggested that sequestration of TSC2 is the mechanism of mTOR repression,[356] whereas a third study found no evidence for constitutive activation of mTORC1 in cells lacking PC-1.[357] Throughout these studies, mTOR has been separately connected with cilia[315,357] and with polycystins,[225,355] but not with polycystins in cilia. In fact, a recent study of cilia-dependent Lkb1 regulation of mTOR actually excluded regulation by PC-2 signaling.[315] Given this uncertainty in the preclinical data and two negative clinical studies (see the following), consideration of the use of mTOR inhibitors in ADPKD requires further investigation.[358]

Cyclic Adenosine Monophosphate (cAMP)

Elevated levels of intracellular cAMP in polycystic kidneys are believed to promote cyst growth and disease progression (Fig. 16.6).[359,360] cAMP levels are regulated by the balance of adenylate cyclase (AC) synthetic activity and phosphodiesterase (PDE) degradation. AC activation occurs following ligand interaction with heterotrimeric GPCRs. A subset of ACs and PDEs are calcium responsive, resulting in the refinement of cAMP activity by local subcellular calcium levels. Increases in cAMP levels above a threshold results in the activation of PKA, which acts as an effector for cAMP signaling. Finally, the subcellular microdomain localization of cAMP signals is achieved by the scaffolding activity of A kinase anchoring proteins (AKAPs), which confine PKAs to regions in close proximity to AC and its GPCR receptor, as well as PDE.

Elevated cAMP levels have been found in animal models of ADPKD.[224,361] The mechanisms underlying the elevated cAMP levels are uncertain. Increased levels of circulating vasopressin as well as of the vasopressin type 2 GPCR (V2R) in the collecting duct in polycystic kidneys have been hypothesized to enhance cAMP production.[362] It is notable in this regard that the largest number and size cysts in ADPKD appear to be derived from collecting duct segments that express V2R.[224,363] In addition, the hypothesized reduction in cellular calcium resulting from the loss of the activity of the PC-1/PC-2 channel complex may result in increased activity of calcium inhibitable ACs and decreased activity of calcium-activated PDEs. Recent work may have shed additional light on the mechanistic relationship of polycystins and cilia to cAMP activity. Cilia contain an interacting protein complex comprised

of adenylate cyclase 5 (AC5), A kinase anchor protein 150 (AKAP150), phosphodiesterase-4C, and PC-2.[364,365] PC-2 is required for the ciliary location of AC5/6, which are calcium inhibitable, and the loss of either Pkd2 or of cilia through Kif3a mutation results in elevated cAMP perhaps due to the loss of AC5/6 inhibition.[364] Although the GPCR associated with this complex is unknown, V2R has been found in the cilia.[366] cAMP has also been implicated in determining cilia length and functioning in a negative feedback loop whereby longer cilia produce reduced responses to flow, which in turn decreases intracellular cAMP. This flow-mediated adaptive response is lost in the absence of polycystins.[367]

Several mechanisms have been proposed by which increased cellular cAMP fosters progression in ADPKD. In vitro, cAMP inhibits the proliferation of normal kidney epithelial cells but paradoxically increases the proliferation of ADPKD cyst cells. The latter occurs by a Ca^{2+}-dependent activation of the B-Raf/MAPK/ERK pathway.[347,368] cAMP-dependent activation of PKA and the consequent activation of the cystic fibrosis transmembrane regulator (CFTR) chloride channel is thought to be responsible for the chloride-dependent transepithelial fluid secretion that drives cyst enlargement.[369–371] In support of this, Pkd1 mutant kidney explants treated with cAMP analogs develop cysts, and this process can be blocked by inhibitors of CFTR.[372] CFTR inhibitors have also shown some efficacy in a mouse model based on Pkd1.[373] V2R antagonists that were effective in preclinical trials[224,361] and that are currently in human clinical trials are thought to act by reducing intracellular cAMP levels. The reduction of both cAMP production and cAMP-dependent fluid secretion remain attractive targets for therapeutic trials in ADPKD.

Calcium Signaling

The importance of calcium signaling in PKD was initially suggested by the identification of PC-2 as a nonselective calcium-permeable cation channel of the TRP family and by evidence that point mutations that abrogate PC-2 channel activity are pathogenic in human ADPKD.[135,179,180,374] This association was bolstered by the finding that the mechanical deflection of primary cilia result in cellular calcium transients[310,375] that are dependent on normal expression of PC-1 and PC-2 (Fig. 16.6).[311] The molecular mechanisms underlying these whole cell calcium changes have been challenging to define in large part because the local calcium effects in cilia have not been directly measured. In addition, the whole cell calcium results are confounded by the function of PC-2 as an ER calcium channel[188,311] and perhaps as a channel on the cell surface.[376,377] It has been difficult to define the precise disease-associated signaling processes because it is not known whether the polycystin-dependent calcium effects relevant to ADPKD are very localized (e.g., in cilia), or generalized in the whole cell.

A direct demonstration of the role of calcium in most of the putative ADPKD-effector signaling pathways has also been elusive. Some effector pathways have shown a response to fluid flow, and fluid flow has been associated with cellular calcium transients; therefore, a role for calcium has been inferred but not shown. For example, fluid flow in ciliated cells was implicated in modulating inversin levels, which in turn determine the balance between canonical and noncanonical Wnt signaling.[323] The inference that flow induced calcium transients in mediating inversin action remains speculative. The role of calcium in the regulation of cAMP levels through calcium-dependent AC and PDE as discussed in the previous section has remained inferential. The activation of MAPK/ERK attributed to changes in cAMP levels are linked to cellular calcium homeostasis by in vitro studies.[368] The limitations of these indirect associations are illustrated by the case of cilia and flow-dependent mTORC1 signaling in which PC-2–dependent calcium transients were specifically excluded, whereas a novel role for ciliary Lkb1/AMPK was identified.[315]

Aside from calcium changes following mechanosensitive activation of PC-1/PC-2 receptor channel complex, PC-2 functions as a calcium-activated intracellular calcium release channel.[179] The calcium sensitivity of PC-2 activity is modulated by phosphorylation at serine 812. PC-2 and PC-1 interact with and modulate the activity of the major epithelial ER calcium release channel, the inositol 1,4,5-triphosphate receptor (IP_3R).[378–380] PC-2 also regulates calcium signaling by the other major ER calcium release channel, the ryanodine receptor.[381] The ER t-SNARE protein syntaxin-5 interacts with the COOH-terminus of PC-2 and inactivates the channel to prevent calcium leak from ER stores.[188] PC-2 has also been proposed as a GPCR or receptor tyrosine kinase (RTK)–operated cell surface channel. An epidermal growth factor (EGF) treatment of epithelial cells stimulates PC-2–dependent increases in cellular calcium.[376] This response is augmented by an overexpression of PC-2 and is attenuated by the siRNA knockdown of PC-2. It has also been suggested that PC-2 is activated downstream of GPCR and PLC signaling.[377] This activity requires heterotrimeric channel formation between PC-2 and TRPC1 and is independent of PC-1.

There is compelling evidence based on the structure, function, and mutations in PC-2 that calcium ions play a central role in the pathogenesis of ADPKD. There is a general consensus that the channel activity of the polycystin complex in cilia is related to the pathogenesis of ADPKD. The relevance of polycystin channel activity in the ER and plasma membranes to the disease pathogenesis remains less certain. The difficulty in discovering the detailed mechanistic role of calcium signaling in ADPKD may rest with the fact that the bulk of PC-2 channel activity occurs at sites other than the cilia, yet it is the minute portion of the signal occurring in cilia that is central to disease progression.

Regulation of the Cell Cycle

The profound increase in the cystic cell mass in ADPKD suggests that proliferation is a central feature of the disease phenotype (Fig. 16.6). Whether this proliferative response is primarily connected to polycystin signaling or is secondary

to the multitude of changes occurring as the tissue remodels in response to the loss of polycystins is an area of active investigation. Interfering with the expansion of cystic cell mass has the potential to slow the progression of PKD. The first study to directly address the role of polycystins in cellular proliferation showed that the overexpression of full-length PC-1 in MDCK cells resulted in cell cycle arrest at the G0/G1 phase.[242] PC-1 was proposed to interact with JAK2, which increased STAT1 expression, which in turn upregulated $p21^{wafl}$ to inhibit cell cycle progression. This process is dependent on the ability of PC-1 to interact with PC-2. Although these studies were cell based, in vivo support was provided by the demonstration of the altered expression of STAT1 and $p21^{wafl}$ in $Pkd1^{-/-}$ embryos.[242] PC-2 has been proposed to interact with and sequester Id2 in the cytosol, thereby preventing translocation of the latter to the nucleus where it can stimulate proliferation.[382] The extension of this mechanism to PC-1 was suggested by the increased nuclear localization of Id2 in $Pkd1^{-/-}$ mice and by the ability of RNAi knockdown of Id2 to decrease proliferation in $Pkd1^{-/-}$ cells in culture. $Pkd2^{-/-}$ cells in culture also exhibit increased proliferation and increased propensity toward branching morphogenesis in a three-dimensional culture.[348] Additional proliferative effects from the loss of polycystins may be mediated through the activation of either the MAPK/ERK or the mTOR pathways, as discussed previously.

The proliferative response in kidney tubule cells following the loss of polycystins is likely to be highly context dependent. As discussed, the early inactivation of polycystins during postnatal development in vivo results in rapid cystic expansion, whereas adult inactivation of polycystins results in the more indolent progression of cystic disease.[220] The proliferation of cyst cells in vivo likely results from a combination of cell autonomous effects due to reduced or absent polycystin function coupled with noncell autonomous effects that can foster an enhanced proliferative milieu. The latter can result from ongoing development,[220] acute injury and regeneration,[234,236–238] or stimulation by mediators of inflammation entering the kidney.[248] The most specific agents for therapy targeting proliferation in ADPKD would be directed at the earliest cell autonomous changes resulting from the loss of polycystins. On the other hand, the earliest available therapies may in fact be targeted toward the noncell autonomous secondary processes that foster the enhanced growth of cyst lining cells and the progression of PKD.

KIDNEY DISEASE PROGRESSION AND TOTAL KIDNEY VOLUME IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

ADPKD is characterized by the development and expansion of renal cysts, resulting in increased kidney size and TKV. Combined kidney weights can exceed 10 kg once ESRD is reached. The initiation and growth of cysts over time cause increased kidney size, compression, and remodeling of normal renal architecture and vasculature resulting in inflammation, interstitial fibrosis, and renal failure.[43] Although cyst expansion results in total organ enlargement, the initiation of cyst formation and growth is restrictive and focal in nature. Microdissection studies of early stage ADPKD kidneys showed focal cyst formation involving less than 5% of nephrons and a minor subset of cells within individual tubules.[44] The increase in TKV in ADPKD can be seen in utero, is a consistent finding during childhood, and is predominant throughout adult life. ADPKD is the only hereditary cystic kidney disorder associated with an inexorable increase in kidney size. Although ARPKD is associated with increases in TKV, this is typically greatest during the early postnatal period and either plateaus or diminishes over time as renal function declines.

A striking feature of ADPKD is the prolonged oligosymptomatic period typically spanning 4 decades, during which cyst mass is expanding with an increase in TKV while kidney function remains relatively intact when measured by the glomerular filtration rate (Fig. 16.8). During this phase, all of the common signs and symptoms related to progression in ADPKD, including hypertension, chronic pain or heaviness in the flank or abdomen, hematuria and cyst hemorrhage, urinary tract infections, and nephrolithiasis occur and are directly associated with increased TKV.[62] Despite the preservation of kidney function, the size of kidneys increases from a normal size (150 to 200 mL each) in childhood to

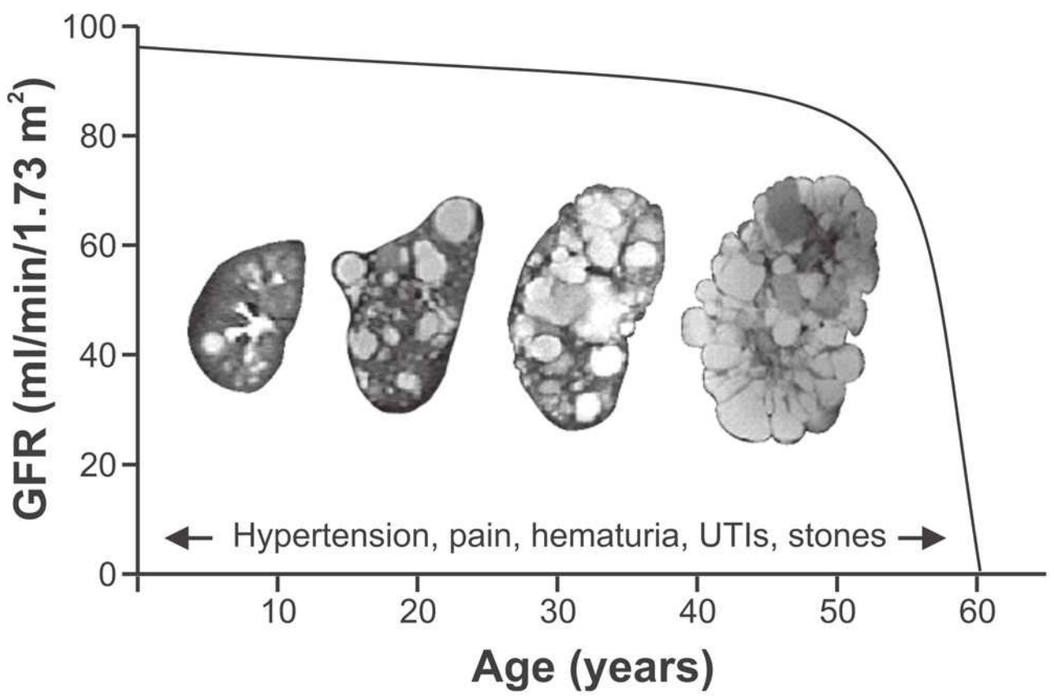


FIGURE 16.8 A proposed relationship between renal cyst burden, age, and renal function in autosomal dominant polycystic kidney disease (ADPKD). Coronal magnetic resonance imaging (MRI) of four ADPKD individuals age 9, 20, 21, and 40 with normal renal function are overlaid on a plot of glomerular filtration rate (GFR) as a function of age in ADPKD patients. Although hypertension, pain, hematuria, urinary tract infections (UTIs), and nephrolithiasis occur throughout the course of ADPKD, renal function remains largely intact until total kidney volume reaches a size where renal reserve no longer can compensate. Thereafter, renal function inexorably declines to end-stage renal disease. (Adapted with permission from Torres V, Scheinman S. Polycystic kidney diseases. *NephSAP*, Jan 2004;3(1):22.)

greater than 1500 mL by the third to fourth decade.[383] The relentless expansion of TKV due to the growth of cysts is the hallmark of ADPKD and leads inexorably to deformation of normal renal and vasculature architecture, inflammation, interstitial fibrosis, tubular atrophy, and finally, progressive kidney dysfunction.

Measurement methods using ultrasound, CT, and MRI have been developed to quantify TKV in ADPKD. Ultrasound imaging uses the formula for a modified ellipse to determine TKV [4/3π × ½(anterior–posterior diameter + width) × ½(length)].[31,384] Obtaining accurate longitudinal, axial, and depth measurements requires significant training and appropriate alignment through the cystic kidney. Ultrasound measurements are accurate in individuals with relatively small kidneys, which can be measured in a single imaging window. Significant variability occurs in individuals with extremely large kidneys, or in those with concomitant significant polycystic liver disease. Additional variability is introduced with different operators, motion artifact, and respiratory variation. Ultrasound determinations of TKV tend to underestimate TKV measurements obtained by MRI by approximately 25% in healthy controls.[384] Despite these limitations, ultrasound ellipsoid-based and MR-based stereology estimates of TKV are highly correlated (r = 0.89). The reproducibility or the coefficient of variation differs greatly with ultrasound (21% to 35%) compared to MRI (2.1% to 2.5%).[384] Importantly, kidney length is the most reproducible measurement using ultrasound. These observations indicate that ultrasound is not an appropriate imaging tool for short-term longitudinal monitoring in ADPKD, but may have a screening role for risk stratification in young individuals or could be used in individuals followed over long periods (more than 5- to 7-year intervals).

Recently, the National Institutes of Health (NIH)–sponsored CRISP has evaluated the relationship between GFR progression and TKV expansion in ADPKD. MRI acquisition methods that can accurately, reproducibly, and reliably determine TKV in ADPKD individuals have now been developed. Using both T2- and T1-weighted MRIs, interobserver and intraobserver variability, as well as day-to-day variability of TKV measurements, are all less than 2.5%.[385] Both gadolinium-enhanced and non–gadolinium-based MR measures of TKV are accurate, with a slightly greater TKV (1% to 6%) measurement seen in postgadolinium studies.[386] Given the previous reports of systemic nephrogenic fibrosis related to gadolinium exposure, CRISP no longer uses gadolinium contrast agents during image acquisition. Total cyst volume measurements in CRISP are more variable than TKV measurements, but they remain highly correlated with TKV (r = 0.99), indicating that renal cystic expansion accounts for the overwhelming majority of the increase in TKV seen in ADPKD. CRISP has now had the opportunity to prospectively follow 241 nonazotemic patients for 8 years with MRI examinations to measure the progression of kidney and cyst volumes.[385,387] The mean rate of increase in TKV over 3 and 8 years was 5.3% and 5.2% per year, respectively, which is consistent with an independent cohort from the SUISSE pre-randomization studies (5.8% per year). On average, CRISP participants increased their TKV 55% from baseline at the end of 8 years of follow-up. This rate of change is roughly equivalent to a 75 to 90 mL per year increase in TKV, which is equivalent to half of a normal adult kidney size and which is easily detectable by current imaging methodologies.

In CRISP, the overwhelming majority of ADPKD individuals demonstrated detectable increases in TKV over relatively short (6- to 12-month) periods (Fig. 16.9).[387] Men had higher rates of kidney and cyst growth than women. These differences diminish when TKV is indexed for measures of body size, including height (htTKV). In the CRISP study, baseline TKV correlated with a subsequent rate of increase in TKV, and an initial TKV above 1500 mL (approximately 5 times greater than normal) was the primary predictor of declining glomerular filtration rate in the first 3 years of study. Importantly, receiver operator characteristic curves

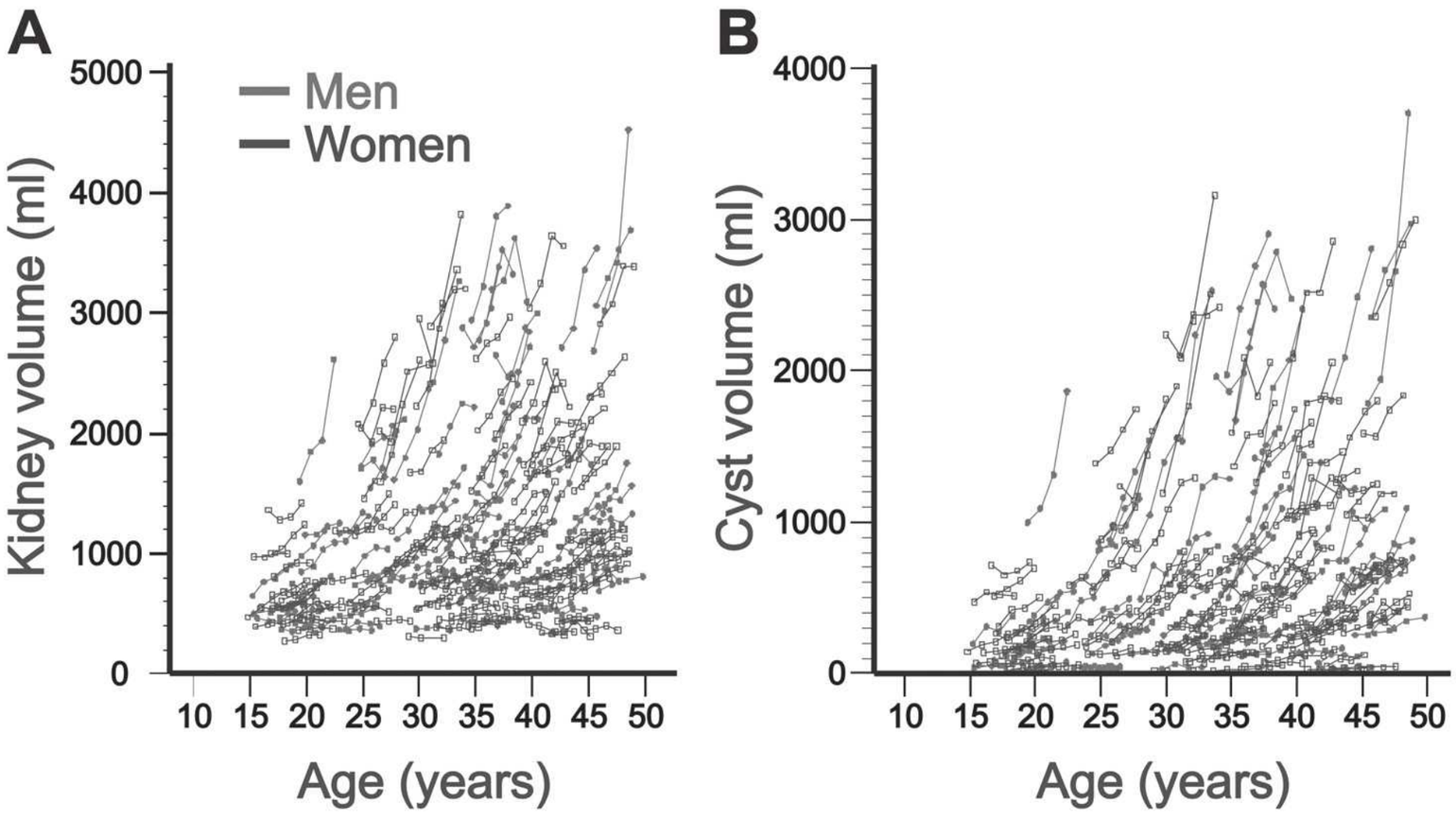


FIGURE 16.9 Total kidney volume **(A)** and total cyst volume **(B)** in relation to age in women (*blue*) and men (*red*) imaged annually over 3 years during participation in the Consortium for Radiologic Imaging studies in Polycystic Kidney Disease (CRISP). (From Grantham JJ, Torres VE, Chapman AB, et al. Volume progression in polycystic kidney disease. *N Engl J Med.* 2006;354:2122–2130. Copyright (c) 2006 Massachusetts Medical Society.) (See Color Plate.)

(ROC) demonstrated that htTKV reliably and accurately predicts the development of chronic kidney disease (CKD) stage 3 within 8 years of measurement with a cut point of 600 mL per meter (equivalent to a TKV of approximately 1200 mL). The htTKV-based prediction of CKD stage 3 was independent of variables known to associate with renal insufficiency in ADPKD, including genotype, gender, race, and age. A multivariate analysis further indicated that for each 100 mL increment of htTKV at baseline, the odds of reaching a CKD stage 3 end point within 7.9 years increases 1.48-fold.

PKD2 participants in CRISP demonstrated significantly smaller TKVs than PKD1 participants, but nonetheless showed similar rates of increase in TKV.[227] Mathematical modeling of TKV data from CRISP participants to estimate TKV at age 18 were accurate, indicating a relatively constant rate of increase in TKVs accounting for the observed exponential rate of kidney size growth.[388] Computational modeling integrating cyst surface area, volume, and an assumed overall constant rate of cyst growth (assuming variability across cysts within an individual) as shown by TKV change in the CRISP study suggested that cysts that developed early in life were the main contributors to TKV.[388] These inferred data were interpreted as showing that there are periods of accelerated cyst growth and/or that the initiation of cyst burden is mostly established by the time of birth.

The relationship between clinical symptoms of ADPKD and GFR is highly variable, which is likely due to the striking dissociation between the expansion of TKV and GFR observed early in the course of the disease. A significant time lag between the increase in TKV and the decline in GFR and an increasingly negative correlation between baseline TKV and GFR measured in subsequent years was demonstrated in CRISP.[389] The stability of GFR results from hyperfiltration of the surviving nephrons. The finding of stable GFR when ADPKD kidneys are dramatically enlarged, distorted by multiple cysts, and fibrotic provides false reassurance as to the stability of the disease progression. Once GFR begins to decline the progression is inexorable, with an average rate of decrease of approximately 4.4 to 5.9 mL per minute per year, a faster rate than in other types of progressive renal disease.[390] Currently, changes in GFR (frequently estimated from changes in serum creatinine) are considered the gold standard for quantifying the progression rate in most chronic renal diseases. However, given that these changes are seen only once the kidney architecture has been grossly and irreversibly distorted, GFR may not be suitable as a primary end point for clinical studies testing early interventions in ADPKD.[383] Risk factors associated with a worse prognosis in addition to TKV include male gender, a first episode of hematuria before the age of 30, PKD1 genotype, the onset of hypertension before the age of 35, dipstick-positive proteinuria, elevated low density lipoprotein (LDL), decreased high density lipoprotein (HDL), increased dietary sodium intake, and decreased renal blood flow.[391,392] All of these variables, with the exception of sodium intake and dyslipidemia, are specifically related to TKV. The CRISP study has shown that kidney and cyst volumes are the strongest predictors of renal functional decline,[389] and it is anticipated that this measurement will find its way into clinical practice.

RANDOMIZED CLINICAL TRIALS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

The availability of an extensive array of orthologous and nonorthologous animal models for PKD has permitted the preclinical evaluation of a broad spectrum of potential therapeutic agents. Many of these agents have shown promising results in one or more rodent models, but only a handful have been deemed sufficiently promising to enter human clinical trials.

Vasopressin V2 Receptor Antagonists

Arginine vasopressin (AVP) increases cAMP levels in the collecting duct, and evidence for the role of cAMP in cystogenesis has provided the rationale for preclinical trials of V2R antagonists in a variety of hereditary renal cystic diseases. Inhibition of the AVP V2 receptor and the subsequent decrease in adenylyl cyclase activity may help to compensate for alterations in intracellular calcium homeostasis and may affect the normalization of intracellular cAMP levels, thereby reducing the proliferative phenotype of cystic epithelium.[393] The V2R antagonists OPC-31260 and OPC-41061 (Tolvaptan) reduce renal levels of cAMP and markedly ameliorate cyst development in models of ARPKD (pck rat), NPHP (pcy mouse), and ADPKD ($Pkd2^{WS25/-}$).[224,394] High water intake by itself, possibly by suppressing the release of vasopressin, has demonstrated a protective effect on the development of PKD in pck rats.[395] The genetic deletion of the vasopressin gene in pck rats by intercrossing them with Brattleboro rats leads to lower renal cAMP levels and an almost complete inhibition of cystogenesis.[396] The cystic phenotype can be recovered following the administration of the exogenous V2 receptor agonist, desmopressin (dDAVP). Activation of the predominant endothelin receptor subtype in the collecting tubules, the endothelin-1 endothelin B (ETB) receptor, inhibits AVP action. An antagonist of this receptor increases renal cAMP levels and aggravates renal cystic disease in a mouse model of ADPKD, presumably by interfering with the inhibition of AVP activity.[397] In aggregate, the data support the hypothesis that the pharmacologic inhibition of the V2R pathway is a logical strategy to inhibit the development and expansion of renal cysts in ADPKD.

Based on these preclinical studies, the Tolvaptan Efficacy and Safety in Management of PKD and Outcomes (TEMPO) program was initiated. Tolvaptan is an orally effective, relatively short-acting, nonpeptide arginine vasopressin V2 receptor antagonist. It is currently approved in the United States and the European Union for the treatment of hyponatremia associated with hypervolemic states (e.g., cirrhosis, congestive heart failure) and euvolemic syndrome of inappropriate

antidiuretic hormone (SIADH) states, and in Japan for the treatment of cardiac edema that is resistant to diuretics. Tolvaptan is also being studied in the United States and in Europe as an adjunct therapy for volume overload in patients with heart failure. A phase IIa dose ranging study to determine the response to increasing doses of tolvaptan in patients with normal renal function has been completed.[398,399] An international phase III clinical trial for the treatment of ADPKD is ongoing at the time of this writing (Table 16.3). A primary therapy study to delay the progression of ADPKD (NCT00428948) has enrolled over 1400 relatively young (< 50 years) ADPKD patients with preserved kidney function (creatinine clearance > 60 mL per minute) but with TKVs greater than 750 mL as measured by MRI. Based on this enrollment, regardless of the final study outcome, this trial will leave open the question of the potential role for V2R antagonists in patients with more advanced kidney disease and the potential efficacy of these drugs at lower doses, which are associated with reduced side effects of polyuria and nocturia and with improved tolerance.

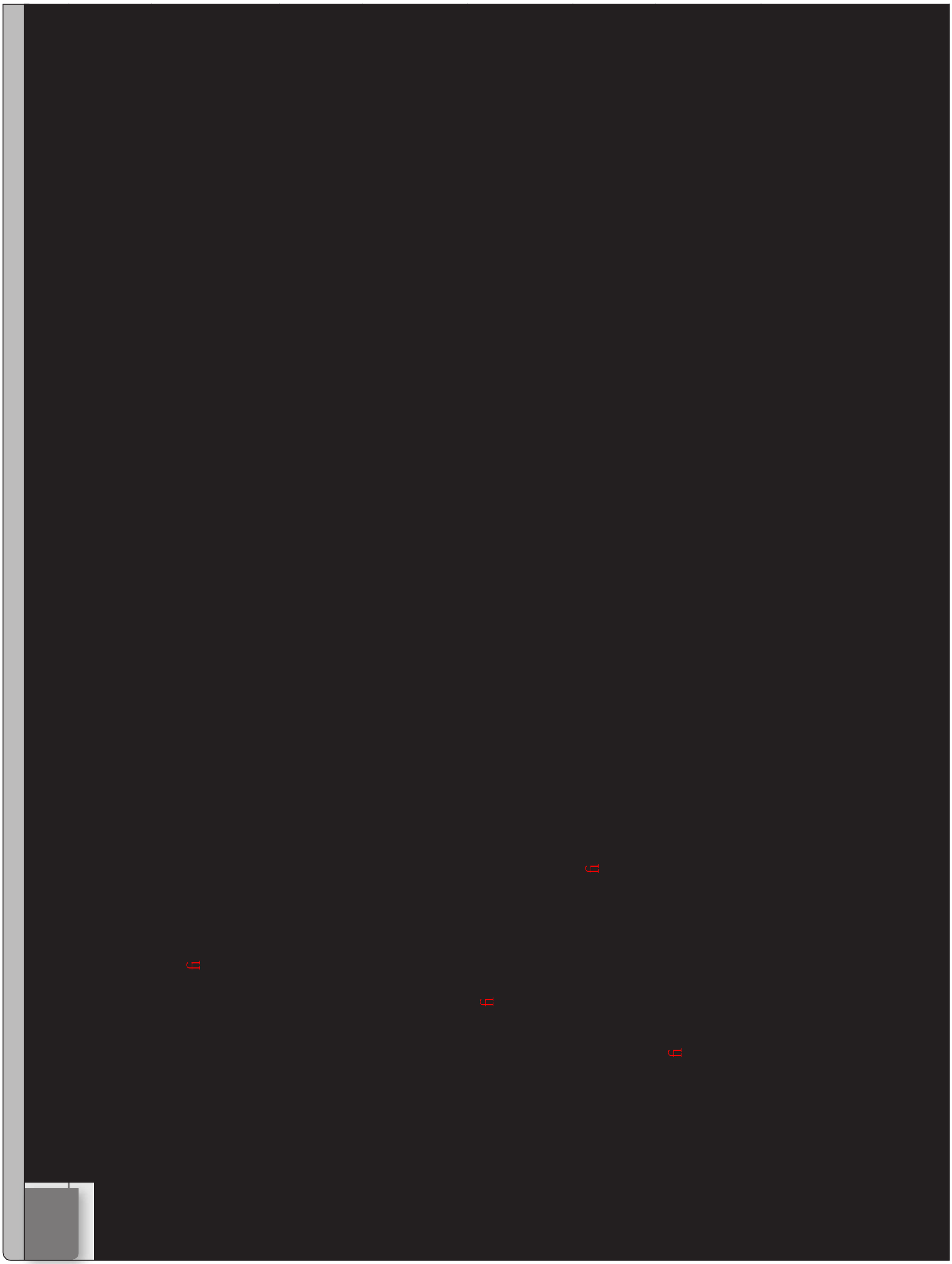
Somatostatin Analogs

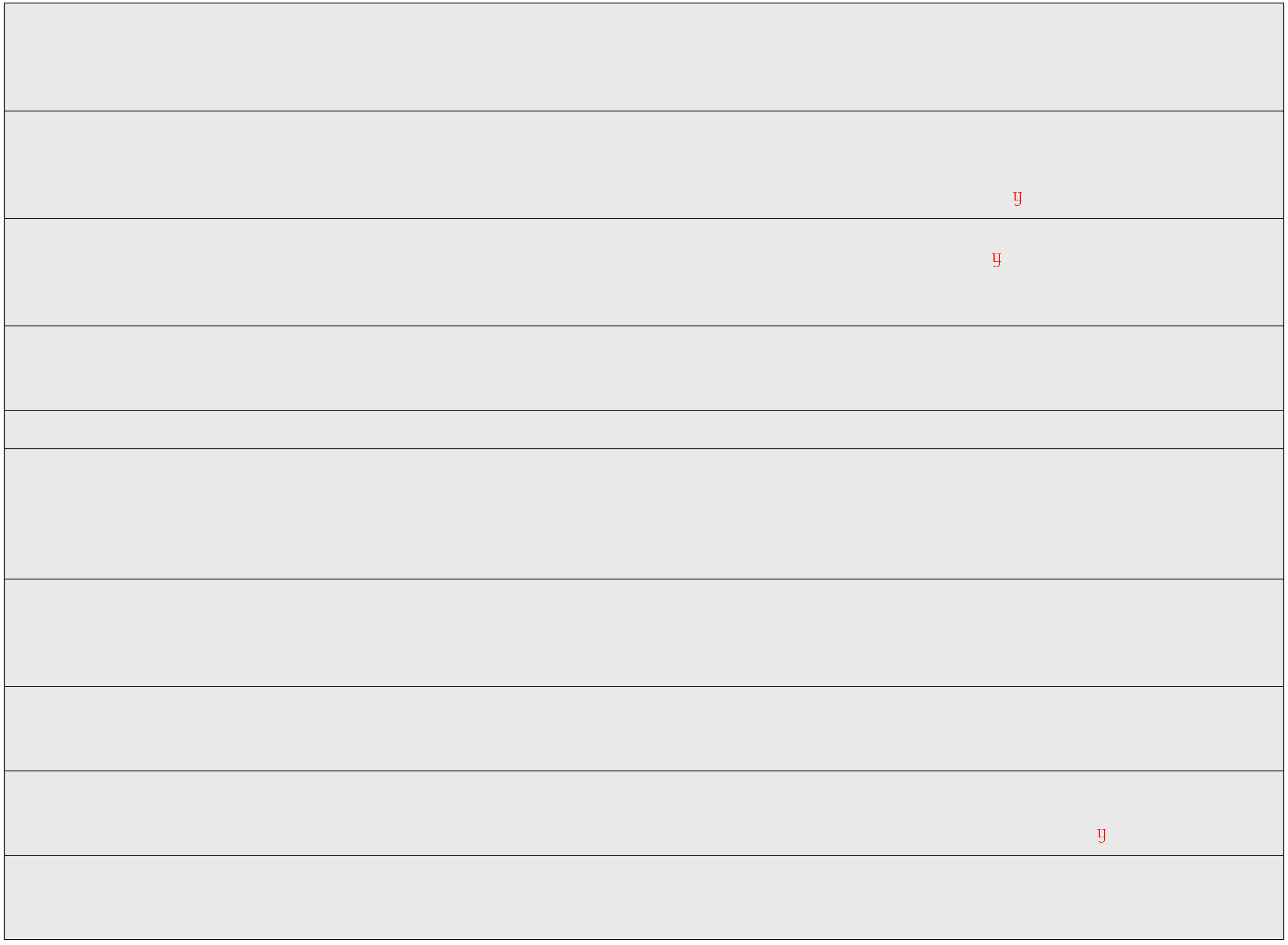
A similar pharmacologic inhibition of cAMP accumulation has been established with the administration of somatostatin, which acts on somatostatin receptor 2 (SST2) receptors in the kidney and liver.[400] Octreotide, a metabolically stable somatostatin analog, halted the expansion of hepatic and renal cysts in ARPKD model pck rats. These observations are consistent with the reduction in total kidney volume in a pilot study of long-acting octreotide for human ADPKD. Presently, there are a number of ongoing clinical trials of octreotide and lanreotide for PKD and liver disease (NCT00309283, NCT00426153, and NCT00565097). Pilot studies evaluating the feasibility of increasing the fluid intake in the form of water have also begun in ADPKD patients.[401] These are based on the assumption that decreasing the urinary osmolality to less than 300 mOsm per kilogram in the steady state will result in the deactivation or inhibition of the V2R. The tolerability of increasing fluid intake is reasonable; however, long-term compliance and 24-hour monitoring of urinary osmolality is a potential concern. Measures of disease progression under these conditions have not yet taken place.

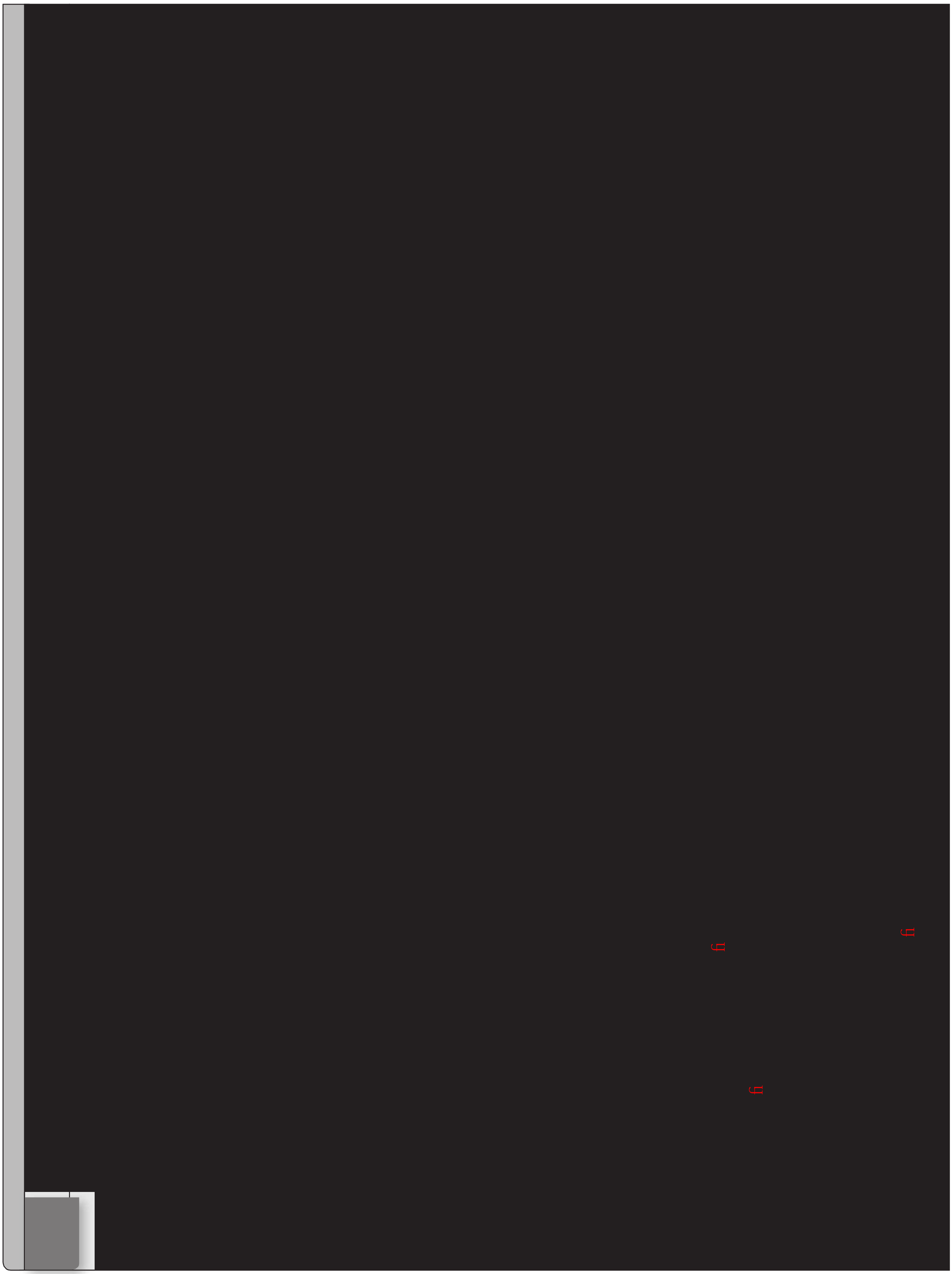
Inhibition of the Mammalian Target of Rapamycin

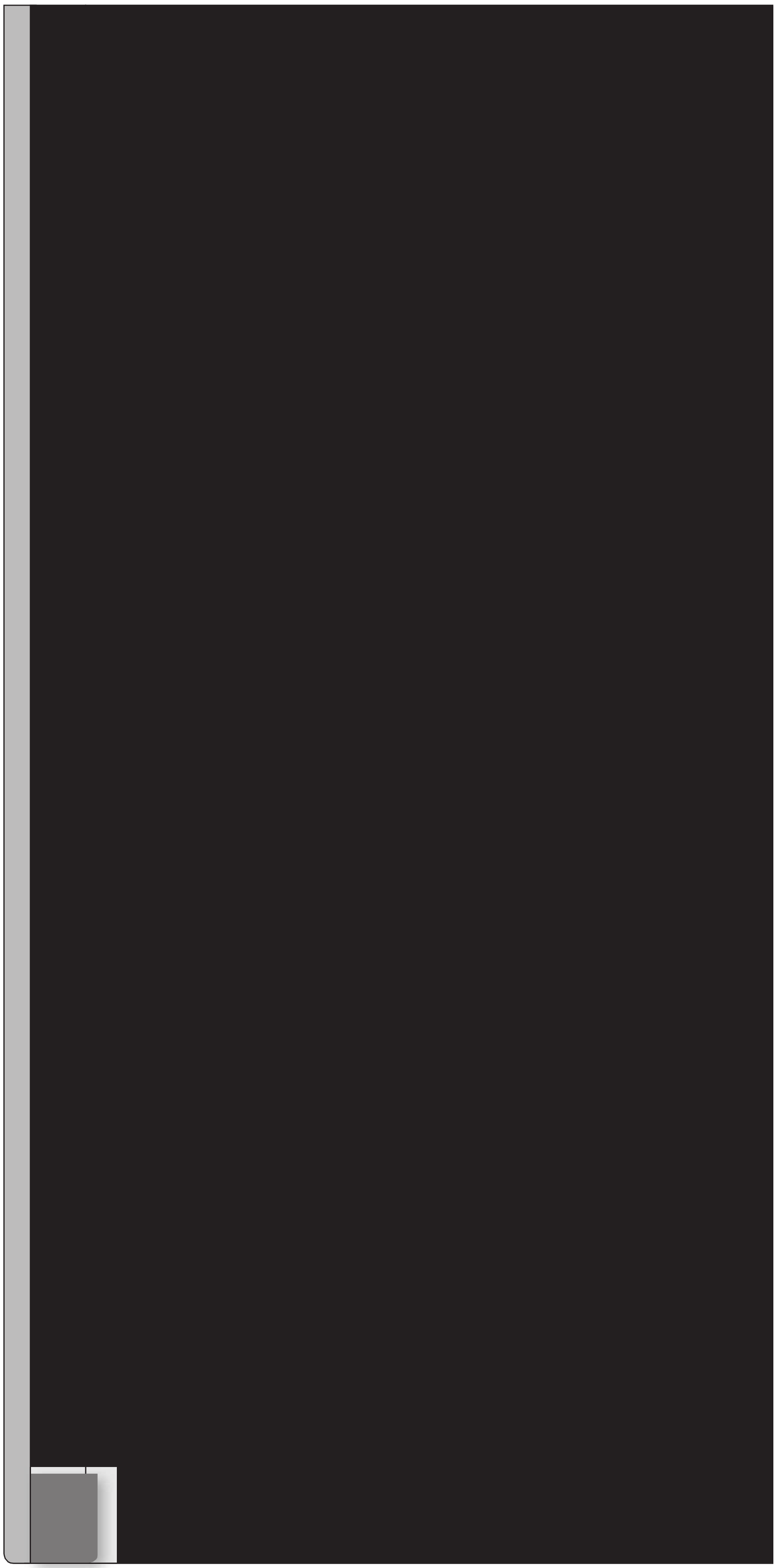
mTOR, a serine-threonine kinase that is involved in the coordination of cell growth and proliferation, is inappropriately activated in cysts lining the epithelial cells of kidneys from mice and humans with ADPKD (see previous).[225,402,403] Studies in animal models of cystic kidney disease have shown that mTOR inhibition suppresses cyst growth. Sirolimus or everolimus significantly reduced kidney volume growth in the nonorthologous Han:SPRD rat model by reducing cyst expansion with a sustained effect with prolonged treatment.[404,405] Sirolimus also had some effect in an animal model of NPHP and in an orthologous mouse model with the conditional inactivation of Pkd1.[354,406] These studies used higher doses of mTOR inhibitors than can be achieved in humans and only tested their preclinical potential in early onset, rapidly progressive orthologous gene mouse models of Pkd1.[354] A small scale, 6-month, randomized trial comparing eight patients on sirolimus with eight control patients showed reduced kidney volume growth in the sirolimus-treated group.[407] Collectively, these preclinical and clinical observations offered a rationale for clinical trials of mTOR inhibitors in ADPKD.

Two major prospective randomized clinical trials using sirolimus or everolimus have since been completed (Table 16.3). The studies differed with respect to patient number, subject characteristics, and dose range. The two studies reported negative results. Walz et al.[408] randomly assigned 433 patients aged 18 to 65 years with ADPKD and an estimated GFR > 30 mL per min per 1.73 square meter (chronic kidney disease stage II/III) to receive either placebo or the mTOR inhibitor everolimus (2.5 mg twice daily) for 2 years. Everolimus treatment was associated with a marginal slowing of the increase in TKV, reaching statistical significance at 1 year and marginal significance at 2 years. The finding that increases in TKV were persistently lower in the everolimus group suggests that everolimus may be able to limit the growth of cysts in patients with ADPKD. The perceived lack of a positive effect on glomerular filtration rate and the progression of chronic kidney disease may be due to the reduced power of the trial resulting from the fact that approximately one-third of the patients dropped out, largely because of drug-related adverse effects. In a complementary trial, the SUISSE ADPKD study treated 100 patients aged 18 to 40 years with preserved renal function with sirolimus (2 mg per day) for 18 months.[409] At the end of the study period, the median increase in TKV was similar in the placebo group to the sirolimus group (97 mL versus 99 mL, respectively). The study used a relatively low target dose of sirolimus, and the dose delivered was approximately 25% lower than the intended dose because of adverse effects. The average sirolimus dose normalized by mean patient body weight was approximately 0.020 mg per kilogram. ROC curve analyses of dose finding studies identified 0.049 mg per kilogram of body weight as the cut-off threshold for sirolimus dosage that predicted a reduction or reversal of total cyst volume growth. The negative findings from these studies contrast the positive results from preclinical studies and the results from smaller clinical studies of shorter duration.[407] Taken together, mTOR inhibitors require further investigation to determine any potential to favorably impact changes in TKV in ADPKD individuals. Efficacy is limited by their toxicity, and the impact of mTOR inhibitors appears to be greatest during the most proliferative stages of disease type. It remains to be seen if this class of agent can still be used in patients during the more aggressive phases of cyst growth and expansion, potentially in concert with other disease modifying agents.









3-Hydroxy-3-Methyl-Glutaryl-CoA (HMG-CoA) Reductase Inhibitors

Lovastatin therapy in the heterozygous male (but not the female) Han:SPRD rats resulted in decreased cystic kidney size and improved renal function.[410] These effects may be due to the decreased formation of farnesyl pyrophosphate, an intermediate in the conversion of acetyl-coenzyme A (CoA) to cholesterol that is also required for the activation of Ras, which in turn may be an important factor in cell proliferation. Three clinical trials of statins lasting from 4 weeks to 2 years in small numbers of ADPKD patients have shown improved renal hemodynamics, including increased GFR and effective renal plasma flow, improved endothelial function, and longer term kidney function and urinary protein excretion.[411–413] Currently, a phase III randomized trial comparing pravastatin to placebo over 5 years is under way in 107 children and young adults aged 8 to 22 years and treated with angiotensin-converting enzyme inhibitors. The end points in this trial include TKV, renal function, and urinary protein excretion levels.[414]

Triptolide

Triptolide is a natural compound derived from the Chinese herb Thunder God Vine. It is a potent inhibitor of nuclear factor-kappa B (NF-κB) and nuclear factor of activated T cells (NFAT)-mediated transcription, which thereby decreases inflammatory and proliferative cellular responses. Triptolide also promotes increased PC-2–mediated calcium release and reduces cyst formation in Pkd1 mouse models.[415–417] Current clinical trials using triptolide are under way in approximately 300 patients at the University of Nanjing to evaluate its effects on kidney function and TKV.

Epidermal Growth Factor Receptors and Src Inhibitors

Epidermal growth factor receptor (EGFR) inactivation has been shown to slow cyst growth in nonorthologous recessive models of polycystic kidney disease,[418,419] but not in a rat model orthologous to human ARPKD.[224] EGFR tyrosine kinase inhibitors, one of which is an Src inhibitor, play an intermediary role in cAMP pathways.[420] Src activity has been shown to be associated with the progression of disease in bpk mice and pck rats.[421] A truncated EGFR-like protein, Bosutinib, which is a Src inhibitor, has been valuable in treating breast cancer.[422] Most of the EGF receptor tyrosine kinase inhibitors are relatively nonspecific and have significant side effects, which suggest that long-term use at normal antineoplastic doses will not be feasible in ADPKD individuals. However, they may be extremely useful at a reduced dosage level with other disease modifying agents in appropriate subsets of ADPKD individuals. A clinical trial of 400 ADPKD individuals is under way using the receptor kinase inhibitor Bosutinib SKI606 (NCT01233869). This trial is designed to inhibit Src and to increase MAPK activation. Both renal function and TKVs are being evaluated in this study.

HALT Polycystic Kidney Disease

Substantial evidence has implicated the RAAS in the pathogenesis of ADPKD and associated hypertension. However, evidence that treatments inhibiting the RAAS are superior to other treatment strategies or are at all beneficial are, to date, inconclusive. Limitations of previous studies due to small sample size, short periods of follow-up, and the study of patients at relatively late stages of the disease without a complete blockade of the RAAS have hampered the development of evidence-based guidelines for the treatment of hypertension in ADPKD. Because of the importance of hypertension in ADPKD and the uncertainties surrounding its treatment, the NIH/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) has funded two distinct, multicenter, double-blind, randomized clinical trials adequately powered to assess the effect of RAAS blockade on renal progression in early and late stages of the disease (NCT00283686). HALT PKD consists of two ongoing randomized trials with the largest cohort of systematically studied patients with ADPKD to date. Study A is designed to compare the combined treatment with an angiotensin-converting inhibitor and receptor blocker to the inhibitor alone at standard compared to low blood pressure targets. There are 558 ADPKD early stage patients with an eGFR over 60 mL per min per 1.73 square meters. Study B is comparing angiotensin-converting enzyme inhibitors and receptor blockers to the inhibitor alone at standard blood pressure level in 486 patients with more progressive renal disease and an eGFR between 25 and 60 mL per minute per 1.73 square meters. An initial evaluation of this study population[95,423] demonstrates a significant association between eGFR, urinary albumin excretion, body surface area, and TKV. These studies are due to be completed in 2014.

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CHAPTER

17 Alport Syndrome, Fabry Disease, and Nail-Patella Syndrome

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Hereditary glomerular diseases have diverse and often covert patterns of inheritance and varied clinical presentations. They are more common than is generally appreciated. Vigilance and awareness of these subtleties are important for prompt and accurate diagnoses, which allow for optimal management and timely genetic counseling.

ALPORT SYNDROME

Alport syndrome (AS), formerly called hereditary nephritis, is a disorder characterized by hematuria, proteinuria, progressive renal failure, variable sensorineural hearing loss, and ocular abnormalities. On a histologic examination, irregularities of the glomerular basement membrane (GBM) constitute the primary disease feature. Cecil Alport's archetypal kindred[1] had dominantly inherited kidney disease that was characterized in both sexes by hematuria and urinary erythrocyte casts, variable proteinuria, and by hearing loss and renal failure in males. Affected males died in adolescence of uremia, whereas females lived to old age.

The Present Definition of Alport Syndrome and the Molecular Defect Definition

For this chapter, AS will be defined as progressive, hereditary, hematuric, nonimmune glomerulonephritis that is characterized ultrastructurally by progressive irregular thickening, thinning, and lamellation of the GBM and genetically by a mutation in COL4A3, COL4A4, or COL4A5.

The Genetic Classification of Alport Syndrome

Between 2% and 5% of males with end-stage renal disease (ESRD) and probably less than 1% of females have AS. Various estimates place the gene frequency between 1:5,000 and 1:53,000, respectively.[2] The true prevalence may be higher because affected patients with subtle hearing loss are easily overlooked. There are three main genetic types of AS: X-linked Alport syndrome (XL-AS), autosomal recessive Alport syndrome (AR-AS), and autosomal dominant Alport syndrome (AD-AS), which are classified based on the mode of inheritance as described in Table 17.1. The severity of symptoms varies from person to person and with age and gender. Large kindreds show modes of inheritance and kindred-specific phenotypes that clearly reflect the genetic heterogeneity of AS. Thus, although the demonstration of a family history of glomerulonephritis among affected persons within a kindred may be helpful to ascertain, demonstration of a causative mutation remains the gold standard for genetic diagnosis.

X-Linked Alport Syndrome Caused by a Mutation in *COL4A5* (OMIM 301050)[3]

XL-AS is the most common form of AS, accounting for 80% of cases. XL-AS results from a mutation in the COL4A5 gene located at Xq22.3, which encodes the α_5 chain of type IV collagen (α_5[IV]). Hematuria from birth occurs in 100% of hemizygous males[4,5,6] and 90% to 100% of heterozygous female carriers of XL-AS.[5,7,8] Disease expression is phenotypically very heterogeneous. ESRD is inevitable in males but occurs at widely different ages in different families. The age of ESRD tends to run true within a family, but even within a family there can be quite wide variability. Knowing the mean age of ESRD in males in a family is useful from a prognostic standpoint. Moreover, extrarenal manifestations, such as hearing loss and ocular defects, tend to occur more commonly, more severely, and at an earlier age in kindreds whose males develop ESRD early.[4]

Because they are late symptoms, it is unwise to equate chronic renal failure and ESRD with Alport gene penetrance. We define penetrance of ESRD as the fraction of a population at risk in whom ESRD eventually develops. For males in each Alport kindred, ESRD penetrance generally coincides with hematuria penetrance. In Figures 17.1 and 17.2, representations of the probability of ESRD and hearing loss in boys and girls with XL-AS are shown. Inactivation of the X chromosome likely explains both incomplete hematuria penetrance among females heterozygous for XL-AS and their low probability of ESRD and hearing loss.[9] Jais et al.[7] found a cumulative prevalence of ESRD of 12% in female carriers of XL-AS by the age of 40 years.

17.1 Genetic Classification of Alport Syndrome

Description	OMIM	Gene	Comment
X-Linked Alport Syndrome	301050	COL4A5	Most common form of Alport syndrome accounting for 80% of cases. A heterogeneous condition with progressive renal insufficiency and timing of ESRD occurring between childhood and adult. Hearing loss and eye problems are common but severity is variable. More severe phenotype in affected males.
COL4A5 and Contiguous Gene Defects			
Alport Syndrome with Diffuse Leiomyomatosis	308940	COL4A5-COL4A6	Alport syndrome with diffuse leiomyomatosis.
Alport Syndrome, Mental Retardation, Midface Hypoplasia, Elliptocytosis (AMME)	300194/95	COL4A5 with FACL4/ AMMECRI	Contiguous gene deletion affecting genes located 3′ of the COL4A5 gene.
Autosomal Recessive Alport Syndrome (ARAS)	203780	COL4A3/COL4A4	This form of Alport syndrome accounts for 15% of cases. Most cases result in the early onset of ESRD. Hearing and eye problems are common, with both males and females being equally affected.
Autosomal Dominant Alport Syndrome (ADAS) without Hematologic Defects	104200	COL4A3 or COL4A4	A milder disease with the later onset of renal impairment, and a lower incidence of hearing loss and eye problems.
Familial Thin Basement Membrane Disease and Benign Familial Hematuria	141200	COL4A3 or COL4A4	A milder disease, with no hearing loss or occurrence of eye problems.

One consequence of X linkage is that twice as many females as males with a nephritis gene will be born if there are no prenatal effects and if reproductive fitness is independent of gender of the gene-carrying parent. In kindreds with XL-AS and early onset of ESRD, most affected children obtain the gene from their mothers, and the sex ratio of gene-carrying newborns approaches 1:1.

X-Linked Alport Syndrome Caused by a Deletion in *COL4A5* with Damage to Contiguous Genes (OMIM 308940 and 300194/5)

In this form, AS is associated with other features caused by an extension of the deletion outside COL4A5. Alport syndrome with diffuse leiomyomatosis (smooth muscle tumors) (Online Mendelian Inheritance in Man [OMIM] 308940)[3] stems from a deletion embracing the 5′ ends of COL4A5 and COL4A6.[2,10] The AMME syndrome (OMIM 300194/5)[3] consists of AS, midface hypoplasia, mental retardation, and elliptocytosis and has been described in several families with deletions of COL4A5 that extend beyond the 3′ end of the gene.[11–14]

Autosomal-Recessive Alport Syndrome Caused by Homozygous Mutations Mutations in *COL4A3* or *COL4A4* (AR-AS, OMIM 203780)

AR-AS is allelic with familial thin basement membrane nephropathy (TBMN). This form of AS accounts for 15% of

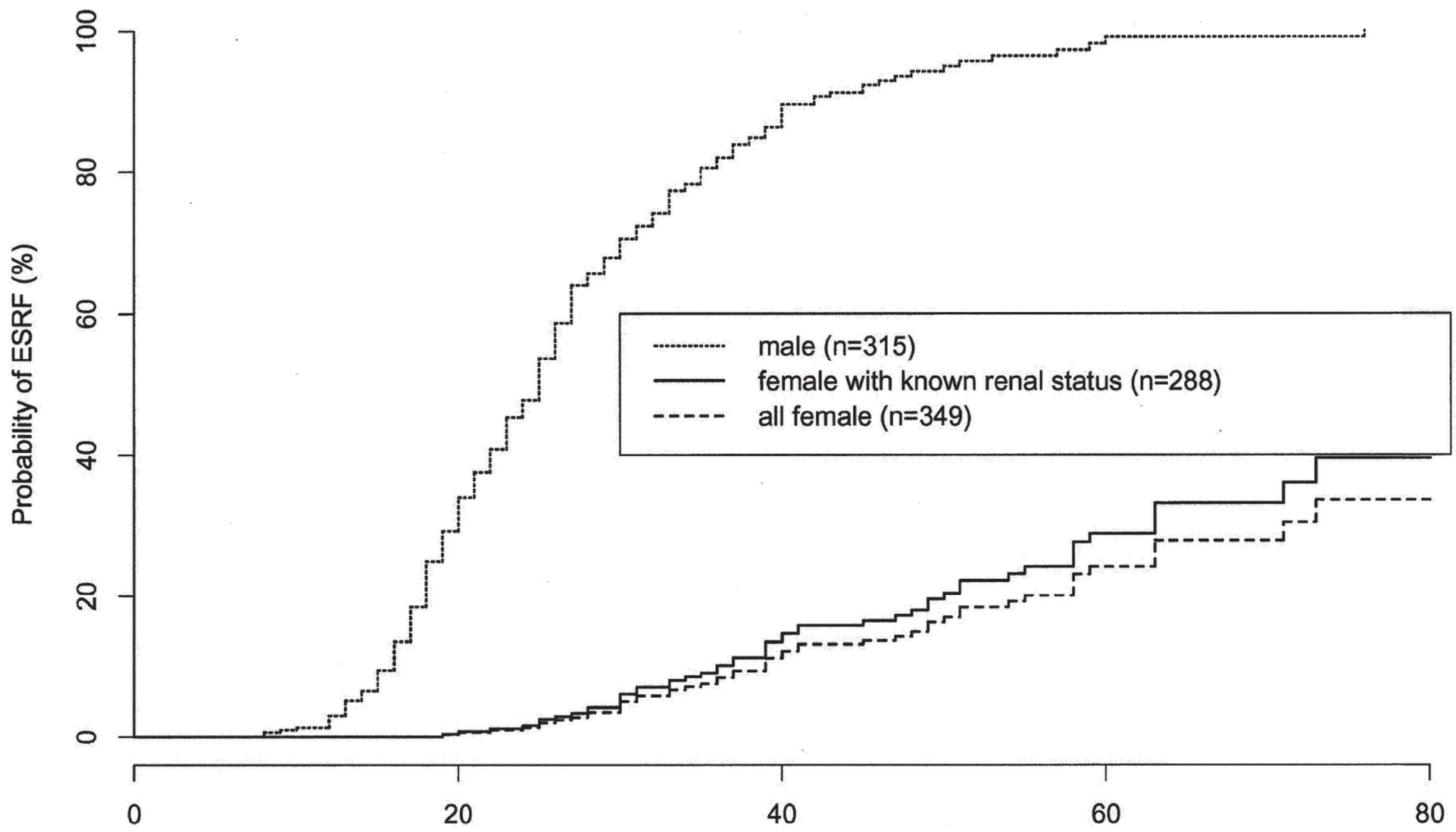

FIGURE 17.1 Probability of end-stage renal disease (ESRD) in 315 boys and men and 288 girls and women with the *COL4A5* mutation. In the third curve, girls and women with incomplete clinical data were added because they were not in ESRD at last follow-up. (Reprinted from Jais JP, Knebelmann B, Giatras I, et al. *J Am Soc Nephrol.* 14:2603-2610;2003 with permission from the publisher, American Society of Nephrology.)

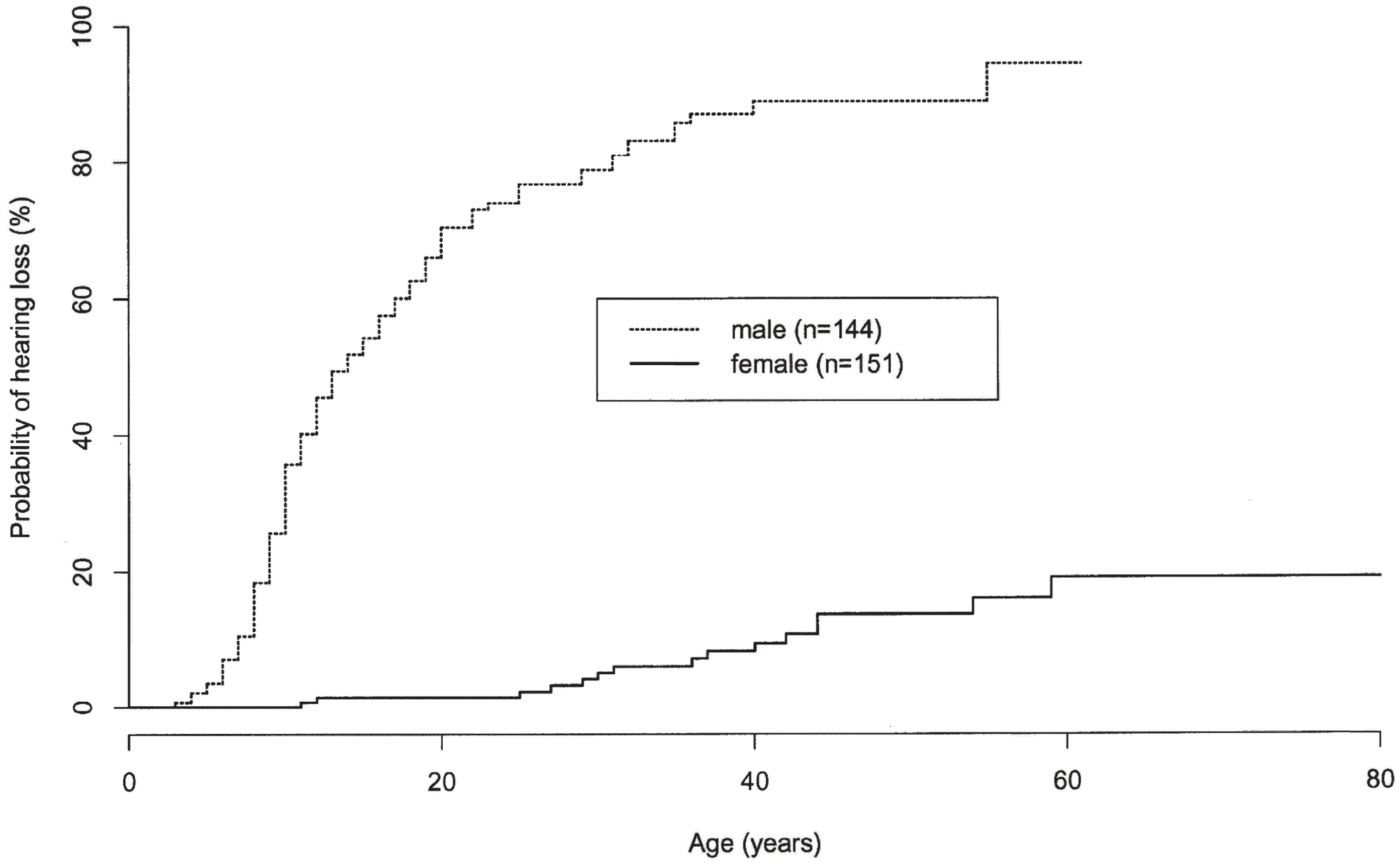

FIGURE 17.2 Probability of hearing loss in 144 boys and men and 151 girls and women with the *COL4A5* mutation. (Reprinted from Jais JP, Knebelmann B, Giatras I, et al. *J Am Soc Nephrol.* 14:2603-2610;2003 with permission from the publisher, American Society of Nephrology.)

AS cases. AR-AS results from mutations affecting both alleles of the COL4A3 gene or the COL4A4 gene. To date, most examples of AR-AS syndrome have resulted in early ESRD, but this may reflect ascertainment bias.[15–17] Males and females are equally severely affected, and hearing and ocular defects are usual. It is not yet clear whether all AR-AS or all TBMN is caused by mutations of these genes.

Autosomal-Dominant Alport Syndrome without Hematologic Defects (AD-AS, OMIM 104200)

AD-AS is a relatively rare genetic form of AS. It is linked with a heterozygous mutation of either the COL4A3 gene or the COL4A4 gene. Only a few families have been described to date. One well described family had relatively mild renal impairment and no hearing loss, eye signs, platelet abnormalities, or leiomyomatosis.[18] The mutation in this family is a splice site mutation in COL4A3.[19] In a study of eight families with heterozygous mutations affecting the COL4A4 gene none had ocular problems, and a low incidence of hearing anomalies was observed.[20] Loss of renal function occurred at a later age than the X-linked form.

Familial Thin Basement Membrane Nephropathy or Benign Familial Hematuria (OMIM 141200)

In 1997, Lemmink et al.[21] linked the occurrence of heterozygous mutations in the COL4A3 and COL4A4 genes with familial TBMN. These conditions are associated with the maintenance of long-term normal renal function and the absence of hearing loss or ocular problems. More recently, Pierides et al.[22] described 11 large pedigrees in which heterozygous COL4A3/COL4A4 mutations were associated with microscopic hematuria before the age of 30 and late development of proteinuria and ESRD due to focal segmental glomerulosclerosis.

Genotype–Phenotype Correlation in Alport Syndrome

COL4A5 Mutations

XL-AS is both clinically and genetically heterogeneous. To date, more than 590 mutations and potential mutations have been described in the COL4A5 gene associated with XL-AS.[23,24] Among affected male patients, the age at ESRD typically ranges between the second and third decades of life. However, in milder cases, ESRD may be delayed until the fifth or sixth decade. Similarly, deafness occurs at variable ages and a wide variety of ocular abnormalities have been reported among patients.[2] Several large studies from Europe, the United States, and China have assessed a genotype–phenotype correlation in XL-AS.[2,4,25,26,27] The COL4A5 mutation type appears to be one factor associated with renal disease severity and extrarenal manifestations. In male-affected patients large deletions, non-sense, and frame shift mutations have been associated with more severe disease manifestations, such as earlier age at ESRD onset, hearing loss, and the occurrence of eye abnormalities compared to patients with missense mutations.[4,25,26] Early renal failure and retinopathy have also been reported to associate with the occurrence of certain specific mutations, including some missense mutations.[28] The position of the mutation and the affected domain of collagen α_5(IV) also appears to affect disease severity. An earlier age at onset of ESRD was shown to associate with a more 5′ gene location in one study.[26] The distance of the mutation from the NC1-domain was shown to affect severity.[25] Glycine substitutions occurring in exons 1 through 20 resulted in a less severe phenotype compared to those affecting exons 21 through 47.[25] This effect was attributed to the fact that the triple helix formation starts at the C-terminal of the NC1-domain and proceeds in a zipperlike manner to the N-terminal end.[29] Similar studies in affected women and girls have failed to demonstrate any genotype–phenotype correlation.[7] However, it should be stated that phenotypic variability is also common among affected family members of the same gender who carry the same germline mutation. This intrafamilial variability may be attributed to both the environment and the effect of other genes.

COL4A3 and COL\4A4 Mutations

To date, 71 mutations or potential disease-associated sequence variants have been described in the COL4A3 gene, and 56 have been described in the COL4A4 gene.[23] Although AR-AS associated with homozygous mutation in either COL4A3 or COL4A4 results in the early onset of ESRD with typical hearing loss and eye problems, heterozygous mutations in the COL4A3/COL4A4 genes are associated with the less severe phenotype associated with benign familial hematuria and familial TBMN. Due to the innate genetic complexity of these disorders, not surprisingly, no genotype–phenotype correlation has been described.

Clinical variability in disease expression has been described for both male and female patients with AD-AS who carry a heterozygous mutation in either COL4A3 or COL4A4. However, no significant genotype–phenotype correlations have been described in AD-AS.[20] This may relate to the small number of families that have been identified with this genetic form of AS.

Pathogenesis

Type IV collagen, a major constituent of basement membranes, is comprised of six chains: α_1(IV) through α_6(IV). The type IV collagen chains assemble into three different heterotrimers in the mammalian basement membrane: $\alpha_1,\alpha_1,\alpha_2$; $\alpha_3,\alpha_4,\alpha_5$; or $\alpha_5,\alpha_5,\alpha_6$, respectively. The $\alpha_3,\alpha_4,\alpha_5$ form is synthesized by the podocytes in the glomerulus, and this heterotrimer of type IV collagen is also the predominant form of collagen found in the basement membrane of the ears, eyes, and lungs.[30] Mutation affecting any of the corresponding genes—namely, COL4A3, COL4A4, or COL4A5—will have a consequential effect on the integrity of the type IV collagen. In the GBM in Alport syndrome, the normal $\alpha_3,\alpha_4,\alpha_5$ collagen network is replaced by the fetal $\alpha_1,\alpha_1,\alpha_2$ network, which

is less resistant to degradation, thus resulting in the gradual deterioration of the GBM and in the clinical features of AS.[31]

Pathology

Kidney

There are no pathognomonic lesions seen with light microscopy in AS.[32,33] Lipid-laden interstitial foam cells are seen in the cortex of some but not all biopsy specimens. Foam cells are typically absent from biopsies taken early in the disease process.[34,35] Direct immunofluorescence is initially negative, whereas a faint deposition of immunoglobulin (Ig)G, IgM, and/or C3 may be observed with the progression of the glomerular segmental lesions.[36] The most definitive diagnostic information is provided by electron microscopy (EM) and by differential immunostaining for collagen α(IV) chains with specific monoclonal antibodies, which is discussed further under immunopathology.

Electron microscopy. The classic ultrastructural lesion of AS is characterized by an irregular thinning and thickening of the GBM, splitting and lamellation of the GBM with the loss of the normal lamina densa, small granules within the GBM, and an irregular outer and inner contour of the GBM (Fig. 17.3).[36] Distortion of the lamina densa may be extreme at times, amounting to a basket weave appearance in which the lamellae branch and rejoin in a complex triangle.[37] Focal or diffuse foot process fusion is common. The ultrastructural changes are common to all variants of AS. However, the extent of GBM thickening and lamellation is gender and age dependent. Moreover, there is considerable variability between affected individuals regarding the presence of these characteristic ultrastructural findings and even between affected individuals from the same family.[36] It should be emphasized that GBM splitting with variable thickness and an irregular outer contour is not specific for AS; such changes may be seen with other renal injuries, as reviewed by Haas.[36] Therefore, the combined use of EM and immunohistology for the detection of the collagen α_3(IV), α_4(IV), and α_5(IV) chains increases the specificity for AS diagnosis. Use of these combined methods permits AS diagnosis in most cases.[36]

Immunopathology. Staining for α_3(IV), α_4(IV), and α_5(IV) collagen chains with monoclonal antibodies distinguishes between the various forms of AS including XL-AS, AR-AS, and TBMN as depicted in Table 17.2. In particular, the absence

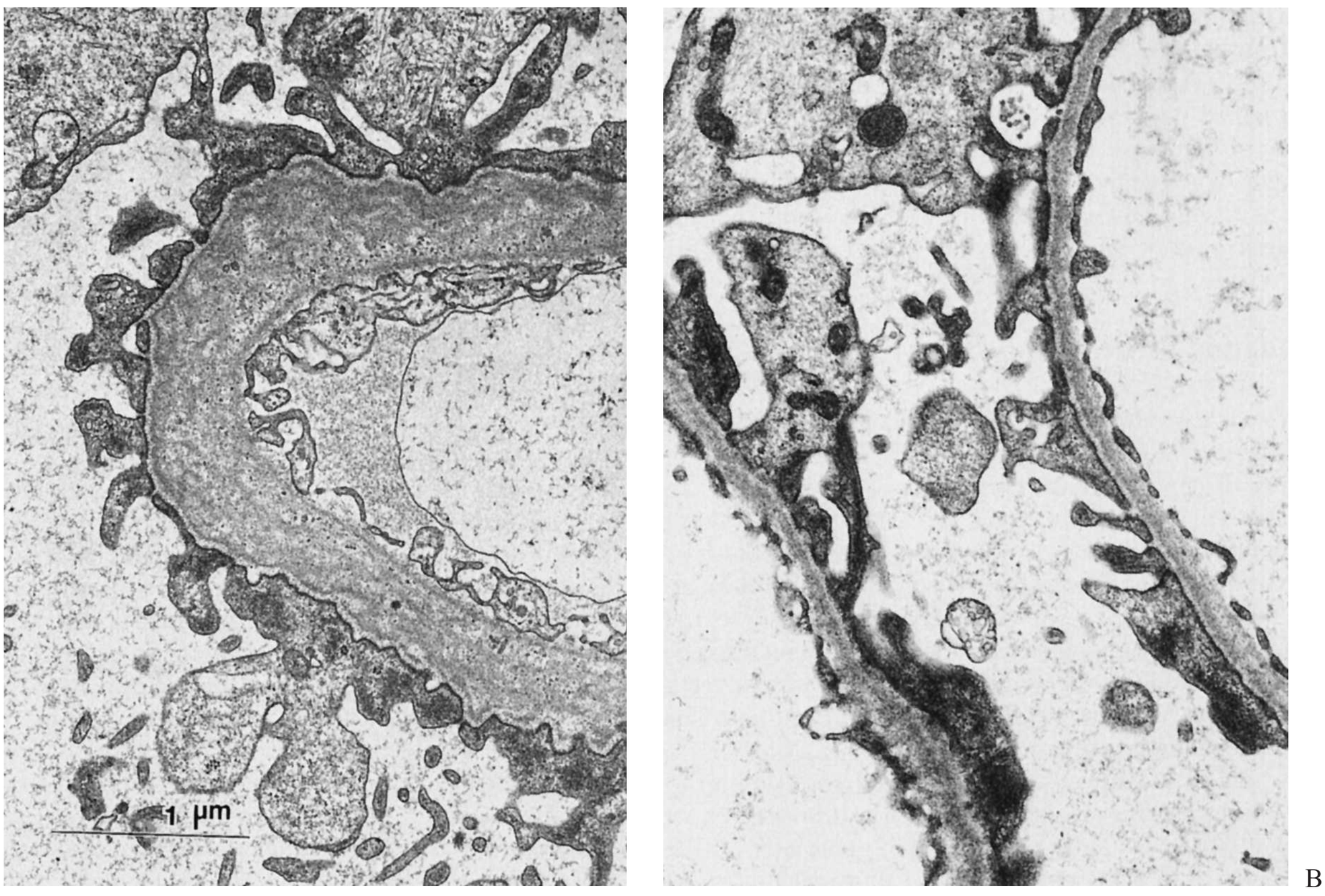

FIGURE 17.3 **A:** Glomerular ultrastructure in Alport syndrome. Electron micrograph of a renal biopsy specimen from a man in Utah kindred M, illustrating a widened lamina densa of the GBM (GBM). The lamina densa is split into several layers, between which may be seen numerous small electron-dense granules. **B:** Electron micrograph, at same magnification as **(A),** from an affected woman with familial thin GBM disease. The uniform thinning of the GBM can be appreciated by comparison with the width of the epithelial foot processes. (Electron micrographs courtesy of Dr. M.E. Hammond, University of Utah, Salt Lake City.)

17.2 Staining for α_3(IV) and α_5(IV) in Thin Basement Membrane Nephropathy (TBMN) and Alport Syndrome Variants

	α_3(IV)			α_5(IV)			
	GBM	BC	TBM	GBM	BC	TBM	EBM
Normal/TBMN	+	+	+	+	+	+	+
Alport variants[a]							
X-linked carrier (heterozygote)	Discont	Discont	Discont	Discont	Discont	Discont	Discont
X-linked male	–	–	–	–	–	–	–[b]
Autosomal recessive	–	–	–	–	+	+	+

[a]Infrequent exceptions to these patterns have been noted on renal biopsies.
[b]Up to approximately 50% of individuals in each of these categories will show normal staining for α_5(IV) on skin biopsy.
GBM, glomerular basement membrane; BC, Bowman capsule; TBM, distal tubular basement membrane; EBM, epidermal basement membrane; Discont, discontinuous staining (mosaic pattern).
Reproduced from Haas M. Arch Pathol Lab Med. 2009;133:224–232 with permission from the publisher American College of Pathologists.

of GBM staining for α_3(IV) and α_5(IV) is indicative of AS: either XL-AS in male subjects or AR-AS in both genders.[36] It should be noted that in 15% to 20% of XL-AS kindreds, α_3(IV) and α_5(IV) staining remains normal. This may result from the presence of certain missense mutations that cause only minimal disruption to the collagen chain structure. Both α_3(IV) and α_5(IV) are normally expressed in TBMN. Readers are referred to a comprehensive overview of differential α(IV) staining patterns for differentiation of the various forms of AS and TBMN by Haas.[36]

Skin

Skin biopsies are far less invasive than renal biopsies and may provide useful diagnostic information based on immunofluorescence analysis in certain patients. A normal epidermal basement membrane (EBM) contains α_5(IV) but not α_3(IV) or α_4(IV) chains. Thus, in AR-AS, α_5(IV) is present in EBM.[38] Absence of staining for α_5(IV) is highly specific of XL-AS in male patients. A pronounced pattern of discontinuous α_5(IV) staining is observed in some heterozygous carriers of XL-AS. However, it should be noted that a significant proportion of XL-AS patients, both male and female, retain a normal EBM staining of α_5(IV).[36] Thus, the diagnostic value of skin biopsies that demonstrate a positive staining for α_5(IV) is limited. Moreover, skin biopsy staining cannot be used for the diagnosis of AR-AS as expression of α_3(IV), and α_4(IV) is normally absent in EBM.

Cochlea

The inner ear is much less amenable to a histopathologic study than the kidney. Specific lesions in AS include a zone of separation between the basilar membrane and the overlying basement membrane of the organ of Corti and the presence of cells filling the tunnel of Corti and the extracellular spaces of Nuel.[39] Cellular infilling of the tunnel of Corti and spaces of Nuel likely represents a persistence of the fetal cochlear structure.[39]

Eye

In the anterior lenticonus, the basement membrane of the anterior lens capsule is thinned and more fibrillar than normal,[40,41] allowing for an anterior bulging of the cortex of the lens, most prominently in the pupillary region. The collagen α_3(IV), α_4(IV), and α_5(IV) chains may be present or lacking in the anterior lens capsule of AS patients.[42]

Another common ocular manifestation is perimacular "dot and fleck" retinopathy, which consists of whitish or yellowish flecks or granulations in a perimacular distribution.[43]

Smooth Muscle

In Alport-leiomyomatosis syndrome, the orderly hyperplasia of smooth muscle may involve the trachea and bronchi, all muscle layers of the esophagus, the clitoris, and the uterus. True leiomyomas, characterized by disorderly smooth muscle proliferation, have been found in the trachea and lungs, the esophagus, the upper part of the stomach, the clitoris, the vagina, the vulva, and the perineum.[10] Malignant transformation has not been observed. Ultrastructurally, basement membranes are normal in the esophageal tissue.[10]

Clinical Features of X-Linked and Autosomal Recessive Alport Syndrome

Renal Symptoms and Signs

Hematuria is the cardinal feature, persistent and present from birth, in 100% of affected males[4] and in 90% to 100%

of heterozygous females.[7] Hematuria is a sine qua non for the diagnosis of AS in males. Single or recurrent episodes of gross hematuria have been reported in 60% to 70% of males.[4] They may follow sore throats or other infections in children and may be the presenting symptom.[44]

Microscopy reveals dysmorphic red cells, renal tubular cells, and red cell casts in the urine. Urinary tract infections are no more frequent than in the general population. Proteinuria develops in 95% of affected males and in 75% of female heterozygous patients and is of variable degrees, ranging from barely detectable in early stages to nephrotic range[4] in some patients in advanced stages. As in other renal diseases, heavy or increasing proteinuria implies a worse prognosis.[4,44] Nephrotic syndrome may occur in severely affected patients. The serum complement concentration is normal.

Renal function initially remains normal for years and then wanes inexorably to renal failure. The age at which ESRD develops is very variable and spans from childhood or adolescence in families with severe (usually truncating) mutations to the fourth or fifth decade in some families with very mild (usually missense) mutations.[4,26] In the past, families in which ESRD developed in males at a mean age of 30 years or younger were classified as "juvenile" Alport families (about 70% of XL-AS), whereas others in which ESRD occurred at a mean age over 30 years were called "adult" Alport families (about 30% of XL-AS).[45] However, even within a family, the age of ESRD may be widely variable. As renal function declines, hypertension develops and worsens. Rarely, crescentic glomerulonephritis has been described and accompanied by rapidly progressive renal failure.[46]

In XL-AS, renal failure is inevitable for affected males, whereas only 30% to 40% of females develop ESRD, generally in later adulthood. In a European study of 195 XL-AS families, 12% of gene-carrying women developed ESRD by the age of 40 years, 30% developed ESRD by age 60, and 40% developed it by age 80 years (Fig. 17.1).[7] However, this may be an overestimate because in that study, about a third of gene-carrying women, possibly the less severely affected, were lost to follow-up. Many females remain asymptomatic carriers, only manifesting microscopic hematuria with or without low-grade proteinuria.[7,9] There is no correlation between the severity of the disease in women and men from the same family, and no genotype–phenotype correlations in women, which is likely due to the random inactivation of X-chromosomes in women.[7,9] The clinical manifestations of AR-AS are indistinguishable from XL-AS, with hematuria, proteinuria, and renal failure developing equally in males and females. ESRD occurs in all affected men and women, usually before the age of 30 years.[2,8]

Sensorineural Hearing Loss

Sensorineural hearing loss occurs in 50% to 80% of males with XL-AS and in 20% to 30% of heterozygous females, as shown in Figure 17.2.[4,7,8] It is never present at birth but starts to develop in childhood; initially, there is high-frequency hearing loss that is detectable only by audiometry, but it progresses and becomes clinically detectable in boys at an average age of 11 years.[2] Deafness develops at varying ages, often concurrently with the progression to ESRD, but in some cases 10 or more years later.[4,47] There is some correlation between the severity of the renal disease and the severity of hearing loss; families without hearing loss appear to have less severe renal disease than those with hearing loss.[44] However, there is also variability within AS families; while some affected family members develop hearing loss, other affected members may have apparently normal hearing even after ESRD.[47] Hearing loss generally occurs less frequently, less severely, and at an older age in carrier females (Fig. 17.2),[7,8,48] although some women and girls may have a profound loss. In the European study of XL-AS families, the risk of deafness by the age of 40 years was only 10% for women, but after the age of 60 years, 20% of the women had developed hearing loss.[7]

There is no anatomic abnormality of the tympanic membrane or ossicular chain; middle ear pressures are normal and air conduction is normal. Hearing loss is usually worse above 1,000 Hz, with an abnormal short increment sensitivity index, negligible tone decay, and normal brainstem auditory evoked responses,[48] thus proving cochlear rather than neural dysfunction. Caloric test results are normal, but more subtle testing reveals impaired vestibular function[48]; flat intensity function curves locate the lesion in the end organ itself. Hearing loss is thought to be due to structural lesions of the capillary basement membrane of the stria vascularis in the cochlea where the collagen α_3(IV), α_4(IV), and α_5(IV) chains are normally expressed.

Renal disease with hearing loss, even if familial, should not be equated with AS. Several genetic diseases affect the ear and the kidney, and chronic renal failure itself has been associated with impaired hearing. Hearing loss from the time of birth is unlikely to be due to AS.[4,47,49]

Ocular Features

A wide range of eye abnormalities have been reported, including anterior lenticonus, dot and fleck retinopathy, corneal endothelial vesicles (posterior polymorphous dystrophy) and erosions, macular holes, retinal detachment, and more recently, bull's eye and vitelliform maculopathy.[50] Eye abnormalities are usually not observed in children but develop in late adolescence and young adults. The most frequent are anterior lenticonus and dot and fleck retinopathy, which appear to be specific for AS.[2,28] The retinopathy consists of yellowish or whitish spots around the macula, sparing the fovea. Loss of the foveal reflex with alterations in macular pigmentation may be observed, as well as more peripheral pigmentary disturbances, either white or dark.[50] Visual acuity is unaffected by the presence of these retinal lesions. Their reported frequency varies and depends in part on whether a thorough eye exam is performed. In a smaller study, the retinopathy was found in 90% of affected males,[2] whereas other studies report it in 50% to 60% of men and in about 15% of women in XL-AS.[4,7,28]

Anterior lenticonus is a conical protrusion on the anterior aspect of the lens due to thinning of the lens capsule; it is less common than the dot and fleck maculopathy, occurring in 20% to 40% of men with XL-AS and is usually, but not invariably, bilateral. It is easy to recognize when it is fully developed. The red reflex is present, but it is impossible to see the fundus clearly because of the severe refractive error. Examination through a dilated pupil with a strong convex lens in the ophthalmoscope reveals an "oil drop" bulging the anterior surface of the lens. Lesser degrees of lenticonus may be difficult to diagnose even by slit-lamp examination.[51] Severe degrees of lenticonus cause grave visual impairment, are not correctable by glasses or contact lenses, and require lens replacement. Lenticonus is usually associated with early onset renal failure and more severe mutations in COL4A5.[4,28] It is rare in women with XL-AS.[7,8] Lenticonus is almost always accompanied by dot and fleck retinopathy,[52] but the retinopathy can be found in the absence of lenticonus. Ocular findings in AR-AS are similar to those seen in XL-AS men.[2] In one study, 91% of subjects with AR-AS had retinopathy and 82% had lenticonus.[8]

Autosomal Dominant Alport Syndrome

AD-AS is characterized by wide intra- and interfamilial variability in severity, but usually it is a milder disease.[2,19,20,53] It has been described in more detail in recent years, and causative heterozygous mutations have been shown in both COL4A3 and COL4A4 genes.[2,19,20,53] Although microhematuria is present in 95% to 100% of gene carriers, proteinuria is observed in about 50% of carriers at an age between 20 and 40 years, and ESRD is observed in 24% at a mean age of 51 years, but increases to 80% among patients older than 60 years.[20] ESRD has not been documented before the age of 31 years, and the overwhelming majority (93%) occurs after the age of 40 years.[20] Manifestations are similar in men and women. Hearing loss occurs in about 20%, with the onset usually after the age of 40 years. Ocular lesions have so far not been observed in AD-AS.[20]

Esophageal and Genital Leiomyomatosis

In several kindreds and in isolated patients, hematuric nephropathy was associated with striking muscular hypertrophy or leiomyomas of the esophagus.[54–57] In females, there was also hypertrophy of the clitoris, vulva, and adjacent structures. Hearing loss and cataracts were common, and anterior lenticonus was occasionally present.[57] Cataracts, which are not a feature of AS alone, were frequently severe and of early onset, and were sometimes congenital. Alport-leiomyomatosis syndrome has been comprehensively reviewed.[55,57]

Inheritance is X-linked dominant with deletions having been shown in the adjacent 5′ ends of COL4A5 and COL4A6.[56,58–60] In cases examined by electron microscopy, lamellation and granulation were seen in renal but not in esophageal basement membranes.[55] Clinically, affected patients suffer from dysphagia, odynophagia, regurgitation with respiratory symptoms, and bleeding. Occasionally, esophageal leiomyomatosis may be asymptomatic. The renal disease is similar to that in XL-AS men or women.[55]

Aortic Abnormalities

Aside from isolated case reports of aortic disease in males with AS, recently five males with a severe form of XL-AS were described who manifested thoracic aortic dissection at ages 25 and 32 years (two cases), ascending aortic aneurysm with rupture at age 32 (one case), aortic insufficiency requiring a replacement of the aortic root and valve at age 23 (one case), or asymptomatic dilatation of the ascending and descending aorta at age 21 years.[61] All five patients had ESRD by age 20 years, three had sensorineural deafness, and two had anterior lenticonus. Supporting the contention that aortic disease is a manifestation of AS was the finding of absence of collagen α_5(IV) from the aortic media in transgenic mice with XL-AS. A ruptured abdominal aortic aneurysm at age 36 and a ruptured intracranial aneurysm at age 14 years had been previously reported in two males with XL-AS.

Rare or Chance Associations

Many strange associations with AS have been described. Often, the diagnosis of AS was insecure, and in others, a coincidence noted on a few cases was not confirmed in larger studies. Further examples are needed to confirm these associations.

Diagnosis

The path to the correct diagnosis lies through a carefully collected, extended family history and a personal examination of the urinary sediment, specifically for hematuria. The proband will often be a child with unexplained hematuria or an adolescent to middle-aged male with ESRD with a vague history of kidney disease in brothers or relatives on the maternal side. A systematic urinalyses may reveal several relatives with hematuria.

There are several points to remember:

Microscopy of urine sediment—do it yourself!

Family history—extend to as many generations and collaterals as possible.

Age of ESRD in males helps to clarify the phenotype.

Female gene carriers—look for hematuria, although not all carriers have it.

When a member on the line of descent in a well-studied kindred is found to have hematuria, a renal biopsy is generally superfluous. The poorer the family history and the more remote the nearest affected relative, the stronger the case for a biopsy. Typical extrarenal features, specifically anterior lenticonus or retinal pigmentary changes in the patient or family, strengthen the presumption of AS and diminish the need for a biopsy. Linkage studies can identify gene carriers with near certainty in large families, but this is frequently

not practical for routine diagnosis due to the need to study several affected family members. About 15% of XL-AS cases are due to new mutations and, therefore, the family history will be negative.[2,62] In these patients, AS is diagnosed or suspected based on a renal biopsy performed for unexplained hematuria with or without proteinuria.

Molecular genetic testing is now generally available for mutation detection in the COL4A5, COL4A4, and COL4A3 genes. Acceptable samples for analysis include buccal swabs or blood samples. Detection of COL4A5 mutations by targeted mutation panel screening identifies the most commonly occurring mutations in this gene with almost 100% detection efficiency.[62] In addition, whole gene sequence analysis is available and has an efficiency of approximately 80% for mutation detection. Detection of large COL4A5 deletions or duplications requires more complex testing, which is offered by specialized clinical laboratories. Carrier testing and prenatal diagnosis may be performed once a mutation has been identified. Molecular genetic screening of both COL4A3 and COL4A4 genes by direct sequence analysis is likewise currently available through several clinical laboratories with an estimated efficiency of 80% for mutation identification. Screening for large deletions/duplications in these genes is less readily available outside of research laboratories.

Treatment

Kidney Disease

No specific treatment is known to affect the underlying pathologic process or to alter the clinical course of kidney disease. One uncontrolled series reported a surprising benefit of cyclosporine[63]; however, this was not confirmed in other studies. Despite a reduction of proteinuria with cyclosporine, renal function deteriorated and significant lesions of cyclosporine nephrotoxicity were seen on repeated renal biopsies.[64,65] Angiotensin converting enzyme inhibitors (ACEI) have been used in both hypertensive and non-hypertensive Alport children with proteinuria.[66,67] They variably reduced proteinuria and appeared to stabilize the decline of GFR. Although there is no proof of efficacy of these agents in AS, they are being widely used to suppress proteinuria in AS children.[68] Control of hypertension is necessary on general grounds and should follow the guidelines for children with renal disease. Patients with deteriorating renal function are monitored and treated as the general population with chronic kidney disease. When ESRD occurs, dialysis and transplantation pose no particular problems.

Transplantation is the treatment of choice for these young and otherwise healthy patients and has had excellent outcomes. Recurrent disease does not occur, but a small proportion of males (3% to 5%) developed de novo anti-GBM glomerulonephritis, which usually does not respond to plasmapheresis and cyclophosphamide and results in graft loss in 90% of cases.[69–71] The autoantibodies are directed against the collagen α_3(IV) and/or α_5(IV) chains, which are missing in the native kidneys. Why anti-GBM disease develops only in a minority of transplanted patients is unknown, but may depend on the specific mutation. It is not possible to predict which patients will develop this complication, but once this has occurred, the risk of recurrence in a subsequent transplant is very high.[71]

Hearing and Vision

Great care should be taken to avoid adding insults from drug cytotoxicity to the advancing aural injury. Improvement or stabilization of hearing loss in AS patients has occasionally been noted after transplantation[44,72]; others noted no benefit.[73] An interpretation of these findings is difficult because dialysis and the uremic state have been associated with reversible hearing loss. There is fair success with hearing aids. When hearing loss worsens, the patient will become more dependent on lip reading and other visual cues. Visual acuity should be monitored at intervals in those with or at risk of lenticonus, and consideration should be given to early lens extraction and intraocular lens implantation. Steroid doses should be kept low after transplantation, and patients should be monitored regularly for cataracts; poor vision is a disproportionate handicap to the deaf.

Genetic Counseling

Inheritance is X-linked dominant in 80% to 85% of families, is autosomal recessive in about 15%, and is autosomal dominant in 1% to 5%.[62] As a group, men with AS have about 30% fewer children than do men without AS; many men with more severe disease will sire no offspring.

An incomplete penetrance of AS in females must always be kept in mind.[74] In kindreds with X linkage, daughters of affected males will all be gene carriers regardless of their urinalysis results. Each clinically normal daughter of dominant gene carriers (mothers in kindreds with X linkage, and parents of either sex in kindreds with autosomal dominance) has a 50% chance of having an undetected Alport gene. Information from genetic tests or from urinalyses of the next generation may help decide whether these females have inherited a gene.

Differential Diagnosis

Conditions with Hearing Loss

Many conditions affect both the ear and the kidney, perhaps because of simultaneous embryogenesis or because of structural and physiologic homology.[75] For instance, hearing loss and proteinuria with a histologic picture of focal segmental glomerulosclerosis (FSGS) have been described in patients with mitochondrial cytopathies.[76] Here we discuss the most important hereditary conditions that might be confused with AS.

Autosomal-Dominant Alport-Like Syndrome Caused by Mutations in *MYH9*

Epstein and colleagues[77] first described a syndrome (OMIM 153650) that looked like a variant of AS with nephropathy,

sensorineural hearing loss, thrombocytopenia, and giant platelets. Subsequently, several kindreds have been described. When there were inclusions in leukocytes and cataracts in addition to nephropathy, deafness, and giant platelets, the term Fechtner syndrome (OMIM 153640) was used.[78] Other families had giant platelets, thrombocytopenia, and leukocyte inclusions but no nephropathy or deafness; this disorder was called May-Hegglin anomaly after the first descriptions in 1909 and 1945 (OMIM 155100). Other families have autosomal dominant hereditary hearing loss without any other manifestations (DFNA 17, OMIM 603622). All of these seemingly different disorders are caused by mutations in the MYH9 gene on chromosome 22q11-13, which encodes for the nonmuscle myosin heavy chain IIA (NMMHC-IIA), and are therefore variable expressions of the same disease.[79,80] Mutations in the motor domain of NMMHC-IIA are associated with a severe phenotype with severe thrombocytopenia and a high risk of glomerulopathy, ESRD, and deafness before the age of 40 years, whereas mutations in the tail domain are milder and often cause only giant platelets with mild thrombocytopenia, with a low risk of developing cataracts or hearing loss late in life.[79,80] However, as in other genetic disorders, there is significant intrafamilial variability in the severity of manifestations, possibly mediated by modifying genes or environmental factors.[80]

Clinical and Laboratory Features of MYH9 Disorders

The hallmark of this disease is macrothrombocytopenia from birth; all patients also have neutrophilic inclusions, although in some cases they are small and difficult to see on routine peripheral blood smears. The bleeding tendency is generally mild and depends on the degree of thrombocytopenia; platelet counts are usually between 25,000 and 100,000 per microliter. Bleeding times may be normal or substantially prolonged.

Glomerulopathy develops in 30% to 70% of patients with MYH9-related disorders at a mean age of 23 years.[80,81] Patients present with proteinuria, which is in the nephrotic range in more severe cases, and/or microhematuria, and often progress to ESRD before the age of 40 years.[80] When a renal biopsy was performed, it usually showed nonspecific light microscopic findings, including mesangial cell and matrix expansion, focal segmental, or global glomerulosclerosis and tubulointerstitial fibrosis.[80,82] Electron microscopy showed a focal and segmental effacement of foot processes and a loss of the interpodocyte slit diaphragm, as well as irregular thickening, splitting, thinning, and even a basket weave appearance of the GBM,[80] which may lead to confusion with AS. A renal biopsy is not necessary for the diagnosis of an MYH9-related disorder.

Sensorineural hearing loss and, sometimes, cataracts usually develop in parallel with the renal disease in patients with severe mutations (in the motor domain or at position 702 of the NMMHC-IIA gene), similar to AS.[79] The differentiation is made clinically by the finding of giant platelets, thrombocytopenia, and autosomal dominant inheritance. Individuals from families with milder mutations (in the tail domain of the gene) may develop presenile cataracts and hearing loss later in life.

Treatment

The treatment or prevention of bleeding complications is with platelet transfusions, with or without adjunctive desmopressin (DDAVP). Corticosteroids, intravenous immunoglobulin, and splenectomies have no role because this is not an autoimmune-mediated disease. The management of the renal disease is nonspecific; drugs that block the renin-angiotensin system are used for patients with hypertension and proteinuria. Drugs that are potentially harmful to the inner ear, lens, or kidneys must be avoided. Subjects who progress to ESRD are good candidates for renal transplantation.

Hereditary Interstitial Nephritis with Hearing Loss

One large kindred with autosomal-dominant inheritance of high-tone hearing loss, proteinuria, casts, and pyuria, but without hematuria, has been described.[83] Biopsy findings in four siblings were interpreted as interstitial nephritis. Renal impairment was generally mild, although renal failure supervened late in life in four men and in one woman. Familial reflux nephropathy was not excluded as a possible cause of this disorder.

Conditions with Normal Hearing

Familial Thin Basement Membrane Nephropathy (Benign Familial Hematuria, OMIM 141200)

Familial hematuria does not always have an ominous prognosis, and large families in which renal impairment never occurred have been described. Rogers and colleagues[84] delineated an entity characterized by uniform thinning of the GBM and prolonged survival without the deterioration of renal function. Familial TBMN is the best name for this condition, which occurs in about 1% of the general population.[36,85,86] In several reports, about 40% of cases have been shown to result from heterozygous mutations in the COL4A3 or COL4A4 genes.[21,85–88] It is not clear whether other genes can cause TBMN or whether the failure to detect mutations in these genes in some families resulted from incomplete penetrance, concurrent conditions causing hematuria, or the limitations of mutation analysis.

TBMN displays autosomal-dominant inheritance[89,90] and represents the carrier state for AR-AS.[22,36,85,86] Clinically, it manifests hematuria from childhood, usually microscopic and continuous, but it may be punctuated by episodes of visible hematuria, particularly during or after an upper respiratory tract infection. Hearing is normal and ocular lesions do not occur. In most individuals, there is no or only minimal proteinuria, blood pressure is normal, and renal

function remains normal.[84,85,91] Flank pain occurs in some patients,[85,91] and TBMN appears to be one of the causes of the so-called loin pain–hematuria syndrome.[92,93] Although familial TBMN does not lead to ESRD in most cases, a few families have been reported in whom significant proteinuria, hypertension, and ESRD developed in older individuals (>50 to 60 years of age), without hearing loss or ocular manifestations.[22,94,95] Because these cases of late onset ESRD were also caused by COL4A3 and COL4A4 mutations, these observations blur the line between benign familial hematuria and AD-AS, in which hearing loss occurs in only some individuals late in life and ocular changes are not seen.[20,36,53] Moreover, recently, five families with a clinical diagnosis of benign familial hematuria have been found to carry a heterozygous missense mutation in the COL4A5 gene, whereas that same mutation caused AS with late onset ESRD in other families,[96] possibly due to the effect of other modifier genes.

In classical TBMN, renal biopsy specimens, apart from the presence of erythrocytes in the Bowman space and in tubules, appear normal by light microscopy.[36,84] Immunofluorescence examinations also reveal normal findings.[84] The characteristic ultrastructural finding is uniform with diffuse thinning of the lamina densa of the GBM. The overall width of the GBM is reduced from 300 to 400 nm to approximately 200 nm.[36,84] Breaks may be seen in the GBM through which red cells can cross.[84] There is no widespread splitting or lamellation of the GBM, although very localized (<5% of GBM length) splitting may be observed.[36]

Even with adequate material for high-resolution electron microscopy, the distinction from AS is not always clear. Biopsies in early stages of AS can show uniform thinning while lacking abnormally thick areas and lamellation, which develop later in the course of the disease.[36,62] In these cases, immunofluorescence staining for α_3(IV) and α_5(IV) may allow the differentiation. These chains are absent from the GBM in about 80% of AS but are always present in TBMN, although at reduced levels.[36,62] Renal biopsies from individuals with late onset proteinuria and chronic renal failure have shown focal-segmental and global glomerulosclerosis in addition to diffusely thin GBM.[85,94,95]

In practice, in the absence of genetic testing, features that help distinguish TBMN from AS include male-to-male transmission, normal hearing and longevity of several affected family members, and characteristic biopsy findings in at least one member of the family. In small families or patients with new mutations, only long-term follow-up will allow for a precise diagnosis.

Familial Immune Glomerulonephritis

A familial incidence is occasionally noted in many forms of glomerulonephritis, including IgA nephropathy,[97] systemic lupus erythematosus, membranous nephropathy,[98] IgM mesangial proliferative glomerulonephritis,[99] focal segmental glomerulosclerosis,[100,101] membranoproliferative glomerulonephritis,[102] and partial lipodystrophy.[103] Congenital complement deficiencies, most commonly C3, predispose an individual to membranoproliferative nephritis.[104] The distinction from AS is evident on a renal biopsy, although the clinical features can be very similar in children or young adults presenting with hematuria with or without proteinuria.

Hereditary Interstitial Nephritides

Familial Juvenile Hyperuricemic Nephropathy (FJHN, OMIM 162000). This entity is a chronic interstitial kidney disease with autosomal dominant inheritance. It is characterized by decreased uric acid excretion, hyperuricemia in childhood and clinical gout beginning in adolescence, the development of chronic kidney disease in the third or fourth decade of life, and slow progression to ESRD by the fourth to seventh decade.[105,106] Besides hyperuricemia, decreased urine concentrating ability is observed early in life, and children may present with polyuria and enuresis.[105,106] Hypertension develops later, but proteinuria is absent or slight and the urine sediment is normal or contains only a few urate crystals or epithelial cells. Renal ultrasound shows normal size or small kidneys, sometimes with multiple small medullary cysts, in which case autosomal dominant medullary cystic kidney disease (MCKD) type 2 was diagnosed until advances in molecular genetics showed that both FJHN and MCKD type 2 are caused by mutations in the uromodulin gene on chromosome 16 and therefore are the same disease.[107] Chronic interstitial nephritis is histologically nonspecific. Sometimes intratubular crystal deposits and a thickening of the tubular basement membranes are seen. There is no specific treatment for this renal disease, but allopurinol will help prevent the development of progressive tophaceous gout.[106]

A similar autosomal dominant interstitial nephropathy with early onset hyperuricemia was recently found to be caused by mutations in the renin (REN) gene on chromosome 1 (OMIM 613092).[106,108] Individuals with REN mutations have decreased renin production, predisposing them to hypotension, hyperkalemia, and acute kidney injury, which is similar to patients receiving ACEI. Children with these mutations also suffer from anemia due to decreased renin and angiotensin production.[108] Gout is common in these families and is due to decreased urate excretion. The pathogenesis of both disorders involves the accumulation of abnormally produced uromodulin or renin in tubular cells, leading to tubular cell death and subsequent tubular atrophy.

There is a third form of autosomal dominant chronic interstitial kidney disease with unknown mutations that is not associated with gout or anemia. Some of these families have been linked to chromosome 1, but REN mutations have not been found.[106]

FABRY DISEASE (OMIM 301500)

Fabry or Anderson–Fabry disease was identified over a century ago, in 1898. This inborn error (also called angiokeratoma corporis diffusum, ceramide trihexosidosis) is a rare

metabolic disorder that particularly affects vascular endothelium, leading to renal, cardiac, and cerebrovascular manifestations and early death. Fabry disease is the only known X-linked sphingolipid storage disease. Lack of α-galactosidase, a lysosomal hydrolase crucial in glycosphingolipid metabolism, causes an accumulation of neutral glycosphingolipids in many tissues.

Epidemiology

The disease is panethnic, and estimates of incidence range from about 1 in 40,000 to 60,000 males,[109,110] but may be higher.[111] Fabry disease affects males more severely than females, although many carrier (heterozygous) females also have symptoms and some have severe organ manifestations because of random X-chromosomal inactivation.[112,113]

Genetics

Inheritance is X-linked and the gene coding for α-galactosidase A (GLA) is situated at Xq22.1 on the long arm of the X chromosome, just centromeric to the AS locus. The GLA gene is a relatively small gene of ~12-kb containing seven exons. To date, over 600 mutations that cause Fabry disease have been identified, including missense, nonsense, small deletions and insertions, large gene rearrangements, and splice mutations.[23] The mutations are spread throughout the entire GLA gene, and most are "private," occurring in one or a few affected families. An analysis of data from the Fabry Outcome Survey (FOS) has suggested a correlation between genotype and clinical severity.[114] Most mutations result in the typical phenotype, but several missense mutations produced signs and symptoms confined to the heart, called "cardiac variant," or produced no symptoms at all.[115] A higher incidence of a splice mutation, IVS4-919G→A, has been reported in Japanese and Taiwanese patients with a late onset cardiac phenotype.[116,117] Moreover, patients carrying this splice mutation have recently been shown to also have a higher incidence of renal and ocular abnormalities, suggesting that the effects of this mutation are not restricted to the heart.[117]

Genotype–phenotype correlations are complex in Fabry disease because the same mutation can lead to both classic and atypical disease, even within the same family.[118,119] Patients with atypical variants of Fabry have been found to exhibit missense mutations that lead to a reduction but not an absence of α-galactosidase A activity.[120] In contrast, an increased incidence of GLA loss of function mutations has been demonstrated in children with ocular manifestations of disease.[121]

The complexity of genotype–phenotype interactions in Fabry disease is further complicated by the potential influence of modifier genes. Polymorphisms in the endothelial nitric oxide synthase gene (NOS3) have been associated with left posterior wall thickness of the heart in patients with Fabry disease and the cardiac phenotype.[122] Polymorphisms in the vitamin D receptor gene (VDR) have also been associated with variability in the Fabry phenotype.[123]

Pathogenesis

α-Galactosidase hydrolyzes neutral glycosphingolipids with terminal α-galactosyl residues. If the enzyme is defective, several glycosphingolipids, particularly globotriaosylceramide (GL-3, or ceramide trihexoside), will accumulate in many cell types, especially vascular endothelial and smooth muscle cells and also in cardiac myocytes, renal glomerular and tubular cells, and cardiac conduction fibers. Patients with a reduction in α-galactosidase A activity have a less extensive accumulation of GL-3 than patients with an absence of activity.

Pathology

Kidney

In early stages (children and adolescents), light microscopy may only show a vacuolization of podocytes and distal tubular epithelia.[124] As the lipids deposited in these cells are dissolved out with routine processing, the cells that contained them appear as foam cells (Fig.17.4). However, foam cells are not specific for Fabry disease and can be seen in other lysosomal storage diseases or in nephrotic syndrome. The accumulation of GL-3 starts in utero and affects all glomerular cell types but is greatest in podocytes.[125] Deposits can also be seen in distal and, to a lesser degree, proximal tubular epithelial cells, as well as in endothelial and vascular smooth muscle cells of arteries and arterioles.[124] In more advanced stages, light microscopy shows focal segmental and, later, global glomerulosclerosis with tubular atrophy and interstitial fibrosis, all of which are nonspecific findings.[124] However, electron microscopy reveals striking stacks or whorls of dense, flat, osmiophilic inclusions in the lysosomes of blood vessels and of glomerular and tubular epithelial cells with a periodicity of 35 to 50 Å (Fig. 17.5). These myelin bodies are 1 to 3 μm in diameter, showing a characteristic "zebra" or "onion-skin" appearance, and are strongly suggestive of a diagnosis of Fabry disease.[126,127]

Nonrenal Tissues

The most striking changes are in blood vessels and are similar to those just described for the kidney. Thromboses can occur in many organs, leading to tissue infarction, and seem to occur as a result of platelet aggregation on areas of sphingolipid accumulation in the endothelium and vascular smooth muscle cells. Aside from ischemic sequelae, the heart may show extensive glycosphingolipid deposition in myocytes and valvular fibrocytes. A left ventricular endomyocardial biopsy can reveal severe hypertrophy and a vacuolization of myocardial fibers, in some cases surrounding normal appearing arterioles.[128] On electron microscopy, lamellar inclusion bodies can be seen in vacuoles, similar to the zebra bodies in renal epithelial cells.[128] All four chambers of the heart may enlarge, the mitral and tricuspid valves thicken, and the mitral valve may prolapse. In advanced stages, prominent cardiac fibrosis develops. Cerebral vessels are strikingly involved, leading to stroke in young subjects. Glycosphingolipid accumulation in neural tissue is confined

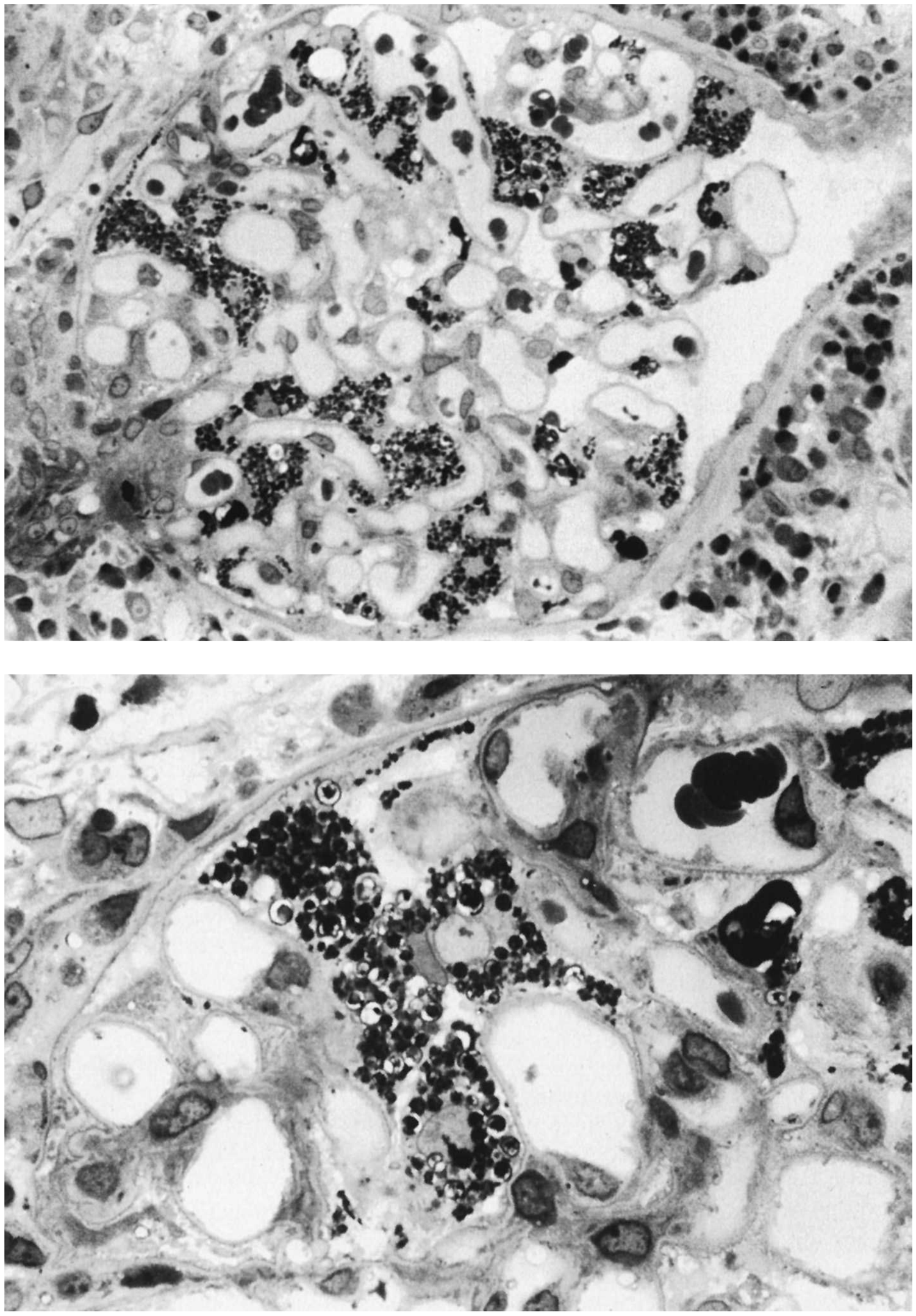

FIGURE 17.4 **A:** Foam cells in the renal glomerular epithelium in Fabry disease. (Magnification ×400.) **B:** Lipid deposits in the renal glomerular epithelium. (Magnification ×1,000.) (The glomerulus was embedded in epoxy resin and was stained with toluidine blue.) (Photographs courtesy of Dr. Melvin M. Schwartz, Rush-Presbyterian-St. Luke's Medical Center, Chicago.)

to the perineurium of the peripheral nerves, autonomic neurons, dorsal root ganglia, and some primary somatic afferent neurons. Accumulation also occurs in the cornea.

Clinical Features and Course

Extrarenal Manifestations

In childhood and early adolescence, affected individuals experience agonizing pain in the limbs, more marked distally.[109] Pain can be chronic and/or acute, with episodic crises often precipitated by changes in temperature, exercise, or stress. They occur in 80% to 90% of male patients in the first decade and in about 10% to 70% of female patients, often in the later course of the disease.[129,130] A typical pattern of pain is acroparesthesia or burning sensations in the palms of the hands or soles of the feet. They become less intense or may even disappear in later life. In severe attacks, pain radiates proximally and may even simulate an acute abdomen. The pain is a result of damage to small nerve fibers caused by the accumulation of GL-3 in the nerve axons and dorsal root ganglia.

Hypohidrosis is a nearly constant feature, leading to heat and exercise intolerance. Some patients suffer from hyperhidrosis. Other common nonspecific complaints in children and adults are abdominal cramps and diarrhea, which are likely caused by autonomic neuropathy. Progressive sensorineural hearing loss, often associated with tinnitus or vertigo,

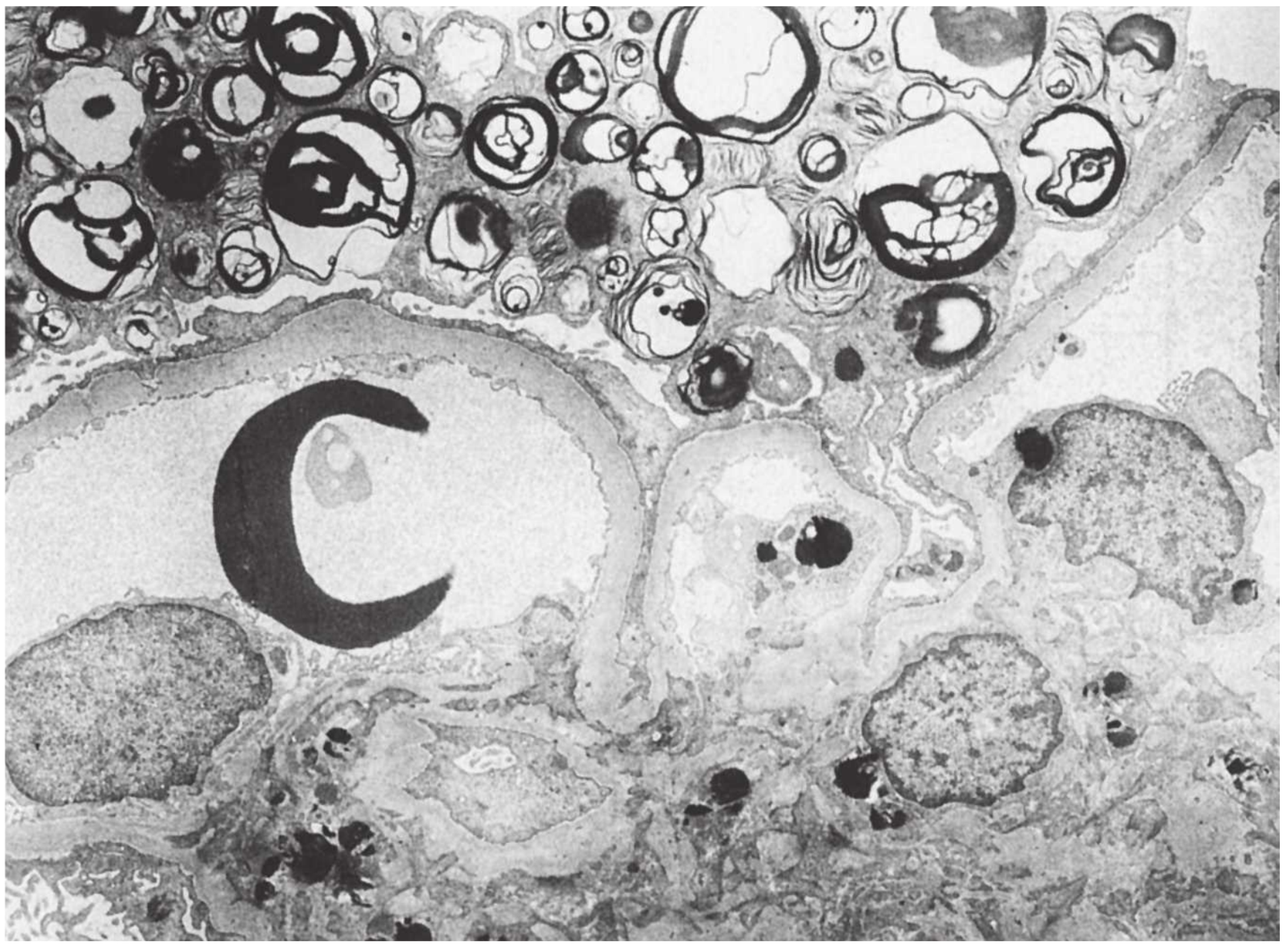

FIGURE 17.5 Myelin figures in Fabry disease. An electron micrograph of glomerular capillary loops with ultrastructural changes characteristic of Fabry disease. (Magnification ×5,500.) Many whorled "myelin figures" or "zebra bodies" are visible in the glomerular visceral epithelial cells. (Photograph courtesy of Dr. Daniel Terreros, Salt Lake City VA Medical Center, Salt Lake City, Utah.)

has also been reported to occur commonly in young male and female patients; its severity appears to be correlated with the peripheral nerve manifestations of the disease.[131,132]

Angiokeratomas, apparent as slightly raised, cherry red to black, nonblanching macules or maculopapules 1 to 3 mm in diameter, are small hyperkeratotic areas of dilated blood vessels. They appear in adolescence and progressively increase on the lower trunk and back, being particularly marked on the scrotum and bathing suit area (Fig. 17.6). Usually, they appear in groups or in generalized forms but they can be isolated. About 30% of carrier females have at least minimal angiokeratomas.[133] Angiokeratomas are not specific for Fabry disease because they also occur in other lipid storage diseases, and their absence does not rule out a diagnosis of Fabry disease.[134]

Cardiac involvement manifests as left ventricular hypertrophy, and some patients are erroneously diagnosed with idiopathic hypertrophic cardiomyopathy.[135] Left ventricular hypertrophy usually develops in the third and fourth decades of life and in the absence of significant hypertension.[113,135,136] In one echocardiographic study, left ventricular hypertrophy was found in 61% of men and in 18% of women who were older than 30 years of age.[135] Patients may present with congestive heart failure and/or severe mitral regurgitation.[134] The accumulation of glycosphingolipids in the cardiac conduction

FIGURE 17.6 Angiokeratomas of the anterior abdomen of a man hemizygous for Fabry disease.

system leads to arrhythmias or conduction defects requiring pacemaker and/or defibrillator implantation.[131] Cardiac and cerebral ischemic episodes can occur at an early age, in the third and fourth decades of life, often preceding the diagnosis of Fabry disease.[137] Therefore, Fabry disease is an important consideration in young patients with an unexplained stroke. One retrospective chart review of 447 Fabry patients reported that cardiac events, strokes, or transient ischemic attacks had occurred in 49% of males at a mean age of 36 years and in 35% of females at a mean age of 44 years.[131]

Cardiovascular events become more common in patients with ESRD. The Fabry registry, a voluntary international registry of 2,712 subjects with Fabry disease, reveals that after the onset of renal replacement therapy, 50% of men experienced a cardiac event or stroke (by a mean age of 48 years) compared with 20% of men without the need for renal replacement therapy (with a mean age of 36 years).[138] In this registry, there were significantly fewer women receiving renal replacement therapy (N = 27, versus 186 men), but 10 of them (37%) had a cardiac event or stroke by a mean age of 51 years. Cardiac involvement is the most common cause of death in both men and women.[139] In this recent study, the median age of death was 54.3 years for men and 62.0 years for women.[139]

Cornea verticillata is a distinctive whorled corneal opacity that is very similar to the opacities that can occur with prolonged chloroquine or amiodarone therapy.[140,141] It is the most common ocular manifestation of Fabry disease, occurring in about 70% of adult patients and 50% of child patients.[121] Opacities appear within the first few years of life and are generally asymptomatic; they are diagnostic of Fabry disease (unless the patient is on long-term treatment with chloroquine or amiodarone). Posterior radial cataracts eventually occur in 50% of patients but scarcely interfere with vision. Retinal vascular tortuosity is observed in about 50% of males, 22% of females, and 27% of children, but by itself it is not diagnostic of the disease.[121] Retinal vascular occlusion and ischemic optic atrophy may cause a visual loss.[142,143]

Female heterozygotes have very variable and often milder manifestations, but some are as severely affected as males. In the Fabry registry mentioned previously, 70% of the 1,077 enrolled females had symptoms and signs of Fabry disease with a median age at symptom onset of 13 years.[113] Twenty percent of females experience a major cerebrovascular, cardiac, or renal event at a mean age of 46 years. Although more severely affected women are more likely to have enrolled in the registry, these data clearly show that a significant number of affected women have serious disease manifestations.

Renal Manifestations

Urinary concentration defects may be the earliest functional manifestation of Fabry renal disease, leading to polyuria and nocturia. A nephrology referral is more typically initiated because of the development of proteinuria. Proteinuria may begin in the teenage years and becomes more frequent when patients reach their 20s and 30s, and may be in the nephrotic range.[144] Microscopy of the urine sediment may show red blood cells, renal tubular cells, casts, and lipids. Lipid droplets with their characteristic "Maltese cross" appearance under polarization microscopy may be found even when proteinuria is slight. Electron microscopy of the urine sediment may show "myelin bodies" morphologically identical to those seen in the lysosomes of renal tubular cells on renal biopsy specimens (Fig. 17.7). Renal ultrasound studies have revealed an increased incidence of renal sinus and parapelvic cysts compared to healthy controls; the pathophysiology is unclear.[145]

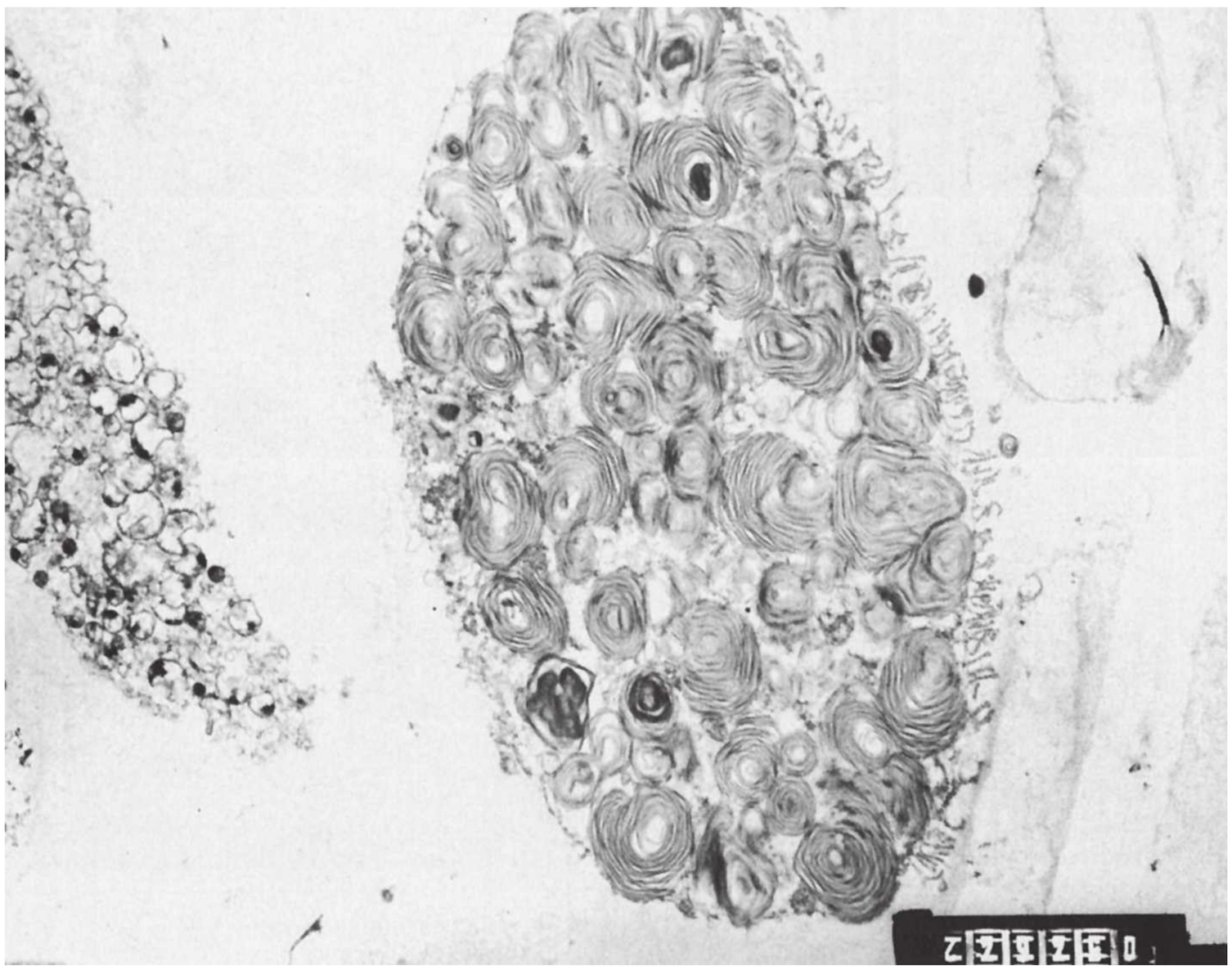

FIGURE 17.7 Urinary myelin bodies. An electron micrograph of a cell in the urine of a female heterozygous for Fabry disease. It is filled with myelin figures. (Uranyl acetate and lead citrate stain; ×20,000.) (Photograph courtesy of Dr. Melvin M. Schwartz, Rush-Presbyterian-St. Luke's Medical Center, Chicago.)

The progression to ESRD is near universal in males in their third to sixth decade of life, but occasionally occurs in their teenage years.[144,146] The mean age at the onset of ESRD in a study of 105 males was 39 years, and all patients who survived to age 55 developed ESRD.[144] Fewer women develop ESRD, and often at an older age than males,[131,147,148] although some of them develop ESRD as early as men.[113] The strongest predictor for a more rapid decline in GFR is the degree of proteinuria. Data on the natural history (i.e., the disease course before enzyme replacement therapy) comes from the Fabry registry and shows that men with a urine protein to creatinine ratio of 1.5 or greater lose GFR at a rate of 5.6 mL/min/1.73 m^2 per year, compared to 1.3 mL/min/1.73 m^2 per year for those with lesser degrees of proteinuria.[148] In the same study women had a slower decline, averaging 1.3 mL/min/1.73 m^2 per year if their urine protein to creatinine ratio was greater than 1.2; women also were about 5 years older at enrollment, and fewer women exhibited the higher levels of proteinuria.[148] Hypertension is a late manifestation, usually developing after GFR starts to decline, and is also associated with faster GFR decline, at least in men.[144,148]

Diagnosis

The most important step is to consider the diagnosis of Fabry disease. The index patient in most families goes undiagnosed for years or decades. In affected males with fully expressed disease, the constellation of symptoms and signs is highly suggestive. The diagnosis is confirmed if there is low α-galactosidase A activity in plasma or leukocytes. The test in leukocytes is more sensitive and is therefore preferred. The enzyme activity in Fabry leukocytes is usually under 4% of normal, and often undetectable in men, but it may be normal in women.[113,131] Therefore, direct genetic analysis is often required to make the diagnosis in women. A biopsy of the skin or kidney that demonstrates the characteristic glycolipid deposits may establish the diagnosis if no other means is available. Sometimes a kidney biopsy is performed for the evaluation of proteinuria, and the finding of "zebra bodies" in glomerular cells leads to the diagnosis of Fabry disease.[149] In such cases, family screening should be done in order to identify additional gene carriers with or without symptoms who might benefit from therapy (see the text that follows).

Prenatal Diagnosis

The severity of Fabry disease in males and in a proportion of females is the principal motivation for prenatal diagnosis. An analysis of α-galactosidase A activity in fetal cells obtained from chorionic villous tissue can confirm the disease in a male fetus; genetic mutation analysis should also be done, particularly in female fetuses because they can have residual enzyme activity. A mutation analysis can be performed on chorionic villi or cultured amniocytes.[150]

Treatment

Until recently, Fabry disease management was limited to symptomatic and palliative treatment, but this has changed with the availability of the recombinant human α-galactosidase A enzyme, agalsidase. Two different intravenous agalsidase formulations have been obtained: one from human fibroblasts (agalsidase α), and one from Chinese hamster ovary cells (agalsidase β). Both preparations underwent clinical trials that documented the feasibility, efficacy, and safety of the treatment. Based on observations made during these trials, both agalsidase α (Replagal) (0.2 mg per kilogram every other week) and agalsidase β (Fabrazyme) (1.0 mg per kilogram every other week) have been approved in Europe, but only agalsidase β was approved in the United States.[151,152]

A recent review identified 48 prospective clinical studies of enzyme replacement therapy (ERT), 22 each for agalsidase α and β and 4 with pooled treatments.[132] The longest reported treatment duration and follow-up was 4.5 to 5 years.[132,153] Overall, these studies showed that treated patients had a sustained decrease in plasma GL-3 levels and sustained endothelial GL-3 clearance as seen on skin, kidney, or heart biopsies.[153,154] The safety profile was favorable. Neuropathic pain and quality of life usually improved, even in dialysis patients. However, no effect on the incidence of strokes could be demonstrated in any study.[132] The effect on renal disease progression depended on the stage at which ERT was begun. If patients had more than 1 g proteinuria per day and/or decreased GFR ($<$ 60 mL/min/1.73 m^2), ERT was usually unable to halt the progression.[136,147,153] However, there was long-term stabilization of renal function in patients who were treated before they developed significant proteinuria. Similarly, a reduction in left ventricular hypertrophy was achieved only in patients without significant cardiac fibrosis, arguing for an early initiation of ERT.[132] A point to keep in mind is that ERT is extremely expensive.

Supportive treatment is also important for patients with Fabry disease. The debilitating pain may be considerably eased by phenytoin or carbamazepine. Excess glycosphingolipids can be removed by plasmapheresis with a temporary symptomatic benefit.[155] Given the limited benefit of ERT in patients with proteinuria, aggressive antiproteinuric therapy with angiotensin converting enzyme inhibitors and/or angiotensin receptor blockers in addition to ERT is both feasible and recommended.[136,156] The goal is to reduce proteinuria to less than 500 mg per day and to reduce the rate of GFR decline to less than 1 mL/min/1.73 m^2 per year.

When ESRD supervenes, both hemodialysis and peritoneal dialysis can be carried out with no specific problems. In studies from both the United States and Europe, the 3-year survival of dialysis patients with Fabry disease was worse compared with nondiabetic dialysis patients of similar age.[157] Renal transplantation is the treatment of choice for Fabry patients. In the most recent study of 197 kidney transplant recipients with Fabry disease, 5-year graft survival was similar to that of a matched cohort of recipients with other causes of ESRD, but 5-year patient survival was worse (81% versus 90%).[158] The most common reported cause of death was myocardial infarction. Few patients, if any, in these reports had received ERT. Although ERT is safe in kidney transplant

recipients and small studies show symptomatic benefit, it is currently unknown whether ERT will improve the prognosis of Fabry patients with ESRD.[157] Graft loss due to recurrent Fabry nephropathy has so far not been documented.[157]

Variants and Diseases Related to Fabry Disease

Recently, a variant form of Fabry disease was identified with manifestations primarily limited to the heart[128,159]; these "cardiac variants" lack the classical disease symptoms, and present in the sixth or seventh decade of life with left ventricular hypertrophy (LVH) and/or cardiomyopathy. These patients may also have proteinuria, but their renal function is typically normal for age. Of note, cardiac variants have residual α-galactosidase A activity due to missense mutations and lack the systemic vascular endothelial glycosphingolipid deposition characteristic of classically affected patients. Screening of 230 consecutive Japanese male patients with LVH and 153 British male patients with hypertrophic cardiomyopathy by plasma α-galactosidase A assays revealed that 3% and 3.9%, respectively, were previously unrecognized cardiac variants.[128,159]

There is mounting evidence that renal disease with ESRD can also occur in the absence of other typical organ manifestations. Nakao et al.[160] screened 514 male Japanese hemodialysis patients and identified 6 with exclusively renal manifestations. Interestingly, these subjects had residual α-galactosidase A activity, but reached ESRD at an age similar to patients with full-blown disease. These may be examples of a renal variant.

Angiokeratoma corporis diffusum with glycopeptiduria is an autosomal-recessive disorder resulting from a deficiency of α-N-acetylgalactosaminidase.[161,162] Features are similar to Fabry disease, with angiokeratomas and peripheral neuropathy. Both a severe infantile onset and a milder adult onset form of the disease have been described.

Finally, renal ultrastructural lesions identical to those in Fabry disease have been described in patients on long-term treatment with hydroxychloroquine, chloroquine, and amiodarone.[163] These drugs can inhibit several lysosomal enzymes, including α-galactosidase A. This complication of drug therapy has been called iatrogenic phospholipidosis and seems to be limited to the kidney and cornea. After the removal of the offending agents, a variable improvement in renal parameters has been reported.[163]

NAIL-PATELLA SYNDROME (NPS) (OMIM 161200)

Nail-Patella syndrome (NPS) is a rare autosomal dominant disorder first described by Little in 1897, which is characterized by the tetrad of dysplastic nails, hypoplastic or absent patellae, iliac horns, and deformities of the elbow. Other names include hereditary onycho-osteodysplasia (HOOD), Fong's syndrome, Turner-Keiser syndrome, and Osterreicher-Turner syndrome. It shows wide variability in phenotypic expression and organ involvement within and between families. Renal involvement is observed in about 40% of cases and appears to cluster in certain families.[164,165] Renal failure has been reported to occur in 1% to 15% of patients.[164,165]

Epidemiology and Genetics

The incidence of NPS has been reported to approach 1 in 50,000 live births.[165–167] The condition was linked to the ABO blood group locus as early as 1955[168]; fine mapping established the locus at 9q34.1.[3,169,170] The gene was identified in 1998 as the LMX1B gene,[170] which codes for a transcription factor that is essential for a wide range of developmental processes including dorso-ventral patterning of the limb, differentiation of dopaminergic and serotonergic neurons, patterning of the skull, and normal development of the kidney and eye as recently reviewed by Dai et al.[171] These developmental processes apparently require the function of two normal LMX1B genes because a mutation in one of them results in NPS, which is likely due to haploinsufficiency.[172,173]

To date, over 160 mutations[23,81] have been reported in patients with NPS. In 10% to 15% of families, no mutation is found; about 12% of cases have no family history and may be either new mutations, subclinical disease in the parent, or due to somatic mosaicism of the parent.[165,174] Most negative studies attempting to demonstrate genotype–phenotype correlations in NPS have been small. One larger study of 106 subjects from 32 NPS families demonstrated that individuals with LMX1B mutations affecting the homeodomain had significantly more frequent and higher values of proteinuria compared to those individuals carrying LIM domain mutations.[164] The genetic basis for the wide inter- and intrafamilial phenotypic variability is unknown but may involve modifier genes or polymorphisms in the target genes of LMX1B.[165]

Renal Pathology

Light Microscopy of renal biopsy specimens is nonspecific and often demonstrates normal findings. The only consistent but nonspecific glomerular feature is focal GBM thickening.[175,176] In more advanced stages, when renal functional impairment is present, light microscopy findings include focal segmental glomerulosclerosis, proliferative glomerulonephritis with crescents, and hyalinization of glomeruli.[175–177]

Immunofluorescence microscopy is negative or shows presumably a nonspecific trapping of IgM and complement.[175] Staining of the GBM for collagen α_3(IV) and α_4(IV) chains, podocin, synaptopodin, CD2AP, α3 integrin, and nephrin is normal.[165]

Ultrastructural changes are characteristic and specific and have been found in all NPS patients. The GBM is irregularly thickened and contains lucent areas and areas of fluffy low-density material, giving the appearance of a "moth-eaten" basement membrane. Overlying pedicles are effaced.

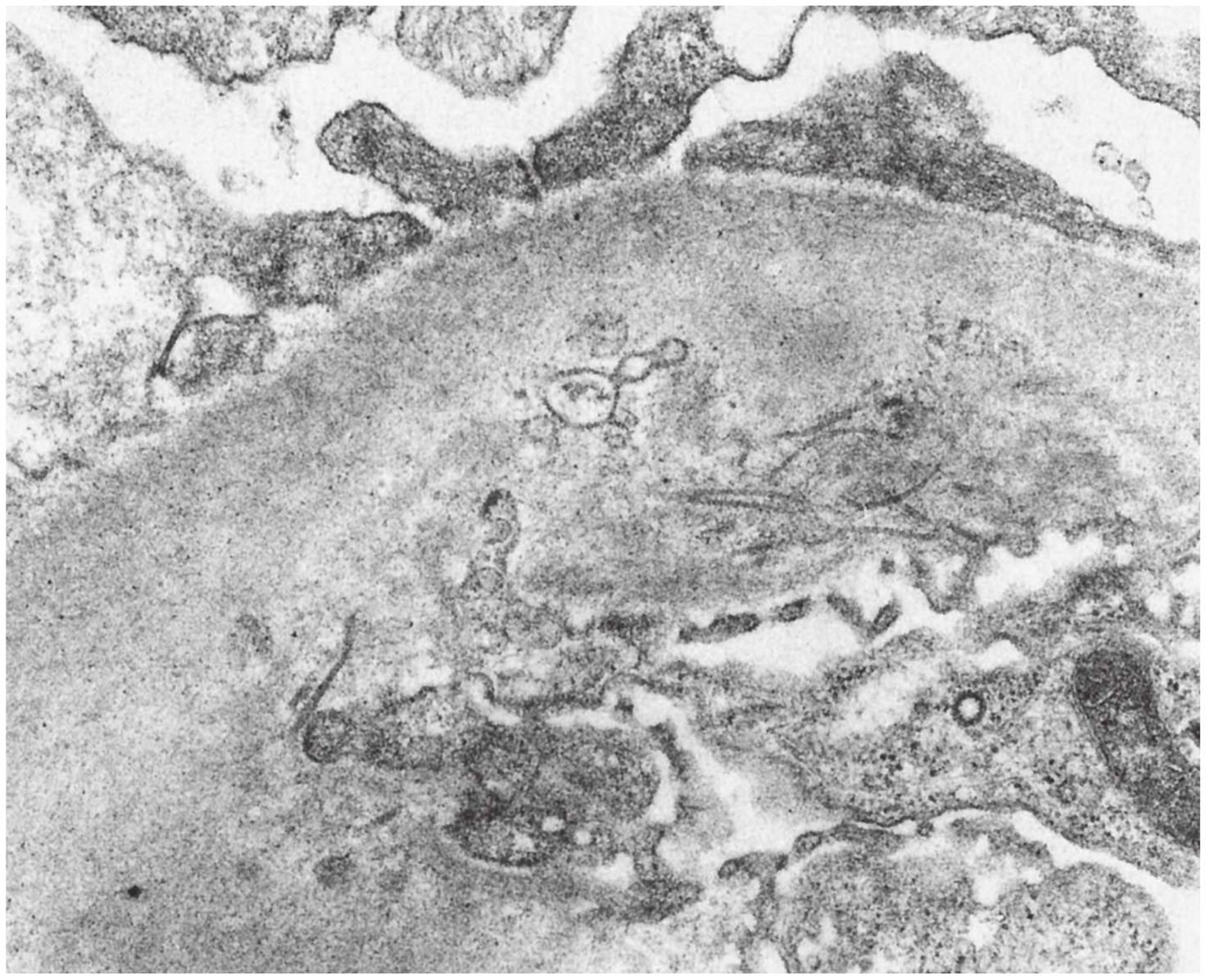

FIGURE 17.8 Glomerular basement membrane (GBM) in nail-patella syndrome (NPS). A high resolution (magnification ×55,000) electron micrograph of the GBM showing fluffy lucencies expanding the GBM and blurring its endothelial margin. Several collagen fibrils are visibly embedded in the GBM. (Photograph courtesy of Dr. Daniel Terreros, Salt Lake City VA Medical Center, Salt Lake City, Utah.)

Patches of dense fibrillar material with a periodicity of collagen are scattered throughout the entire thickness of the GBM (Fig. 17.8).[175–178] The Bowman capsule and tubular basement membranes are not specifically affected, but the mesangium may show fibrillar collagen similar to that in the GBM and is usually accompanied by mesangial cell proliferation. Although similar mesangial lesions have been seen in other conditions such as diabetes, membranoproliferative glomerulonephritis, amyloidosis, and glomerulosclerosis, in these conditions the material is not collagen and can be distinguished by its relatively weak staining with phosphotungstic acid.

NPS has been associated with other glomerular lesions, including membranous nephropathy,[179] systemic vasculitis,[180] IgA nephropathy,[181] Goodpasture syndrome,[182] hemolytic-uremic syndrome,[183] and bilateral renal stones.[184] The significance of these associations is unknown, but perhaps the abnormal structure or abnormal immunogenicity renders the GBM more vulnerable to other insults.

Clinical Features and Course

Extrarenal Manifestations

Patients with NPS have a lean body mass, which is more apparent in adolescents and young adults. This decrease in muscle mass is more prominent in the dorsal parts of the upper arms and in the upper legs. Other abnormalities include lumbar lordosis and scoliosis. The classic tetrad of NPS consists of dystrophic nails (Fig. 17.9), patellar and lateral femoral condylar hypoplasia (Fig. 17.10), hypoplasia of the radial head and capitellum of the elbow (Fig. 17.11), and bilateral iliac horns (Fig. 17.12).

Nail dystrophy is present at birth in more than 90% of those affected and is the most constant feature of NPS.

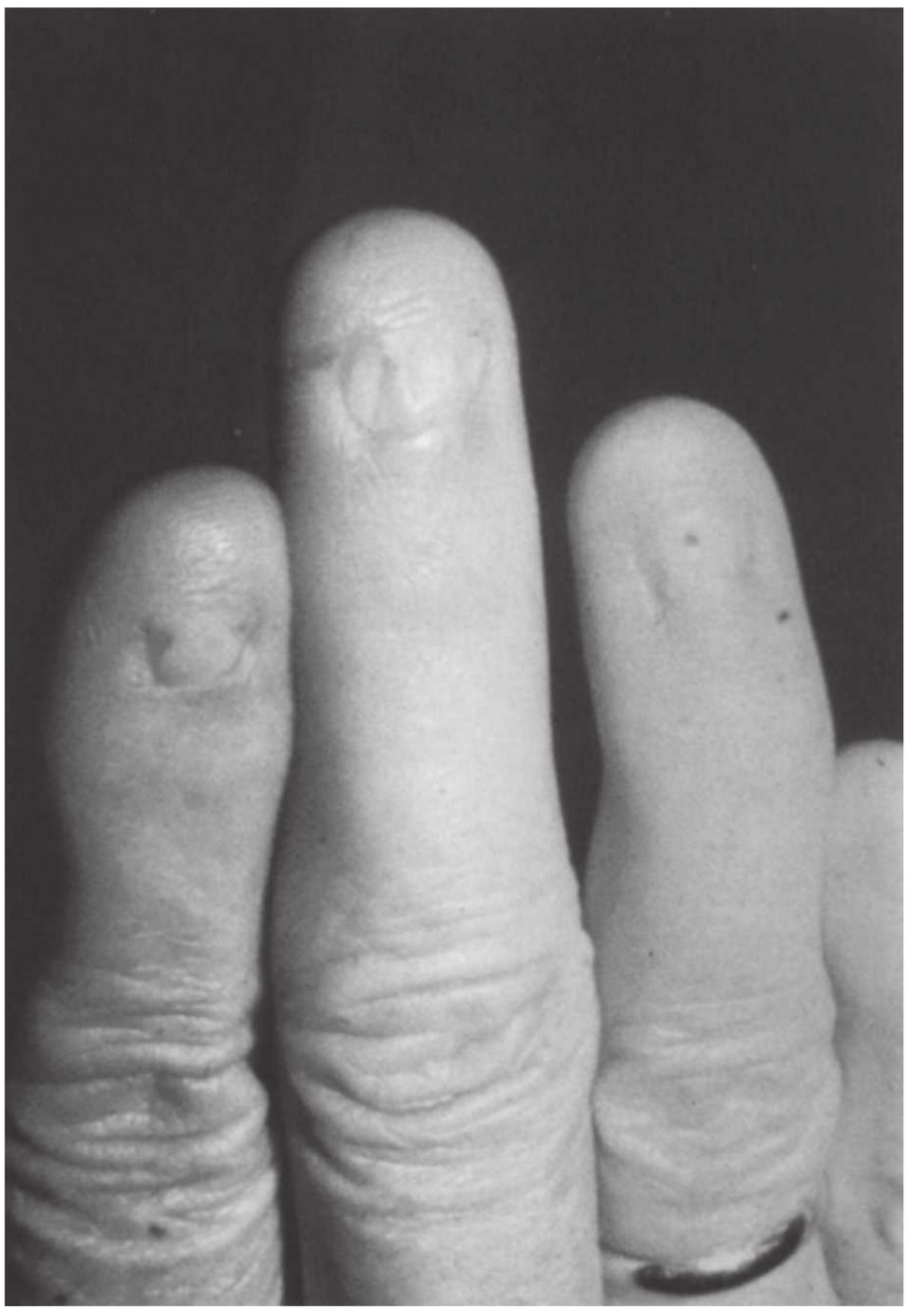

FIGURE 17.9 Characteristic nail changes of nail-patella syndrome (NPS). All the nails are small. The long finger shows the typical central triangular dystrophy with its base at the lunula. Usually, the changes are more marked toward the radial side of the hand.

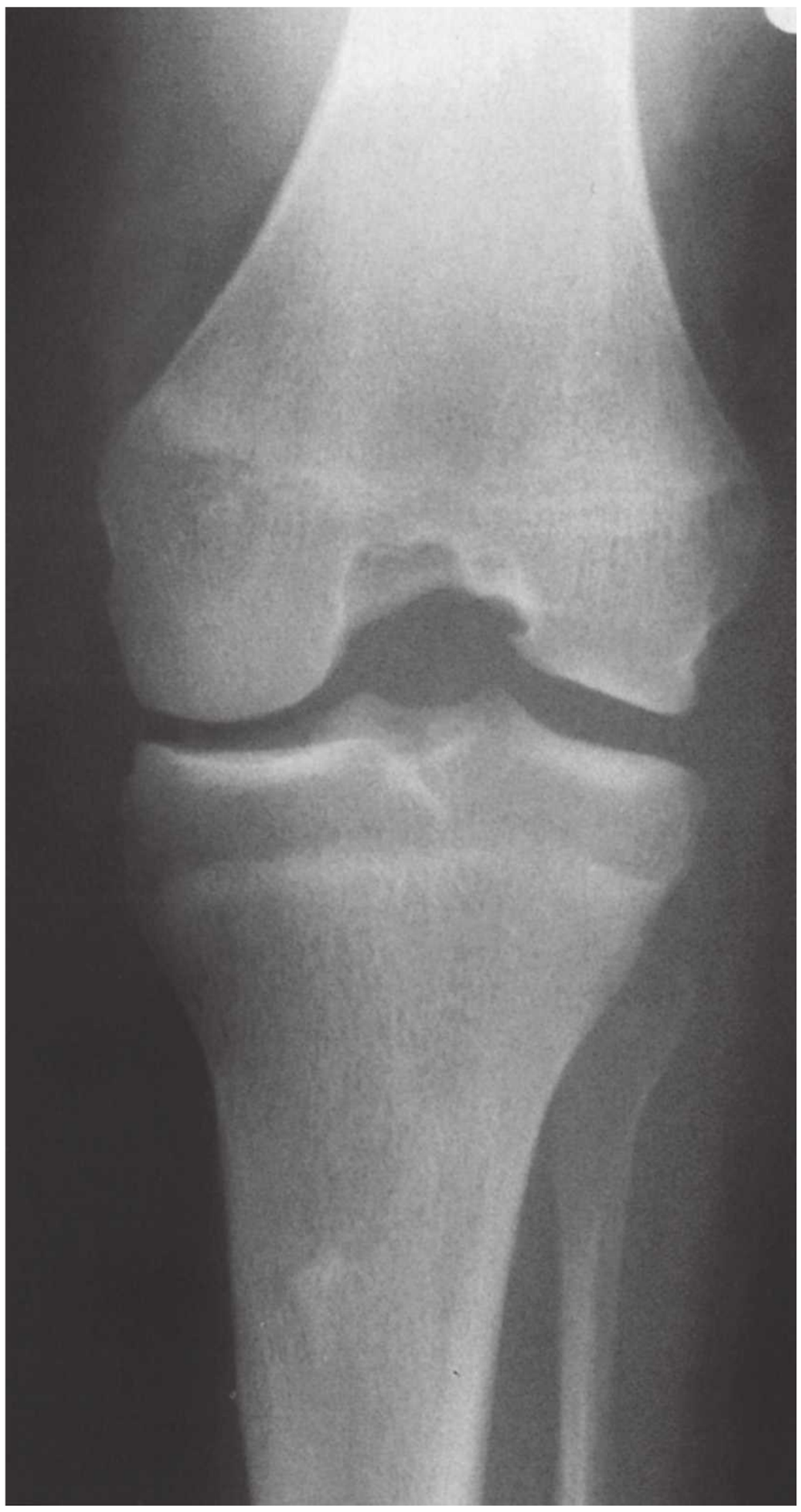

FIGURE 17.10 An absence of patella in nail-patella syndrome (NPS). A radiograph of the left knee of a 19-year-old girl with NPS. The patella is congenitally absent and the lateral femoral condyle is hypoplastic.

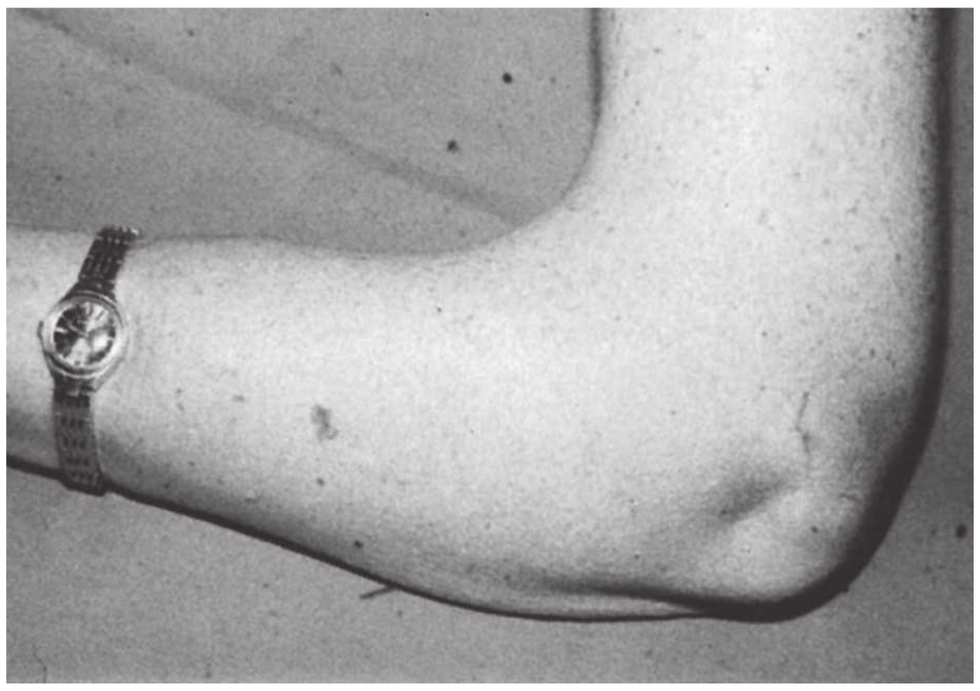

FIGURE 17.11 An elbow contracture in nail-patella syndrome (NPS). The left elbow of a 44-year-old woman with NPS and end-stage renal disease (ESRD) (same patient as in Fig. 17.9). She is holding her elbow as straight as possible. A web of soft tissue fills the antecubital fossa.

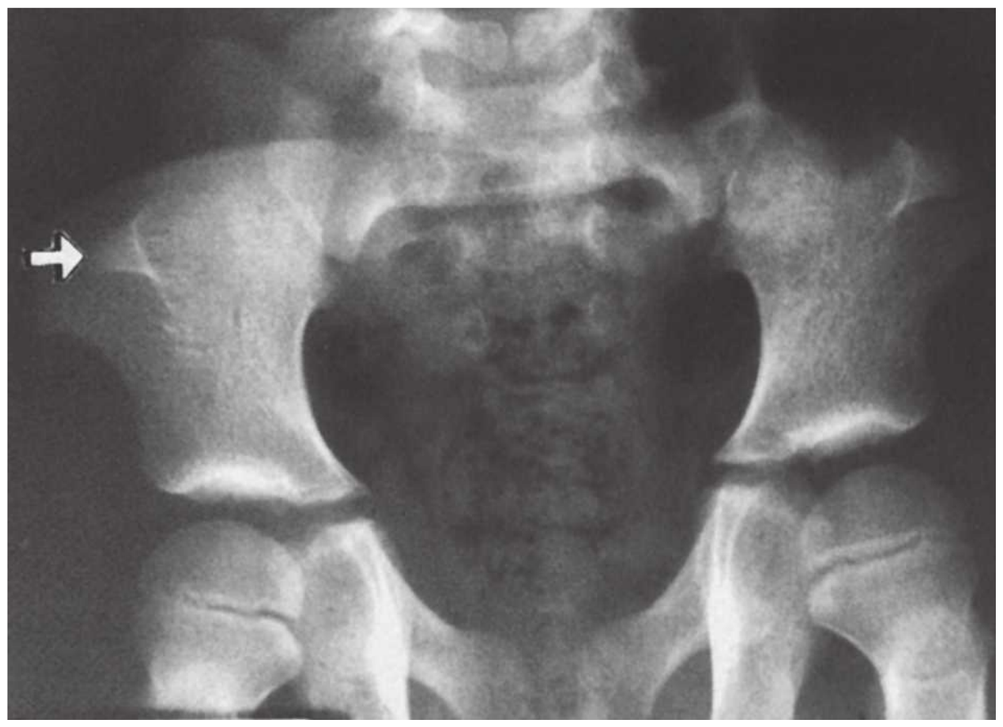

FIGURE 17.12 Iliac horns. A pelvic X-ray film of an 8-year-old girl with nail-patella syndrome (NPS) (same patient as in Fig. 17.10, but 11 years earlier). The *arrow* indicates the characteristic iliac horn, which is visible medial to the outer margin of the blade of the ileum. The radiograph incidentally shows a fusion defect and lumbarization of S1.

The presence of a triangular nail is a pathognomonic sign for NPS. The lunula appears stretched toward the free border of the nail, and the central portion of the nail is deformed. Commonly, affected nails are smaller than normal and may even be absent. Fingernails are more affected than toenails, and thumb and radial-side digits are more affected than ulnar-side digits. Furthermore, longitudinal ridging, splitting, spoon-shaped, and flaky nails have been associated with NPS.

The patella is small, misshapen, or absent in more than 90% of patients (Fig. 17.10). Concurrent hypoplasia of the lateral femoral condyle and poor development of the vastus medialis muscle may lead to recurrent subluxation or dislocation of the patella, knee instability, and knee pain complicated by patello-femoral arthrosis. Involvement of the elbow is almost as common, with hypoplasia of the radial head and capitellum being characteristic. This predisposes the patient to posterior subluxation of the radial head. Synechiae may lock the elbows in a permanent and disabling flexion (Fig. 17.11).

Conical bony horns projecting from the back of the blade of the ileum are seen in about 80% of patients and is considered pathognomonic of NPS. Often palpable in thin subjects, they are readily seen radiographically (Fig. 17.12).

Although not part of the classic tetrad, the most common presentations to orthopedists are foot or ankle deformities (club feet) and/or hip dysplasia.[164,185] Usually, these deformities require surgical correction.

More recently, ocular involvement and sensorineural hearing impairment have been described in patients with NPS. The most common pathologies are glaucoma, isolated glaucomatous alteration of the optic disk, and ocular hypertension, which are found in 35% of individuals in one study.[164] The mean age of these patients was 63 years, and glaucoma was rare under the age of 40 years. Cataracts were found in 8% of subjects,

including congenital cataracts in two siblings. Other anomalies were iris pigmentation (Lester sign) and corneal abnormalities. Unilateral or bilateral hearing impairment was detected by audiometric testing in 46% of patients at a mean age of 47 years.[164]

Renal Manifestations

It is the renal manifestations of NPS that influence mortality, occurring in 12% to 55% of the patients.[166] Between 5% and 14% develop ESRD.[166] The first and most typical sign of renal disease is moderate proteinuria with or without hematuria. Proteinuria may occur at any age and may resolve spontaneously, remain stable, or progress to renal failure at variable ages.[166] Nephrotic syndrome may also sometimes occur.[165,175]

The relationship between the somatic features of NPS, the clinical signs of nephropathy, and the GBM lesion is perplexing. There appear to be families with the somatic features in whom nephropathy does not develop, and other families with clinical renal disease and typical renal ultrastructural findings but no somatic manifestations. In a genotype–phenotype correlation study, Bongers et al.[164] presented evidence that the LMXB1 mutation position is involved with the risk of developing nephropathy. However, even families that clearly express the full spectrum of somatic and renal features, including proteinuria and the progression to ESRD, may have affected individuals without renal clinical manifestations. Other individual patients have shown musculoskeletal features and the typical renal ultrastructure but no clinical renal abnormalities.[177,178,186] Thus, the origins of variable phenotypic expression in NPS remain unclear.

Diagnosis

Typically, NPS is readily recognized clinically. If doubt remains, radiographs of the knees and pelvis usually show absent, rudimentary, or deformed patellae and bilateral iliac horns.

Renal biopsy is rarely required to make the diagnosis of NPS. It may be justified in patients with an atypical disease in whom another potentially treatable glomerulonephritis may coexist.

Prenatal Diagnosis

In families with a defined mutation, chorionic villous sampling can potentially be used for a mutation analysis[187]; in large families without an identified mutation, but with sufficient members to be informative, linkage could be sought with polymorphic markers near 9q34.[188] The typical renal ultrastructural features of NPS were found in an 18-week abortus,[189] and the diagnosis of NPS has been made by intrauterine kidney biopsy.[190] Skeletal anomalies may be recognized by fetal sonography.[191,192]

Treatment

As long as we cannot correct the molecular defect of NPS, treatment for all aspects is supportive. Orthopedic surgery to relieve contractures and to fuse or realign joints confers major benefits. Knee, ankle, and foot surgery is frequently helpful, whereas elbow surgery is rarely needed.[185] The treatment of renal disease is as in other patients with chronic kidney disease. ACEI may be of benefit in patients with proteinuria. Hemodialysis, peritoneal dialysis, and transplantation have been carried out successfully. In three patients who have had a biopsy of the allograft,[193,194] there was no evidence of recurrence of NPS lesions or of anti-GBM nephritis. Most intriguing, in a single patient,[194] nail lesions appeared to improve after transplantation.

Variants and Diseases Related to Nail-Patella Syndrome

More than 20 patients showing the renal ultrastructural features of NPS without the somatic findings have been described.[195–197] In seven families, inheritance appeared to be autosomal recessive. It is possible that these cases were examples of collagen III glomerulopathy, as previously described.[198] There is also some evidence for an autosomal dominant NPS-like syndrome without extrarenal symptoms.[199] These GBM diseases might be due to a partial expression of NPS or another type of hereditary glomerulopathy.[167]

A girl with a heterozygous complete deletion of the COL5A1 gene at 9q34 and underexpression of α_1(V) chains had dysplastic nails, normal patellae, but other mesenchymal and ectodermal features more suggestive of Goltz syndrome than of NPS.[200]

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CHAPTER

18 Isolated Renal Tubular Disorders: Mechanisms and Clinical Expression

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Renal control of acid and base reabsorption and excretion is controlled by the proximal and distal tubules (see Chapter 6). Thus, it is likely that abnormalities in those mechanisms responsible for these processes will result in clinically significant disorders. The transporters for carbohydrates, amino acids, and ions are polarized to either the apical or basolateral membrane, and the clinical abnormalities of fluid, electrolyte, and acid-base balance regulated by these transporters either have a genetic basis or are the result of acquired abnormalities of specific transport proteins. In this chapter, we review the major tubular transport defects of carbohydrates, amino acids, and some of the ions (H^+, K^+, Na^+). We describe the clinical features of these disorders as they appear in humans and summarize some of the insights learned from experimental models.

RENAL TUBULAR ACIDOSIS

Renal tubular acidosis (RTA) is a clinical syndrome characterized, when fully expressed, by a chronic hyperchloremic nongap metabolic acidosis, which results from a defect in urinary acidification.[1] This defect can be localized to the nephron segment responsible for the pathophysiologic expression and may be inherited or, more commonly, acquired by specific diseases or by drug or toxin effects. An incomplete form of distal RTA may be exhibited in family members of patients with genetic abnormalities of specific transporters in this nephron segment. Examples of specific defects involved in the pathogenesis of proximal and classical distal RTA include acquired or inherited abnormalities of the basolateral electrogenic Na^+/HCO_3^- symporter, the apical Na^+/H^+ exchanger, NHE-3, or the enzyme carbonic anhydrase II in the proximal tubule, and the H^+-ATPase and the HCO_3^-/Cl^- exchanger in the distal tubule. Proximal RTA (type 2 RTA) and classical distal RTA (type 1 RTA) are both associated with chronic hypokalemia. In contrast, a more generalized abnormality in the distal nephron is associated with hyperkalemia. The diagnosis of "complete" RTA requires spontaneous nongap metabolic acidosis in association with either a less than maximal urine pH or low ammonium excretion. Incomplete RTA is seen without spontaneous metabolic acidosis, but with evidence of the inability to acidify urine maximally in response to an exogenous acid load. Most inherited forms cause growth retardation or short terminal stature. Both inherited and acquired forms of proximal and classical distal RTA are accompanied by hypokalemia, often sufficiently severe enough to cause periodic paralysis or even seizures.

DIFFERENTIAL DIAGNOSIS OF NONGAP (HYPERCHLOREMIC) METABOLIC ACIDOSIS

A nongap metabolic acidosis is recognized by a low plasma bicarbonate concentration (or total [CO_2]), low blood pH, and a normal anion gap (8 to 10 mEq per L). A compensatory decrease in PCO_2 is typical, indicating the presence of a pure, or simple, nongap metabolic acidosis. The differential diagnosis of a nongap metabolic acidosis includes both nonrenal and renal causes. The differential diagnosis of nongap acidosis is displayed in Table 18.1. The evaluation of a nongap or hyperchloremic metabolic acidosis requires one to appreciate the role of the kidney in the acid-base balance, and to determine whether the kidney is responding appropriately to the prevailing acidosis (Table 18.2), which is to increase ammonium (NH_4^+) production and excretion adaptively. In contrast, ammonium production and excretion are impaired with chronic renal insufficiency, hyperkalemia, and with all forms of nongap acidosis of renal origin, including all examples of renal tubular acidosis.

Loss of HCO_3^- from the Gastrointestinal Tract versus Renal Tubular Acidosis

Diarrhea is a common cause of hyperchloremic metabolic acidosis. Diarrheal stools contain a large amount of HCO_3^- and HCO_3^- decomposed by reaction with organic acids.[2] The HCO_3^- loss and the ensuing volume depletion cause hyperchloremic metabolic acidosis. Hypokalemia develops due to direct K^+ loss in the stool and increased renal K^+ secretion (in the cortical collecting tubule) due to a secondary increase

18.1 Differential Diagnosis of Nongap Acidosis

Extra-Renal Causes
Diarrhea or other GI losses of bicarbonate
    (e.g., tube drainage)
Posttreatment of ketoacidosis (dilutional)
    (occasional: initial DKA)

Renal Causes Not Due to Renal Tubular Acidosis
Ureteral diversion (e.g., ileal loop, ureterosigmoidostomy)
Progressive chronic kidney disease
Toluene ingestion (excretion of hippurate)
Drugs
    With associated hypokalemia
        Carbonic anhydrase inhibitors (acetazolamide and topiramate)
        Amphotericin B
    With associated hyperkalemia
        Amiloride
        Triamterene
        Spironolactone
        Trimethoprim
    With normal potassium
        $CaCl_2$, $MgSO_4$
        Cholestyramine
Exogenous acid loads (NH_4Cl, acidic amino acids, total parenteral nutrition, sulfur)
Posthypocapnic state

Renal Tubular Acidosis
Low $[K^+]_p$
    Type 1 (classical distal) RTA
    Type 2 (proximal) RTA
    Type 3 (mixed proximal and distal) RTA
        (carbonic anhydrase II deficiency)
High $[K^+]_p$
    Type 4 (generalized distal RTA)
        Hypoaldosteronism (hyporeninemic and isolated)
        Aldosterone resistance
        Voltage defect in collecting duct

DKA, diabetic ketoacidosis; GI, gastrointestinal; RTA, renal tubular acidosis.

18.2 Diagnostic Criteria for Causes of Nongap Acidosis

Nonrenal Etiology
    Nongap acidosis expect:
        Increase in NH_4^+ excretion
        Negative urine anion gap
        Acid urine pH (<5.5)—exceptions

Renal Etiology
    Nongap acidosis expect:
        Inability to increase NH_4^+ excretion
        Positive urine anion gap
        Urine pH typically >5.5 but variable in type 4

in elaboration of renin and aldosterone in response to volume depletion. Both hypokalemia and nonrenal metabolic acidosis increase renal NH_4^+ synthesis, which increases urinary buffering capacity and urinary pH. The presence of NH_4^+ in the urine can be used clinically to distinguish hyperchloremic metabolic acidosis due to diarrhea from renal tubular acidosis (Table 18.2). In the latter, NH_4^+ excretion is invariably low. Urine pH, although a time-honored method to distinguish these disorders, is less reliable because urinary ammonium excretion is augmented in chronic metabolic acidosis due to diarrhea, causing urine pH to increase over time.

Other Causes of Nongap Metabolic Acidosis

In addition to diarrhea and RTA there are other less common causes of hyperchloremic metabolic acidosis (Table 18.1). External pancreatic and biliary diversion may lead to the loss of HCO_3^--rich fluid and result in hyperchloremic metabolic acidosis. The excretion of sodium salts of ketones during the recovery phase of ketoacidosis represents the loss of potential HCO_3^- and may result in hyperchloremic metabolic acidosis. Ureteral diversion is commonly associated with hyperchloremic metabolic acidosis because the ileum and colon are both endowed with an apical Cl^-/HCO_3^- exchanger. When chloride from urine comes into contact with the gut, chloride is absorbed in exchange for bicarbonate leading to excretion of bicarbonate, absorption of chloride, and hyperchloremic metabolic acidosis.[3] The degree of acidosis is magnified by stasis and prolonged contact of urine with the HCO_3^-/Cl^- exchanger in the pouch. Because of bicarbonate secretion, potassium secretion is also stimulated and leads to hypokalemia. Finally, the administration of acid or acid equivalent (arginine HCl, lysine HCl, or NH_4Cl) or medications such as cholestyramine, calcium chloride, and magnesium sulfate are associated with hyperchloremic metabolic acidosis.[4] Dilutional acidosis occurs in conjunction with rapid infusion of isotonic saline. In these latter examples, the serum potassium is usually normal.

Progressive renal failure is associated with metabolic acidosis. Hyperchloremic metabolic acidosis is commonly seen when the glomerular filtration rate (GFR) is between 20 to 50 mL per min.[5] As renal failure progresses to a GFR of less than 10 to 15 mL per min, the acidosis converts to the typical high anion gap acidosis of "uremic" acidosis. The

principle defect in advanced renal failure is impaired ammoniagenesis and ammonium excretion.[5] The latter is a result of impaired medullary ammonium transport and trapping of NH_3/NH_4^+ in the outer and inner medulla.

In summary, the defect in renal acidification in RTA may be manifest by one of three clinical syndromes: (1) an acid urine pH and low urine anion gap (UAG) during metabolic acidosis or frank bicarbonaturia and hypokalemia during $NaHCO_3$ therapy (as in proximal RTA); (2) an inappropriately alkaline urine pH, hypokalemia, and a positive urine anion gap (classical distal RTA); or (3) hyperkalemia and a positive urine anion gap but variable pH with aldosterone deficiency, aldosterone resistance, or a "voltage" defect in the collecting tubule (generalized defect in distal nephron) (Table 18.2).

Clinical Laboratory Evaluation

Because the measurement of urinary NH_4^+ concentration may be problematic for the routine hospital clinical pathology laboratory, it is helpful to estimate the urine ammonium concentration by considering the electrolytes present in urine. Because NH_4^+ is a cation, its presence in urine, especially when in large amounts as expected in nonrenal forms of hyperchloremic metabolic acidosis, should be denoted by an increase in urinary anions (Cl^-) in excess of the usual cations ($Na^+ + K^+$). The urine anion gap (UAG) is calculated on a "spot" urine sample as follows (Table 18.3)[6]:

$$UAG = [Na^+ + K^+]_u - [Cl^-]_u \quad (1)$$

18.3 Clinical Application of Urine Anion Gap to Approximate Urine Ammonium Excretion

1. Spot urine electrolytes: $[Na^+, K^+, Cl^-]_u$ in a patient with hyperchloremic metabolic acidosis
2. Calculate urine anion gap:

$$UAG = (Na + K)_u - Cl_u$$

3. Interpretation:

$(Na + K)_u > Cl_u$: NH_4^+ low (ammonium excretion impaired)
$Cl_u > (Na + K)_u$: NH_4^+ adequate (nonrenal hyperchloremic acidosis)

4. Pitfalls: Unusual anions in the urine (perform urine osmolar gap)
   Ketones
   Toluene

18.4 Urine Osmolar Gap to Approximate Urine Ammonium Concentration

Urine Osmolar Gap = Measured Urine Osmolality − Calculated Urine Osmolality

Urine = U_{osm} $0.5[2\,(Na^+ + K^+)_u + \text{urea}/2.8 + \text{glucose}/18][NH_4^+]$

Interpretation: Urine ammonium = 75 mEq/L or > anticipated in acidosis with normal renal tubule function

NH_4^+ is assumed to be present in the urine if the sum of the major cations ($Na^+ + K^+$) is less than the concentration of the major anion (Cl^-). Therefore, a negative urine anion gap denotes the presence of ammonium in the urine and signals a "normal" renal response to acidosis of nonrenal origin (i.e., diarrhea). Hyperchloremic metabolic acidosis of renal origin (i.e., RTA) is supported by the presence of a positive urine anion gap. A positive urine anion gap confirms a deficiency of NH_4^+, and obtains when the sum of the major cations ($Na^+ + K^+$) in the urine exceeds the major urinary anion (Cl^-). A positive urine anion gap, therefore, denotes an "abnormal" renal response to acidosis and is consistent with a defect in net acid secretion. The presence of urinary anions other than chloride can invalidate the UAG. Examples of urinary anions which invalidate this shorthand method of estimating urinary ammonium concentrations include drug anions, ketones, and toxins such as toluene. If these constituents are suspected, urinary NH_4^+ may be estimated reliably by measuring urine osmolality (U_{osm}); the concentrations of $Na^+ + K^+$, urine urea, and glucose (Table 18.4); and calculating the urine anion gap. Urine ammonium (U_{NH4}^+) is calculated as:

$$U_{NH4}^+ = 0.5\,(U_{osm} - [2(Na^+ + K^+) + \text{urea} + \text{glucose}]) \text{ (all expressed in mmol/L)} \quad (2)$$

The fractional excretion of sodium may also be helpful to differentiate hyperchloremic metabolic acidosis due to diarrhea from RTA. The fractional excretion of sodium is typically low (<1%–2%) in patients with diarrhea compared to RTA (2%–3%).

PROXIMAL RENAL TUBULAR ACIDOSIS

Role of the Proximal Tubule in Bicarbonate Reabsorption and Urinary Acid Excretion

The kidney employs two fundamental mechanisms to maintain acid-base homeostasis: bicarbonate absorption and H^+ secretion. Of the 4,000 mEq of HCO_3^- filtered by the kidney

each day, 80% to 90% is reabsorbed in the proximal tubule. Effective HCO_3^- absorption in the proximal tubule is mediated by H^+ secretion. Even though the distal tubule is responsible for the secretion of 50 to 80 mEq of H^+ and final acidification of the urine, the vast majority of H^+ secretion obviously occurs in conjunction with HCO_3^+ reclamation in the proximal tubule.

Bicarbonate absorption is dependent on H^+ secretion across the apical membrane of the proximal tubule in exchange for Na^+ entry into the cell via the Na^+/H^+ exchanger (NHE-3; see Chapter 6).[7,8] A low intracellular Na^+ concentration is maintained by the active extrusion of Na^+ across the basolateral membrane via the Na^+,K^+-ATPase. The enzyme carbonic anhydrase present in the cytoplasm (type II) and on the apical and basolateral membrane (type IV) is critical to accelerate the reaction as indicated here:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O \quad (3)$$

The active secretion of H^+ into HCO_3^--rich glomerular filtrate by the NHE-3 results in the formation of H_2CO_3. Luminal carbonic anhydrase (type IV) facilitates the conversion of H_2CO_3 to CO_2 and H_2O. CO_2 freely diffuses through the luminal membrane and, under the influence of cytoplasmic carbonic anhydrase (type II), forms H_2CO_3 that dissociates rapidly to H^+ and HCO_3^-, which are transported, respectively, across the apical and basolateral membranes. Bicarbonate exits the cell via the electrogenic Na^+-3 HCO_3^- symporter (NBCe1; see Chapter 6). The negative cell potential is the primary driving force for this transport process. In addition to H^+ secretion via the Na^+/H^+ exchanger, an apical H^+-ATPase is also responsible for a small but significant fraction of bicarbonate reclamation in the proximal tubule.

Other Proximal Tubular Functions

In addition to its role in H^+ secretion and HCO_3^- absorption, the proximal tubule is the primary site for glucose, amino acid, phosphate, and organic anion reclamation. Each of these solutes is transported across the apical membrane via an Na^+-cotransport process. Sodium enters the apical membrane down its electrochemical gradient. Low intracellular Na^+ concentrations and the negative intracellular potential are maintained via the basolateral Na^+,K^+ ATPase. Once inside the cell, these solutes are either metabolized or diffuse passively across the basolateral membrane. Citrate is reabsorbed in the proximal tubule in parallel with Na^+- via the NaDC-1 (Na dicarboxylate cotransporter-1).[9] The metabolism of citrate within the cell leads to the generation of HC O_3^-. The presence of citrate in tubular fluid and urine has been shown to be protective in the prevention of calcium oxalate stones and nephrocalcinosis. In the presence of all forms of metabolic acidosis except proximal RTA, citrate is preferentially reabsorbed resulting in hypocitraturia and predisposing patients to nephrolithiasis. In proximal RTA, because this Na^+-coupled transport system is impaired, urinary citrate remains high, even with metabolic acidosis, and nephrolithiasis rarely, if ever, occurs.

Generalized and Isolated Proximal Tubular Transport Defects

Renal tubular acidosis involving the proximal tubule can be divided into two major categories: generalized disorders of proximal tubule reabsorption and isolated abnormalities in renal acidification (Table 18.5). Those potential abnormalities that have been documented to date include, in order of frequency, genetic defects in the basolateral Na^+-3HCO_3^-

18.5 Etiology of Proximal Renal Tubular Acidosis (Type 2) with or without Fanconi Syndrome

1. Primary disorders
   - Inherited—isolated pure bicarbonate wasting
     - Autosomal recessive: Mutations of NBCe1/SLC4A4 (several examples associated with ocular abnormalities)
     - Autosomal dominant: Mutation of NHE-3 with short stature (defect not determined)
   - Familial disorders associated with proximal RTA
     - Cystinosis
     - Tyrosinemia
     - Hereditary fructose intolerance
     - Galactosemia
     - Glycogen storage disease (type 1)
     - Wilson disease
     - Lowe syndrome
2. Acquired disorders
   - Multiple myeloma, amyloidosis, light chain nephropathy
   - Chemotherapeutic agents
     - Ifosfamide
   - Carbonic anhydrase inhibitors
     - Topiramate
     - Acetazolamide
     - Sulfamylon
   - Heavy metals
     - Lead, copper, cadmium, mercury
   - Renal transplantation
   - Paroxysmal nocturnal hemoglobinuria
3. Mixed proximal and distal RTA (type 3 RTA)
   - Carbonic anhydrase II deficiency: osteopetrosis and ocular abnormalities (Guibaud-Vainsel syndrome)

RTA, renal tubular acidosis.

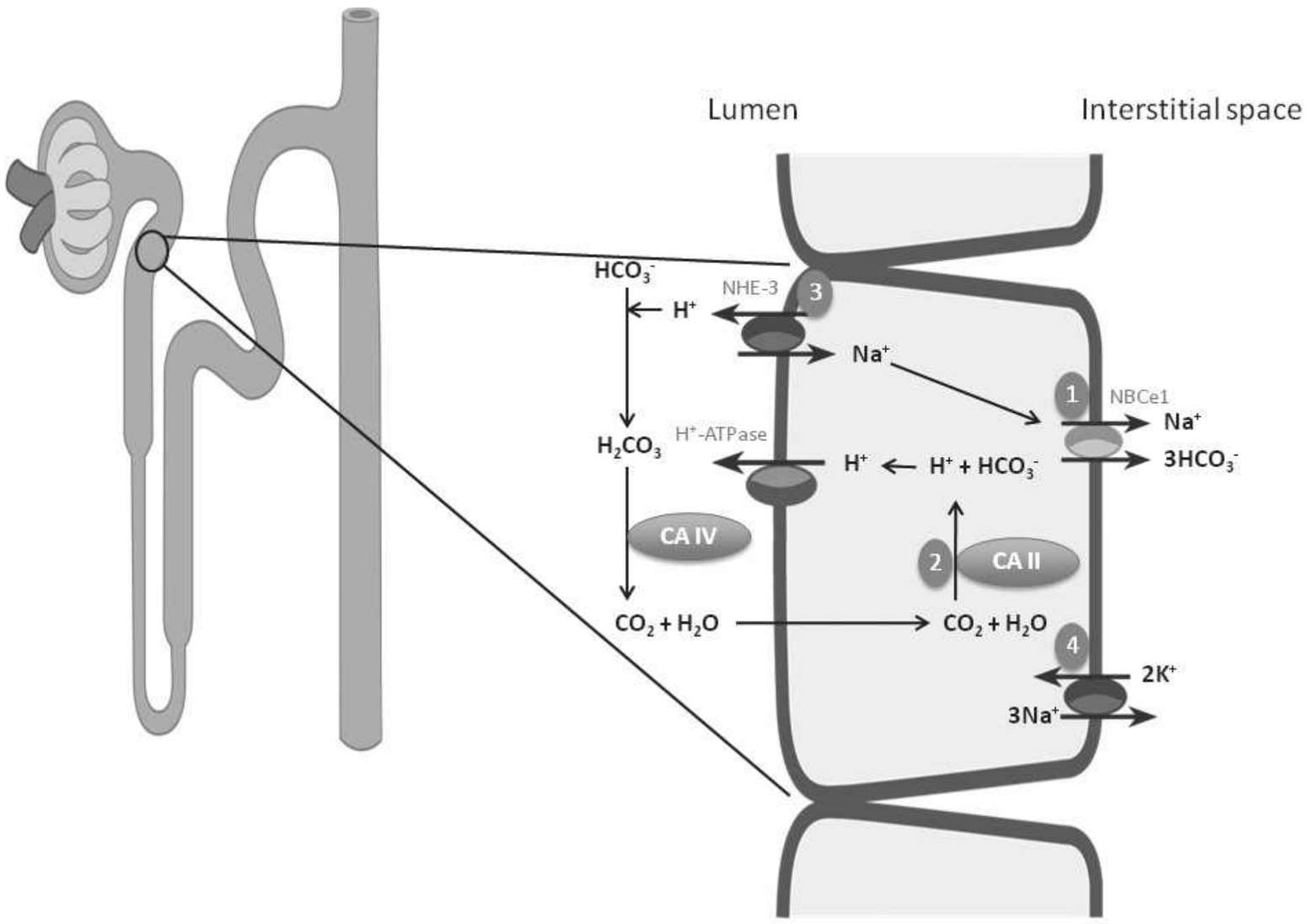

FIGURE 18.1 Pathogenesis of proximal renal tubular acidosis. Model of bicarbonate reabsorption in the proximal tubule showing described inherited defects of genes encoding proximal transport proteins that cause proximal renal tubular acidosis. *1*, defect of basolateral Na^+-HCO_3^- cotransporter; *2*, defect of carbonic anhydrase type 2; *3*, defect of Na^+/H^+ exchanger; 4, defect of Na^+,K^+ ATPase. See text for detail.

symporter or NBCe1, the enzymes carbonic anhydrase type II or IV, and the Na^+/H^+ exchanger (NHE-3) (Fig. 18.1).

Generalized Proximal Tubular Dysfunction

Generalized proximal tubular dysfunction is the more common of the two types of defects, and is appreciated by the co-occurrence of renal tubular acidosis (type 2 RTA), glycosuria, aminoaciduria, phosphaturia, and hypercitraturia. This constellation of symptoms is referred to collectively as "Fanconi" syndrome, which can be either hereditary or acquired. Excessive urinary excretion of glucose occurs, although plasma glucose concentration is normal, and is usually less than 10 g per day. A generalized aminoaciduria also occurs, but because it usually does not result in deficiencies, supplementation is not needed. Sodium and potassium losses occur, which may be massive, resulting in severe secondary hyperaldosteronism and even metabolic alkalosis.

The onset of Fanconi syndrome following an exposure to a triggering agent can vary widely, from minutes (as in the case of patients with hereditary fructose intolerance exposed to fructose[10]), to a few days (in galactosemic patients exposed to galactose[11,12]), to years (following exposure to cadmium[13]). Patients with hereditary fructose intolerance lack the enzyme fructose-1-aldolase, which results in sequestration of intracellular phosphate and is associated with ATP depletion.

Pathophysiologic Mechanisms of Fanconi Syndrome. Most studies suggest that the generalized defect and the defect in transcellular HCO_3^- absorption are due to depletion of intracellular ATP, with inhibition of the Na^+,K^+-ATPase (Fig. 18.1, number 4). Disruption of active HCO_3^-, amino acid, and solute absorption in the proximal tubule due to ATP depletion and inhibition of Na^+,K^+ ATPase has also been observed in an experimental model of cystinosis.[14] Generalized dysfunction of the proximal tubule could occur through three possible mechanisms: (1) an increase in paracellular permeability resulting in backleak of all reabsorbed solutes into the lumen; (2) a generalized defect in proximal tubule absorption, such as ATP depletion; and (3) a defect in basolateral Na^+,K^+-ATPase activity. The second mechanism is the most widely accepted to date.

Regarding paracellular permeability, enhanced efflux causes increased urinary electrolyte and solute excretion, as shown in studies using the maleic acid model (see below). Both mechanisms have been demonstrated in some studies using the maleic acid model of proximal renal tubule acidosis.[15,16] A generalized defect in proximal tubule absorption may be related to the brush-border membrane, such as an abnormality in the sodium-binding domain of the multiple heterogeneous carriers. Alternatively, there may be an abnormality in the way the different carriers are moved to the brush-border membrane. Finally, defects in basolateral Na^+,K^+-ATPase activity can result in abnormal energy generation, as Na^+,K^+-ATPase fuels transport in the proximal nephron. Hereditary fructose intolerance, galactosemia, and cadmium poisoning may result in Fanconi syndrome by reducing Na^+,K^+-ATPase activity. Thus, Fanconi syndrome may be best described as a defect in energy generation in the proximal tubule, with the most important cause being deficient Na^+,K^+-ATPase activity in the basolateral membrane, which decreases sodium-coupled reabsorption due to alterations in the sodium gradient across the luminal membrane. Most of the solutes lost in Fanconi syndrome are those

coupled to apical sodium reabsorption. Fanconi syndrome is also associated with distal nephron dysfunction in some patients. The mechanisms involved are not known, but the evidence suggests that there may be a defect in Na^+,K^+-ATPase throughout the nephron.

Experimental Models of Fanconi Syndrome. Maleic acid is a toxin, relatively specific for the proximal tubule, which results in the best characterized model of Fanconi syndrome. Animals (such as rodents) injected with maleic acid develop proximal RTA and exhibit symptoms similar to those seen in humans with Fanconi syndrome, including decreased activity and expression of Na^+,K^+-ATPase[16] and bicarbonaturia. Maleic acid given to rats also impairs vitamin D conversion.[17]

Because ifosfamide can cause Fanconi syndrome in human patients, it has been used to induce Fanconi syndrome in animals as well. However, the use of this agent in animals is mainly to determine if other agents given with ifosfamide can block the development of Fanconi syndrome without affecting ifosfamide's antitumor activity.[18–22] Heavy metals can also induce Fanconi syndrome in animals and humans. Cadmium, uranium, lead, and mercury all induce Fanconi syndrome in animals, although because cadmium-induced Fanconi syndrome reverses after cadmium administration ceases and because uranium and mercury affect GFR (which is unaffected in people with the syndrome), the study of heavy metal–induced Fanconi syndrome may not necessarily provide accurate information about mechanisms underlying the human disorder.

Dent Disease. Dent disease is a disorder of the proximal renal tubule caused by an X-linked genetic mutation in the CLCN5 gene. Symptoms include proteinuria, nephrocalcinosis, hypercalciuria, and slow progression of renal failure. Because it can also include phosphaturia, aminoaciduria, glycosuria, and rickets, it may be considered a form of Fanconi syndrome. Treatment for patients with Dent disease usually consists of vitamin D to manage rickets and recommendations to reduce hypercalciuria (e.g., thiazide diuretics and citrate supplementation) in order to limit or prevent progression of nephrocalcinosis.[23]

Isolated Proximal Tubule Bicarbonate Transport Abnormalities

Isolated abnormalities of proximal tubular renal acidification in the absence of Fanconi syndrome are less common, but may be associated with depolarization abnormalities or genetic mutations. One model, the infusion of L-lysine in dogs, results in marked bicarbonaturia due to inhibition of HCO_3^- absorption.[24] The presence of luminal L-lysine has been shown to depolarize proximal tubular cells, which could alkalinize the cell by decreasing HCO_3^- extrusion across the basolateral membrane. Mice, in which the gene encoding the renal Na^+/H^+ exchanger (NHE-3) has been knocked out, have metabolic acidosis with proximal RTA[25] (Fig. 18.1, number 3).

Sly et al.[26] have described a group of patients with inherited carbonic anhydrase II deficiency (Fig. 18.1, number 2). These patients develop osteopetrosis, cerebral calcification, and combined proximal and distal RTA.[26] This observation is not unexpected considering the role carbonic anhydrase plays in HCO_3^- reclamation.

Clinical Features of Proximal RTA

Patients with proximal renal tubular acidosis commonly present with hyperchloremic metabolic acidosis, an acid urine pH ($pH<5$) (when systemic acidosis prevails), and minimal HCO_3^- excretion. As bicarbonate is administered to correct the metabolic acidosis, bicarbonaturia occurs and the fractional excretion of HCO_3^- often exceeds 10% to 15% (bicarbonate wasting). This response to alkali therapy, the ensuing increase in potassium excretion in response to the bicarbonate leak into the distal tubule, and the difficulty with which the plasma bicarbonate is corrected are unique features of proximal RTA. Sebastian et al. demonstrated that the level of K^+ excretion correlates directly with HCO_3^- excretion.[27] Decreased NaCl absorption associated with proximal tubular dysfunction enhances K^+ excretion by increasing delivery of Na^+ to the distal nephron and the increase in aldosterone elaboration in response to volume depletion.

The most common causes of proximal RTA in children are acquired either from the administration and toxicity of ifosfamide or from cystinosis.[28,29] Most children present with Fanconi syndrome, but proximal RTA can be limited to an isolated impairment in proximal bicarbonate reabsorption. In contrast, the most common cause in adults is from multiple myeloma or light chain disease. The proximal tubular toxicity related to increased excretion of monoclonal immunoglobulin light chains in patients with multiple myeloma appear to induce a unique biochemical toxicity because of resistance to degradation by lysosomal proteases in proximal tubular cells.[30,31] Accumulation of the variable domain fragments is presumably responsible for the impairment in tubular function. Other causes of proximal RTA that lead to isolated bicarbonate wasting include acetazolamide or the administration of any carbonic anhydrase inhibitor. The most common offending agent currently is topiramate, which is a potent carbonic anhydrase inhibitor.

The majority of cases of proximal RTA are associated with generalized proximal tubule dysfunction (Fanconi syndrome) so that glycosuria, aminoaciduria, proteinuria, hyperphosphaturia, hypophosphatemia, hyperuricosuria, hypouricemia, and hypercitraturia are observed commonly. Table 18.5 lists several familial disorders that may be associated with proximal RTA that result when the abnormal product of metabolism impacts proximal tubule function. Hypercalciuria, a common feature of metabolic acidosis, is absent in

proximal RTA, presumably as a result of enhanced calcium absorption in the distal nephron in response to increased bicarbonate delivery. Rickets, a frequent manifestation of the Fanconi syndrome, is a result of phosphate wasting, not proximal RTA or acidosis. Of particular concern in children with proximal RTA is growth retardation, a direct consequence of acidosis. Because growth retardation will correct with alkali therapy, this complication becomes one of the major indications for correction of the serum bicarbonate concentration.

Isolated proximal RTA without features of the Fanconi syndrome can occur rarely in an autosomal recessive disorder affecting the gene SLC4A4 that encodes for the sodium bicarbonate cotransporter. In addition, a defect in the gene that encodes the Na^+/H^+ exchanger (NHE-3) on the apical membrane has been described in a single family as an autosomal dominant disease.

Management

The primary therapeutic objective in the management of patients with proximal renal tubular acidosis is to maintain a near normal serum HCO_3^- concentration and arterial pH. The bicarbonaturia associated with this disorder, which amplifies potassium excretion, requires administration of a mixture of sodium and potassium salts (e.g., K-Shohl's solution) (Table 18.6). A feature of proximal renal tubular acidosis is the large amount of HCO_3^- required to correct the acidosis, which, in turn, aggravates renal potassium excretion. As an adjunct, the administration of thiazide diuretics has been used to decrease GFR from chronic volume depletion. Sequelae of proximal RTA vary according to cause (generalized vs. isolated). Nevertheless, in children with isolated proximal RTA, stunted growth is normalized by correction of the acidosis. Additionally, the manifestations of isolated proximal RTA in children tend to improve with age, but alkali therapy is usually necessary throughout life.

18.6 Treatment of Proximal Renal Tubular Acidosis

Large amounts of alkali required to correct acidosis:
- 10–20 mEq/kg/day of alkali (typically enhances urinary K loss)

Preparations that include potassium:
- Potassium Shohl's Solution (K-Shohl's:
  - Polycitra-LC: Citric acid 334 mg, sodium citrate 500 mg, and potassium citrate 550 mg per 5 mL [480 mL] [alcohol free, sugar-free])

Thiazides (may be helpful)

DISTAL RENAL TUBULAR ACIDOSIS

Mechanism and Regulation of Distal Acidification (see also Chapter 6)

The role of the distal nephron in maintaining acid-base homeostasis occurs through HCO_3^- absorption and net acid secretion. As discussed previously, the proximal tubule absorbs approximately 90% of the filtered HCO_3^- load, with the distal nephron absorbing the remaining 10%. In addition, the distal nephron is responsible for secreting daily approximately 50 to 80 mEq of hydrogen ions, which matches daily net acid production from metabolism. Thus, excretion of net acid stoichiometrically replaces the bicarbonate lost in extracellular buffering of those acids gained from metabolism of dietary protein. Proton secretion in the distal nephron generates large pH gradients between blood and the lumen. The kidney utilizes an elaborate buffering system to avoid unsustainably large transepithelial H^+ concentration gradients and the ensuing tubular toxicity associated with the expected local acidity which would be necessary to secrete 50 to 80 mEq of H^+ per day without buffering. This buffering system is divided into two components: (1) ammonium and (2) titratable acids. Titratable acids in urine include phosphate, creatinine, and other miscellaneous buffers. Total net acid excretion (NAE) is represented by the sum of titratable acid (TA) and ammonium (NH_4^+) excretion (minus minimal HCO_3^- excretion, if any). Therefore, to maintain acid-base balance, net acid excretion must approximate net acid production.

The Pathophysiologic Basis of Classical Distal Renal Tubular Acidosis

Anatomic and Physiologic Segregation of the Collecting Duct

The collecting duct can be divided into three functional segments: the cortical collecting tubule (CCT), the outer medullary collecting tubule (OMCT), and the inner medullary collecting duct (IMCD). The CCT is a low capacity H^+ secretory segment where the rate of H^+ secretion is modulated by aldosterone, Na^+ and K^+ absorption, and systemic acid-base balance. The CCT has the capacity for both H^+ and HCO_3^- secretion.[32–35] The former function is accomplished by type A intercalated cells, and the latter by type B intercalated cells (Fig. 18.2). The OMCT, in contrast, has a high capacity for H^+ secretion, which is regulated by systemic acid-base homeostasis, the serum K^+ concentration, and aldosterone.[36] Finally, the IMCD is a low capacity proton secretory system. In this segment ammonium transport is regulated by acid-base homeostasis and the serum K^+ concentration.

In each segment of the distal nephron, HCO_3^- absorption is mediated by apical membrane H^+ secretion.[37,38] Because of the negative cell potential, H^+ secretion must occur by an active transport.[39–41] Two ATP-dependent proton pumps, the H^+-ATPase and the H^+,K^+ATPase, have been identified

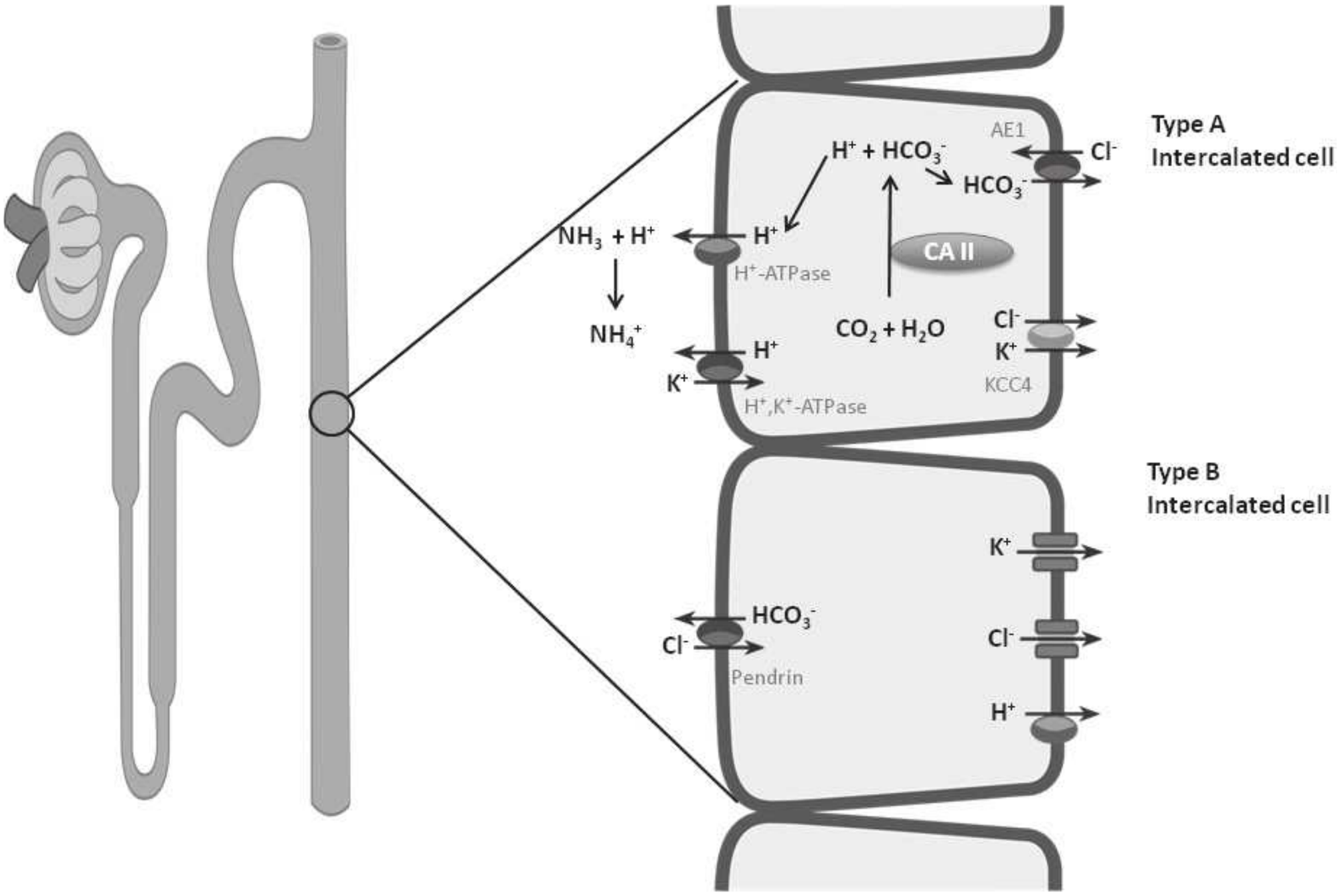

FIGURE 18.2 Type A and B intercalated cells of collecting duct. See text for detail.

in the distal nephron and together are responsible for H^+ secretion[42] (Fig. 18.2). Immunohistochemical studies have localized the H^+-ATPase to the apical membrane of acid secreting cells (type A intercalated cells) in the CCT and OMCT. Both the gastric and colonic H^+,K^+-ATPase subunits are expressed in intercalated cells of the cortical collecting duct and outer medullary collecting duct.[42] These two isoforms of H^+,K^+-ATPase have been designated as the $HK\alpha_1$ ("gastric") and $HK\alpha_2$ ("colonic") subunits. $HK\alpha_1$ is identical to the H^+,K^+-ATPase in gastric parietal cells, whereas $HK\alpha_2$ is homologous to the H^+,K^+-ATPase in distal colon. Several studies have demonstrated that $HK\alpha_2$ mRNA and protein (but not $HK\alpha_1$) are dramatically upregulated by chronic hypokalemia and chronic acidosis.[43] Furthermore, increased H^+,K^+-ATPase activity in the outer and inner medullary collecting duct results in enhanced HCO_3^- absorption.[44]

Apical proton secretion generates HCO_3^- intracellularly, which then exits the cell via the Cl^-/HCO_3^- exchanger (AE-1) present on the basolateral membrane (encoded by the gene SLC4A1).[45] Thus, these three transporters, the apical H^+-ATPase and H^+,K^+-ATPase, and the basolateral HCO_3^-/Cl^- exchanger could be involved, if defective, in the development of an acidification defect in the distal nephron (Fig. 18.3). The reader is referred to Chapter 6 for additional detail. Examples of genetic and acquired abnormalities of the H^+-ATPase and AE-1 have been described and are discussed later.

Additionally, defective net H^+ secretion could occur by the insertion of a "leak" pathway for H^+ into the collecting duct (Fig. 18.3). This abnormality, also referred to as a "gradient lesion," occurs most commonly with amphotericin B nephrotoxicity. Whether this latter abnormality accounts for acidification defects in other forms of inherited or acquired distal RTA has been described in case reports, but has not been established clearly.

Ammonium Production and Excretion

Although ammonium is secreted in several segments of the nephron, the majority of ammonium secretion occurs in the proximal tubule and is regulated by acid-base homeostasis (Fig. 18.4). Ammonium transport involves both ammonia (NH_3) diffusion and ammonium (NH_4^+) transport. NH_4^+ secretion into the proximal tubule lumen occurs via the apical membrane Na^+/H^+ exchanger (NHE-3) through substitution of NH_4^+ for H^+. Ammonium secretion is augmented dramatically by systemic metabolic acidosis. At physiologic pH, α ketoglutarate, a major metabolic product of ammoniagenesis, is converted to HCO_3^- ions, which are transported across the basolateral membrane to the extracellular fluid (ECF). This end product of ammoniagenesis therefore represents "new bicarbonate" when returned to systemic circulation via the renal vein. As mentioned previously, and

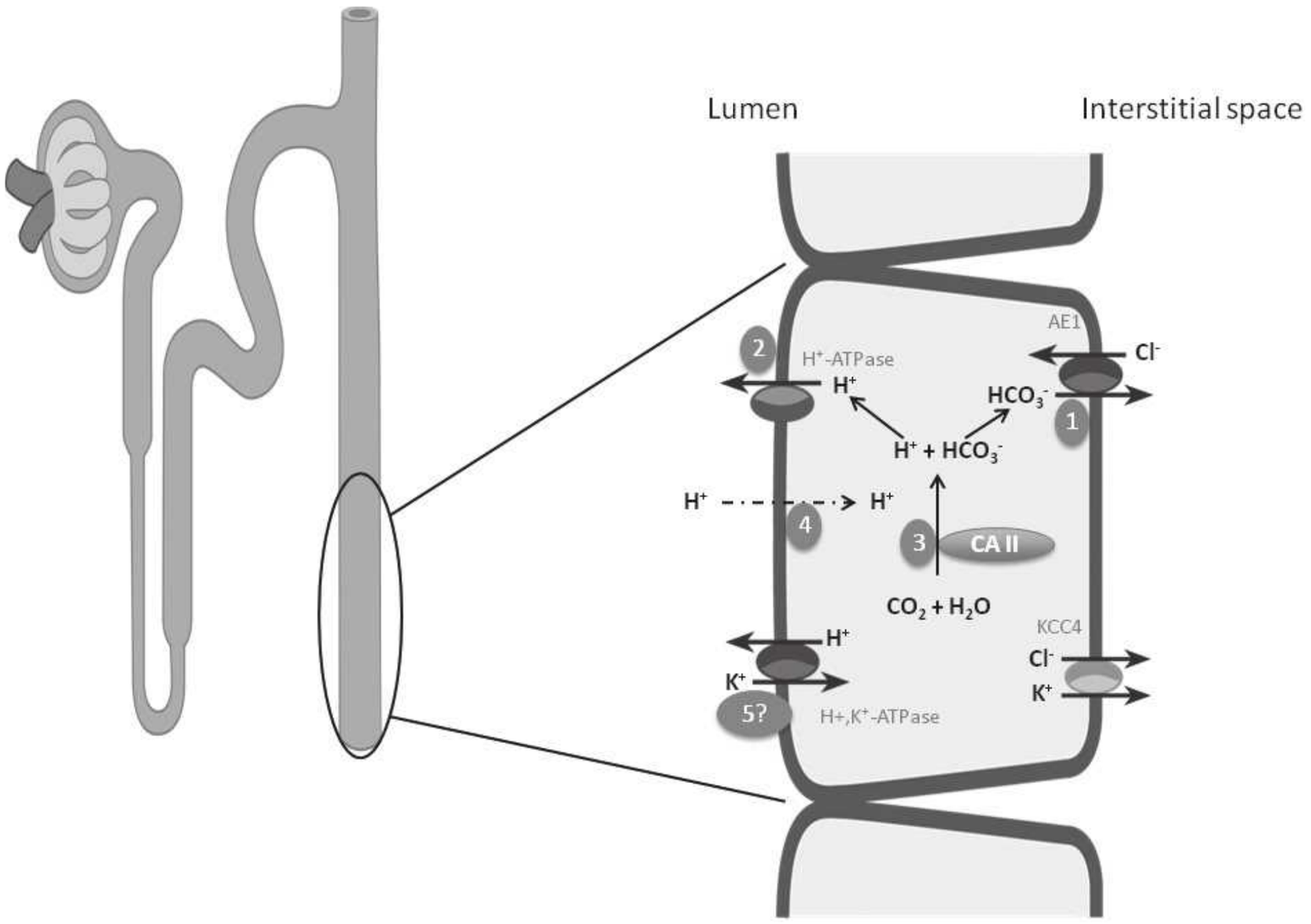

FIGURE 18.3 Pathogenesis of distal renal tubular acidosis (RTA). Model of type A intercalated cell in medullary collecting duct showing described inherited defects encoding distal transport proteins that cause classical distal RTA. *1*, defect of basolateral HCO_3^-/Cl^- exchanger; *2*, defect of specific subunits of H^+-ATPase; *3*, carbonic anhydrase II deficiency; *4*, backleak of H^+ or gradient lesion (amphotericin B and presumed rare inherited abnormalities); *5*, abnormality of H^+,K^+-ATPase (not verified, but may explain endemic [Northeastern Thailand] distal RTA with severe hypokalemia).

discussed in detail in Chapter 6, "new bicarbonate" restores the HCO_3^- lost in the ECF from buffering the acid products of metabolism.

After ammonium enters the proximal tubule lumen, an elaborate system exists to generate high medullary interstitial concentrations of ammonium[46] (Fig. 18.5). First, the HCO_3^- concentration and pH of tubular fluid increases progressively along the thin descending limb of the loop of Henle as a result of water abstraction.[47] This alkaline environment favors NH_3 diffusion out of the tubule lumen. In addition, direct uptake of NH_4^+ is accomplished via the apical Na^+-$2Cl^-$-K^+ cotransporter (through competition for the K^+ site) in the medullary thick ascending limb of the loop of Henle (TALH).[46] Ammonium absorption at this site is stimulated by acidosis and hypokalemia and is impaired by hyperkalemia.[48–52] NH_3 is capable of reentering the proximal straight tubule from the interstitium.[46] Active absorption of NH_4^+ in the TALH allows for trapping of NH_4^+ in the medullary countercurrent multiplication system.[53] The end result of this system is a medullary-to-cortical concentration gradient for ammonium with medullary concentrations exceeding cortical concentrations severalfold. This corticomedullary ammonium gradient is augmented by metabolic acidosis.[50] Ammonium is trapped in the medullary collecting duct by a combination of NH_3 diffusion from the interstitium and active H^+ secretion by the medullary collecting duct (H^+-ATPase and the H^+-K^+-ATPase).[50,54] This process generates high concentrations of ammonium in the final urine. Because NH_4^+ uptake by the TALH is accomplished by the Na^+-K^+-$2Cl^-$ cotransporter, competition between K^+ and NH_4^+ helps explain the association between hyperkalemia and metabolic acidosis.[55] Additional detail on ammonia/ammonium transporters and their regulation is provided in Chapter 6.

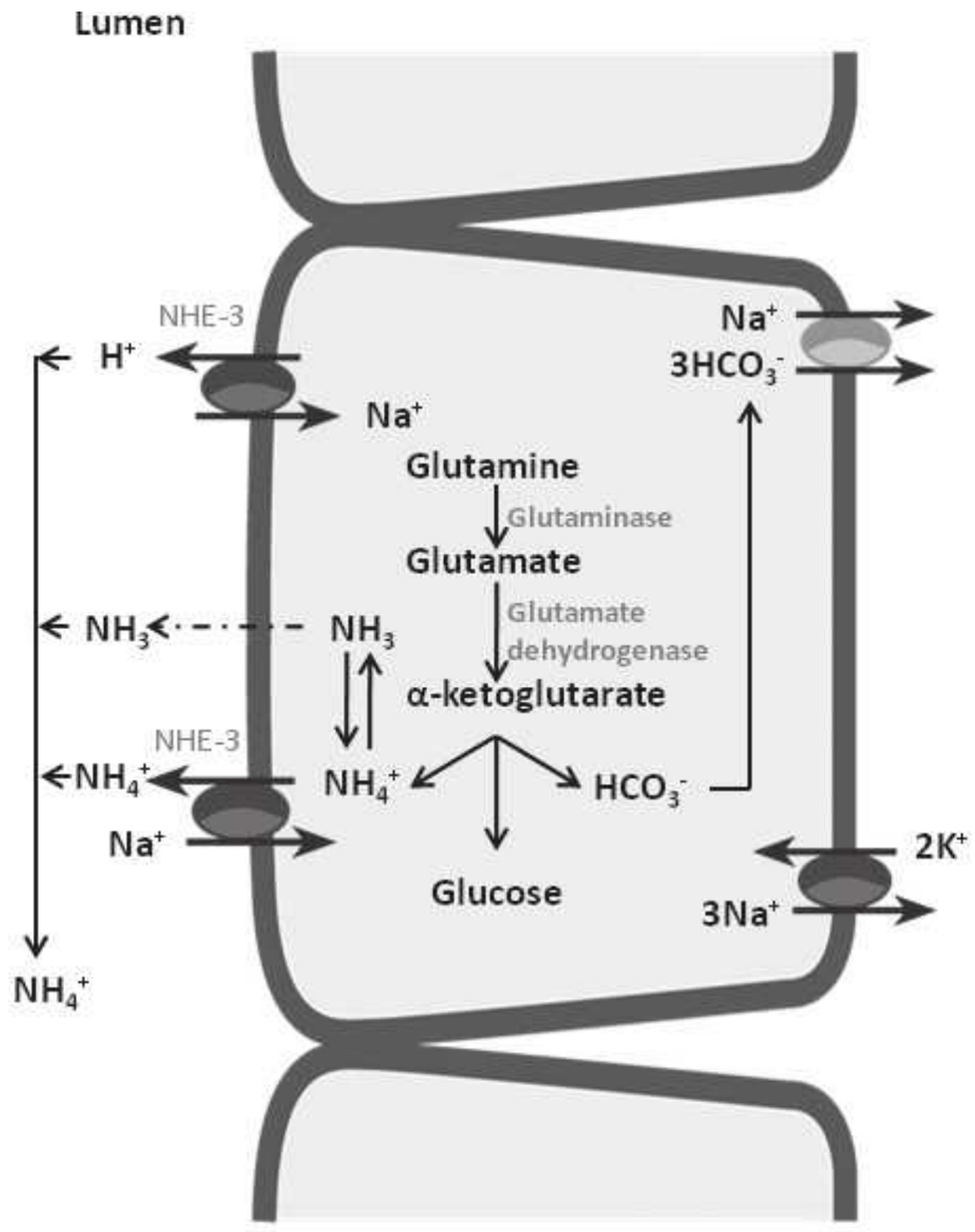

FIGURE 18.4 Ammoniagenesis and transport of ammonia/ammonium in the proximal tubule.

Regulation of Distal Acidification

Apical proton secretion and basolateral HCO_3^- transport together and regulate net HCO_3^- absorption in the distal nephron. The responsible transporters include the

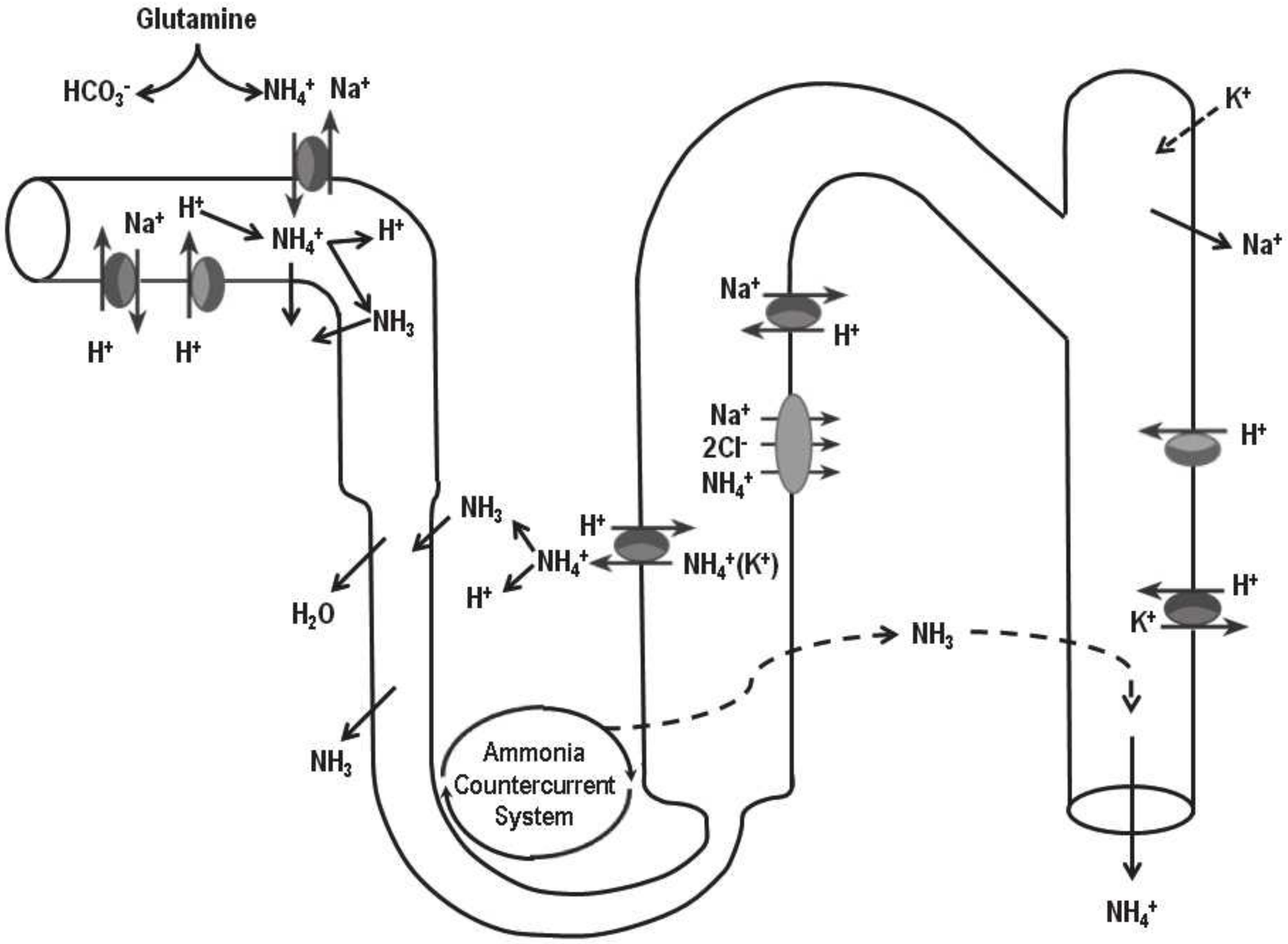

FIGURE 18.5 Summary of ammonia/ammonium transport pathways in the nephron. Possible defective ammoniagenesis and/or ammonium transport associated with distal renal tubular acidosis, discussed in text in detail.

electrogenic H^+-ATPase, the electroneutral H^+,K^+-ATPase on the apical membrane, and the HCO_3^-/Cl^- exchanger on the basolateral membrane (Fig. 18.6). Alteration in the negative transepithelial potential difference, which is dependent on the rate of Na^+ absorption, has a significant secondary impact on proton secretion by the electrogenic H^+-ATPase. Thus, a decline in Na^+ delivery or Na^+ acidity through either impairment of epithelial Na^+ channel (ENaC) function or through absence of mineralocorticoid will secondarily impair H^+ secretion. A defect in H^+ secretion in the CCT in response to a decline in Na^+-transport dependent transepithelial voltage has been termed a "voltage defect." Mineralocorticoids have been demonstrated to be a potent determinant of proton secretion. In the CCT, mineralocorticoids stimulate Na^+ absorption (ENaC) increasing the lumen negative transepithelial potential, which stimulates electrogenic proton secretion secondarily.[56] This early effect of aldosterone on ENaC is reinforced after several hours to upregulate the basolateral Na^+,K^+-ATPase as well. Taken together, mineralocorticoid increases the negative transepithelial potential, thus enhancing Na^+ absorption. Mineralocorticoids have also been shown to stimulate the H^+-ATPase in the cortical, outer, and inner medullary collecting tubules in the absence of Na^+.[40,57] Thus, in summary, both mineralocorticoids and Na^+ absorption in the CCT have important regulatory effects on net H^+ secretion in the collecting duct.

Potassium homeostasis also plays a significant role in the regulation of renal acidification. Clearance studies have suggested that potassium deficiency stimulates distal proton secretion. It has now been established that this regulatory response occurs, at least in part, through upregulation of the H^+,K^+-ATPase. Potassium status can also affect renal acidification indirectly. First, potassium is an important determinant of aldosterone, and as discussed previously, aldosterone is an important determinant of H^+ secretion. Potassium also affects ammonium synthesis and excretion.[52] Chronic hypokalemia stimulates ammonium production while hyperkalemia suppresses ammoniagenesis.[48] Alterations in ammonium production may also affect the medullary interstitial gradient and buffer availability. Hyperkalemia impairs ammonium absorption in the thick ascending limb, also decreasing medullary concentrations of total ammonia and secretion of NH_3 into the medullary collecting duct.[48,49,58]

Pathogenesis of Distal Renal Tubular Acidosis

Classical Hypokalemic Distal Renal Tubular Acidosis. The mechanisms involved in the pathogenesis of hypokalemic distal RTA (DRTA) are not yet completely resolved.

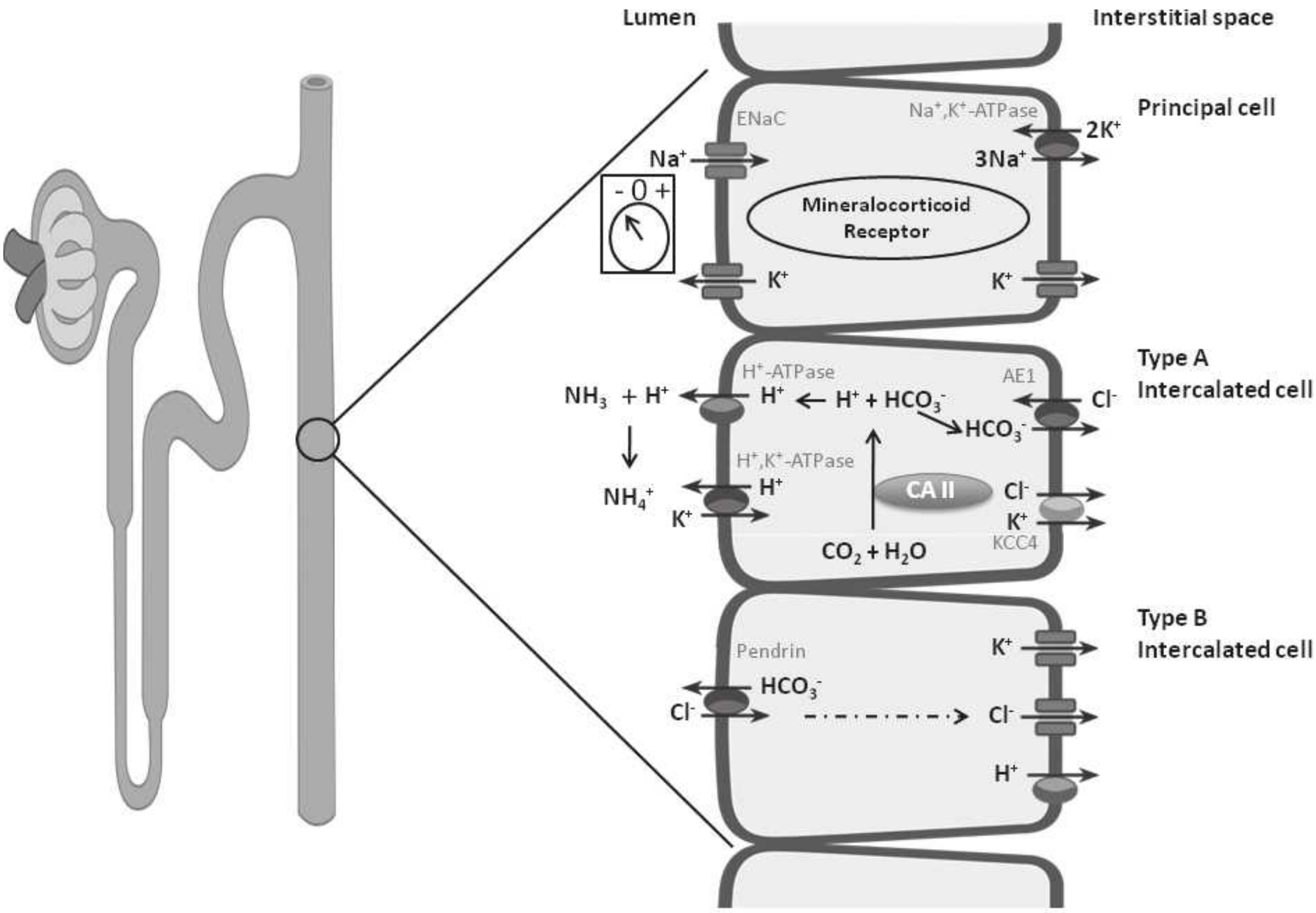

FIGURE 18.6 Three cell types in the collecting tubule: principal cell, and type A and type B intercalated cell. Mechanisms of transport discussed in text.

The occurrence of hypokalemia demonstrates that generalized CCT dysfunction or aldosterone deficiency is not causative. Initially the cause of classical hypokalemic DRTA was considered to be a "gradient lesion." Alternatively, a defect in proton secretion is the most widely accepted explanation for the inability to maximally acidify the urine,[59] in the majority of forms of classical DRTA (Fig. 18.3). The most notable exception of this mechanism is the defect induced by amphotericin B nephrotoxicity, for which insertion of a leak pathway in the apical membrane of the distal tubule by the antibiotic is causative (Fig. 18.3, number 4). An important feature in the determination of the pathogenesis of the acidification defect in these patients is the response of the urine PCO_2 to $NaHCO_3$ infusion. Infusion of $NaHCO_3$ to produce a high HCO_3^- excretion rate results normally in distal nephron hydrogen secretion and the generation of a high CO_2 tension in the urine. The magnitude of the urinary PCO_2 (referred to as the urine-minus-blood PCO_2, or U-B PCO_2) is quantitatively related to distal nephron hydrogen ion secretion.[38,60] A decrease in the rate of hydrogen ion secretion as a result of a defect of one of the H^+ transporters on the apical membrane (H^+- or H^+,K^+-ATPase) or the basolateral HCO_3^-/Cl^- exchanger will lead to a low U-B PCO_2. In contrast, a backleak of H^+, as occurs with a "gradient" defect, has been shown in experimental models to be associated with a normal U-B PCO_2. In patients with classical hypokalemic DRTA, the U-B PCO_2 is usually subnormal, except in amphotericin B-induced DRTA.[60–62] This finding supports the view that most patients with DRTA have a "rate" or "pump" defect (Fig. 18.3, number 1).

H^+-Secretory Defects. The rate of proton secretion could be affected by an abnormality in a specific transporter or mechanism involved in proton secretion. These include the apical H^+-ATPase or H^+-K^+-ATPase, and the basolateral Cl^-/HCO_3^- exchanger, AE-1 (Fig. 18.3, number 2). Impairment of the H^+-ATPase in classical DRTA has been documented in both acquired and inherited disorders. Acquired defects of H^+-ATPase have been demonstrated in renal biopsy specimens of patients with Sjögren syndrome with evidence of classical hypokalemic DRTA. These biopsy specimens revealed trapping of this transporter in intracellular compartments and an absence of H^+-ATPase protein in the apical membrane of type A cells. To further underscore the importance of H^+-ATPase in classical RTA, several

investigators have described autosomal dominant mutations in the ATP6V1B1 gene encoding the B-subunit of the H^+-ATPase in the kidney and cochlea, and these mutations are associated with sensorineural deafness. Another form of classical DRTA, inherited as an autosomal recessive defect, is associated with normal hearing and has been shown recently to be a mutation in the ATP6V0A4 gene that encodes for the A-subunit of this transporter.[63–65]

Alternatively, although abnormalities in the H^+,K^+-ATPase (Fig. 18.3) could result in both hypokalemia and metabolic acidosis, a defect in this transporter per se has not been described to date. An unusually high incidence of hypokalemic DRTA (endemic RTA) in Thailand may be the result of chronic hypokalemia and chronic tubulointerstitial disease, which could, in term, cause the acidification defect.[66] It would be logical to assume a role for chronic hypokalemia and subsequent tubulointerstitial disease in the development of some forms of classical DRTA through compromise of the H^+,K^+-ATPase.

Defects in the HCO_3^-/Cl^- Exchanger. Three groups have independently demonstrated an association between mutations in the AE-1 gene, which encodes the basolateral HCO_3^-/Cl^- exchanger (AE-1) in the collecting duct, and the occurrence of autosomal dominant classical DRTA (Fig. 18.3, number 3).[67–69] The typical clinical manifestations of classical DRTA were associated with heterozygosity for the AE1 point mutation, G1766A.[67–69] This mutation encodes an amino acid substitution, R598H, located at the cytoplasmic end of the AE1 protein in transmembrane span 6. Surprisingly, however, when these point mutations were expressed in vitro, abnormalities in HCO_3^-/Cl^- exchange were not observed. It was hypothesized that misdirection of the HCO_3^-/Cl^- exchanger to the apical, rather than the basolateral membrane might obtain in this disorder, resulting in impaired net H^+ secretion. If correct, enhanced HCO_3^-/Cl^- exchange on the apical membrane would increase rather than decrease urinary PCO_2 during bicarbonate infusion. That an increase in the U-B PCO_2 was demonstrated in patients with inherited classical DRTA supports this view.[70]

Therefore, in summary, genetic evidence exists for abnormalities in two subunits of the H^+-ATPase and misdirection of the HCO_3^-/Cl^- exchanger to the apical membrane as causes of inherited classical DRTA. Another rare mechanism for inherited DRTA includes carbonic anhydrase II deficiency which is associated with osteopetrosis and mental retardation. The diverse pathophysiology of the various inherited and acquired forms of classical DRTA are outlined in Table 18.7.

Impaired NH_4^+ Excretion

Patients with abnormalities in hydrogen ion secretion by the collecting duct in classical DRTA also exhibit uniformly low excretory rates of ammonium.[6,62] In the face of hyperchloremic metabolic acidosis, low ammonium excretion demonstrates that the kidney causes or perpetuates metabolic acidosis. Defective ammonium excretion may occur due to an inability to trap ammonium in the medullary collecting duct as a result of a higher than normal tubular fluid pH, as obtained when H^+ secretion is impaired (e.g., a defect in H^+-ATPase, H^+,K^+-ATPase, or the basolateral HCO_3^-/Cl^- exchanger) (Fig. 18.6). Moreover, urinary concentrating defects from the medullary interstitial disease is common in patients with classical DRTA, and may interfere with the medullary countercurrent multiplier and reduce the corticomedullary concentration gradient for ammonium and thereby reduce ammonium excretion.[50] Although the RhCG channel has been described in the distal nephron and has been shown to be important for ammonia/ammonium transport (see Chapter 6), there is no evidence to date that this transporter is involved in any form of RTA. Since classical DRTA is associated with hypokalemia, it would be anticipated that ammonium production and excretion would be enhanced. Nevertheless, ammonium excretion remains low for the prevailing systemic acidosis and urine pH, most likely as a result of failure of the corticomedullary countercurrent system.

Clinical Phenotype of Classical Distal Renal Tubular Acidosis

Classical hypokalemic DRTA is associated with positive acid balance, a nongap or hyperchloremic metabolic acidosis, and volume depletion with evidence of abnormal urine acidification for the prevailing acidosis (spontaneous or induced). Hyperchloremia is augmented by volume depletion. The positive acid balance and progressive acidosis causes calcium, magnesium, and phosphate wasting and may culminate in metabolic bone disease. Stunted growth is common in prepubertal children. A frequent association is nephrocalcinosis and nephrolithiasis, often with interstitial nephritis with susceptibility to pyelonephritis, which is difficult to eradicate. Both the hypercalciuria associated with DRTA and the hypocitraturia, which accompanies chronic metabolic acidosis, provide a favorable environment for urinary stone formation, chiefly calcium phosphate, and the development of nephrocalcinosis.[71] Nephrocalcinosis associated with nongap metabolic acidosis strongly implicates classical DRTA, because this does not occur with either proximal RTA or generalized distal nephron dysfunction with hyperkalemia.[72] Extra renal manifestations appear to be dependent, in part, on the gene mutated, and can include osteomalacia, rickets, osteopetrosis, deafness, ocular abnormalities, and cognitive dysfunction. The diagnostic features for classical DRTA are displayed in Table 18.8 and the systemic consequences in Table 18.9.

Classical Distal Renal Tubular Acidosis

Classical DRTA may occur as an autosomal dominant or autosomal recessive inherited defect (primary DRTA) or in association with a systemic illness (secondary DRTA). Overwhelmingly, the majority of patients with classical DRTA have secondary DRTA. The different etiologies of classical DRTA are displayed in Table 18.7.

18.7 Disorders Associated With Classical Hypokalemic Distal Renal Tubular Acidosis

Familial

1. Autosomal dominant
   a. Abnormality of the basolateral HCO_3^-/Cl^- exchanger (AE-1) due to SLC4A1 mutation
2. Autosomal recessive
   a. Deficiency or abnormality of the H^+-ATPase
      Autosomal recessive ATP6V1B1 mutation with deafness
      Autosomal recessive ATP6V0A4 mutation with or without deafness
   b. Carbonic anhydrase II deficiency – mixed PRTA-DRTA

Endemic

Northeastern Thailand

Acquired Defect of the H^+-ATPase

Sjögren syndrome

Secondary to Systemic Disorders

Autoimmune diseases
- Hyperglobulinemic purpura
- Cryoglobulinemia
- Sjögren's syndrome
- Thyroiditis
- HIV nephropathy
- Fibrosing alveolitis
- Chronic active hepatitis
- Primary biliary cirrhosis
- Polyarthritis nodosa

Hypercalciuria and nephrocalcinosis
- Primary hyperparathyroidism
- Hyperthyroidism
- Medullary sponge kidney
- Fabry disease
- X-linked hypophosphatemia
- Vitamin D intoxication
- Idiopathic hypercalciuria
- Wilson disease
- Hereditary fructose intolerance
- Hereditary sensorineural deafness

Drug- and toxin-induced disease
- Amphotericin B
- Mercury
- Vanadate
- Hepatic cirrhosis
- Ifosfamide
- Topiramate
- Cyclamate
- Lithium
- Classic analgesic nephropathy
- Foscarnet
- Acetazolamide

Tubulointerstitial diseases
- Balkan nephropathy
- Chronic pyelonephritis
- Obstructive uropathy
- Vesicoureteral reflux
- Renal transplantation
- Leprosy
- Jejunoileal bypass with hyperoxaluria

Associated with genetically transmitted diseases
- Ehlers-Danlos syndrome
- Sickle cell anemia
- Medullary cystic disease
- Hereditary sensorineural deafness
- Osteopetrosis with carbonic anhydrase II deficiency
- Hereditary elliptocytosis
- Marfan syndrome
- Jejunal bypass with hyperoxaluria
- Carnitine palmitoyltransferase I

PRTA-DRTA, proximal renal tubular acidosis–distal renal tubular acidosis.

18.8 Diagnostic Features of Classical Distal Renal Tubular Acidosis

Hypokalemia
Urine anion gap positive with induced or spontaneous metabolic acidosis
Abnormally low NH_4^+ excretion
Urine pH >5.5
Modest bicarbonaturia, <10% $FE_{HCO_3^-}$ >5%
Absence of Fanconi syndrome
Abnormal calcium metabolism (hypercalciuria, nephrocalcinosis, nephrolithiasis, bone disease)
Low urine citrate
Hyperglobulinemia

Inherited Classical Distal Renal Tubular Acidosis. Over 300 individuals from over 60 families have been identified with primary DRTA,[67,68,73,74] and the specific defects are either mutations of the H^+-ATPase (recessive inheritance), or of kAE1/SLC4A1 (dominant and recessive inheritance).[26,67,68,74] Most patients with inherited DRTA have associated hypercalciuria and hypocitraturia predisposing to nephrocalcinosis and bone disease.[1]

Autosomal Recessive Classical Distal Renal Tubular Acidosis Associated with Mutations of the H^+-ATPase. The features of autosomal recessive DRTA include severe metabolic acidosis, an inappropriately alkaline urine, growth failure, rickets, and renal calcification. This constellation of findings may occur with or without sensorineural deafness (see previous text and Table 18.9). Mutations in genes encoding isoforms of two subunits of the H^+-ATPase subunits include, respectively, the V_1 and V_0 domains. Over 20 mutations have been reported for ATP6V1B1 with deafness, all with a recessive inheritance pattern. Progressive bilateral hearing loss occurs with mutations in ATP6V1B1, encoding the B-subunit of the collecting duct apical electrogenic proton pump (H^+-ATPase) and the H^+-ATPase in the cochlea.[64] Over 28 mutations of ATP6V0A4 that encode the a4 subunit have been reported with variable deafness and are also transmitted as an autosomal recessive inheritance.[65]

Dominant and Recessive Distal Renal Tubular Acidosis with Mutations of kAE1/SLCA1**.** Approximately 20 family members with either recessive or dominant classical DRTA have been reported with mutations of SLC4A1/kAE1.[68,69,75] The normally configured kidney isoform of AE1 (kAE1) is a truncated product of the SLC4A/AE1 gene, whereas, in contrast, selective mutations cause DRTA. Dominant DRTA involving kAE1 result in either abnormal intracellular retention of kAE1, which prevents appropriate targeting, or a reversed polarity of the mutant kAE1. Recessive missense mutations of kAE1 encode an unstable protein that results in a null phenotype, such as severe hemolytic anemia, DRTA, and cardiovascular instability. Others have impaired urinary acidification but normal AE1 in red cells. The mutant kAE1, when expressed in kidney cells in culture, can be rescued with glycophorin A. Genetic models in mice ($AE1^{-/-}$) have severe hemolytic anemia, heart failure, and acidosis associated with growth abnormalities, spherocytosis, and poikilocytosis. Because this defect is often fatal, thorough studies have not been performed, but nephrocalcinosis has been reported.[7]

Accompanying Disorders of Distal Renal Tubular Acidosis: Hypokalemia, Nephrogenic Diabetes Insipidus, and Hypercalciuria (Table 18.9). Although the hypokalemia that frequently accompanies DRTA has been assumed to be secondary aldosteronism, more recent evidence implicates the BK Maxi-K channel (Slo1), which interestingly, exhibits pH sensitivity. Acid extracellular pH, presumably in the renal interstitium, may enhance K secretion.[76] However, there are other pH effects on other Na and K channels that may be offsetting and render this possible mechanism both complex and, as yet, not fully solved.

Calcium phosphate nephrolithiasis and nephrocalcinosis are very common in DRTA. The etiology has not been fully elucidated. The consensus has been that, with acidosis, calcium salts are leached from bone, thereby causing a high filtered load of calcium and thus hypercalciuria. However, pH-dependency of calcium handling by the distal nephron may participate. For example, TRPV5, the major mechanism of calcium reabsorption, is inhibited by an acid intracellular pH, a condition that likely prevails in the medullary interstitium of acidotic patients with DRTA.[77] Further work on this mechanism and a better understanding of the cellular basis of nephrocalcinosis is needed as are more effective therapies

18.9 Consequences of Classical Distal Renal Tubular Acidosis

Progression of chronic kidney disease
Bone disease (result of acidosis, hypercalciuria)
Nephrocalcinosis
Nephrolithiasis
    Hypercalciuria and hypocitraturia
        Acidosis leads to hypocitraturia
Progression of renal failure
Hypokalemia – may be severe
Pyelonephritis – difficult to eradicate
Stunted growth
Nephrogenic diabetes insipidus

for this devastating feature of DRTA. Nephrocalcinosis and interstitial fibrosis are a major cause of progressive kidney disease in these patients.

Endemic Distal Renal Tubular Acidosis. A high incidence of DRTA has been reported in northeast Thailand.[78,79] This disorder has a female to male ratio of 3:1 and patients range in age from 18 to 76 years. Associated features include severe hypokalemia resulting in profound muscle weakness, nephrocalcinosis, renal stones, hypocitraturia, osteomalacia, and nocturia. The hypokalemia and the accompanying features are more severe in the summer months and are most prevalent in impoverished groups whose diet consists almost entirely of rice. Therefore, dietary hypokalemia may be causative. Although the precise mechanism involved remains unclear, chronic tubulointerstitial renal disease as a result of chronic hypokalemia appears to be a better explanation than vanadate toxicity, as initially suggested.[80]

Secondary Distal Renal Tubular Acidosis. The disorders associated with acquired classical DRTA are outlined in Table 18.10. Hyperglobulinemia is commonly associated with acquired classical DRTA. Up to 50% of patients with Sjögren syndrome and hyperglobulinemic purpura eventually exhibit an acidification defect.[81–83] These patients may have profound hypokalemia at times. Other autoimmune and hyperglobulinemic states that are associated with DRTA include cryoglobulinemia, thyroiditis and Graves disease, primary biliary cirrhosis, chronic active hepatitis, systemic lupus erythematosus, and HIV nephropathy.[84–86] The co-occurrence of hypokalemic paralysis, a nongap metabolic acidosis and thyrotoxicosis, indicates DRTA rather than hypokalemic periodic paralysis.

18.10 Classification of Major Disorders Associated with Acquired Classical Distal Renal Tubular Acidosis

- Autoimmune disorders
  - Hyperglobulinemia- autoimmune diseases
    - Sjögren's, thyroiditis, primary biliary cirrhosis
- Hypercalciuria and nephrocalcinosis
  - Hypervitaminosis D, hyperparathyroidism, Graves disease
- Drug- or toxin-induced disease
  - Amphotericin B, ifosfamide, topiramate, lead, lithium, tetracycline, toluene
- Tubulointerstitial diseases
  - Minimal change disease, classical analgesic nephropathy

Abnormalities in calcium homeostasis, particularly when nephrocalcinosis is present, are commonly seen in patients with acquired DRTA. Examples of the co-occurrence of DRTA and nephrocalcinosis include: primary hyperparathyroidism, vitamin D intoxication, medullary sponge kidney, hyperthyroidism, idiopathic hypercalciuria, X-linked hypophosphatemia, and type 1 glycogen storage disease (Table 18.7).[87–93]

DRTA can also be caused by various drugs or toxins (Tables 18.7 and 18.10). Examples include amphotericin B,[94] carbonic anhydrase inhibitors (including the anticonvulsant topiramate), lithium carbonate,[95] analgesics,[96,97] ifosfamide, foscarnet,[98] and vanadate. DRTA occurs in the chronic rejection of renal transplantation.[99–101] DRTA with renal transplantation is attributed to defective ammonium excretion and generalized distal tubular malfunction.[100] Renal tubulointerstitial disease can be associated with DRTA. Prominent examples include hyperoxaluria,[102] obstructive uropathy,[103] vesicoureteral reflux,[104] lupus nephritis, leprosy, and pyelonephritis with nephrolithiasis.[105] DRTA is also associated with a variety of inherited disorders not discussed here, including Ehlers-Danlos syndrome, hereditary elliptocytosis, hereditary spherocytosis, sickle cell disease, medullary cystic disease, type I glycogen storage disease, and carnitine palmitoyltransferase I deficiency.[26,106–109]

Diagnosis of Classical Distal Renal Tubular Acidosis (Table 18.8)

The diagnosis of classical DRTA is most often made clinically in a patient with acquired forms of the disease (Table 18.10), indicating the importance of a thorough history and physical examination. These patients usually exhibit spontaneous and chronic nongap metabolic acidosis in association with chronic hypokalemia and an inappropriately alkaline urine pH (>5.5), and a positive urine anion gap, as mentioned previously. Hypokalemia may first bring the patient to medical attention, particularly if severe because it may be associated with flaccid paralysis. Additionally, patients may have a history of nephrolithiasis (calcium phosphate), variable degrees of nephrocalcinosis, or evidence of autoimmune disorders instead (Sjögren syndrome, thyroiditis, or primary biliary cirrhosis) (Table 18.10). Drugs and toxins may cause tubular effects or toxicity (Table 18.10). Conversely, other patients, usually members of families with known inherited classical DRTA, may have one or more of the disorders (e.g., nephrocalcinosis or nephrolithiasis) but not display evidence of chronic nongap acidosis. When such patients are challenged with an oral ammonium chloride load (0.1 gm per kg BW), urine pH does not decrease below 5.5 (as in nonaffected controls). This latter category of patients has what has been designated as "incomplete" classical DRTA. Patients with DRTA, in contrast to patients with proximal RTA, do not have a Fanconi syndrome and the fractional excretion of bicarbonate is lower (usually in the 5% range).

Finally, the urine to blood PCO_2 difference, or gradient, is typically lower than in normal subjects for both complete and incomplete DRTA. The U-B PCO_2, although not performed clinically in the majority of cases, remains the most sensitive diagnostic test for a secretory abnormality (i.e., abnormal H^+-ATPase in collecting tubule). In an alkaline urine, induced by an intravenous infusion of bicarbonate, the urinary PCO_2 typically increases 25 mm Hg above arterial PCO_2 levels. In patients with classical DRTA (as well as the generalized abnormality discussed later), the urine PCO_2 remains at or no more than 10 mm Hg above arterial levels (for review, see Chapter 6).[110]

Treatment of Classical Distal Renal Tubular Acidosis

The primary therapeutic objective for patients with classical DRTA is to correct the chronic metabolic acidosis. This can be achieved by administration of alkali in an amount sufficient to neutralize the daily acid load, or approximately 1 to 3 mEq/kg/day, and to return the plasma bicarbonate concentration to the normal range (Table 18.11). Shohl's solution (15 to 20 mL in water or juice, not as a syrup) may be more readily tolerated than $NaHCO_3$ tablets (325 or 650 mg or 3.9 and 7.8 mEq per tablet, respectively) in some patients. Compliance to either is often limited by taste fatigue with Shohl's solution and gastrointestinal discomfort with $NaHCO_3$ tablets. These and other choices are listed in Table 18.11. Correction of the acidosis reduces urinary potassium and sodium excretion, thus decreasing the need for potassium supplementation.[111,112] If required, potassium can be administered as potassium bicarbonate (K-Lyte 25 or 50 mEq), potassium citrate (Urocit K), or Polycitra (K-Shohl's).

Rarely, patients present with flaccid paralysis due to severe hypokalemia, metabolic acidosis, and hypocalcemia requiring immediate therapy. Because the hypokalemia may result in respiratory depression, increasing systemic pH with alkali therapy may worsen the hypokalemia. Therefore, immediate intravenous potassium replacement should be achieved prior to alkali administration.

Prognosis. The first goal of alkali therapy in DRTA is to normalize the serum bicarbonate and thereby prevent the relentless progression of chronic kidney disease. The GFR should be expected to stabilize with normalization of the serum bicarbonate (into 23 to 25 mEq per L range). This requires modest amounts (1 to 3 mEq/kg/day) of alkali therapy daily in divided doses. Hypercalciuria usually subsides on correction of the acidosis and urinary citrate excretion increases, thus reducing the incidence of urolithiasis. Although it is possible that nephrocalcinosis may improve over many years of alkali therapy, direct evidence for this view is lacking. Potassium homeostasis improves or normalizes in most adult patients and the renal phosphate clearance also improves. In children, growth is usually restored. Therefore, most if not all of the accompanying abnormalities are corrected with sustained correction of the serum bicarbonate.

18.11 Sources of Alkali for Therapy for Renal Tubular Acidosis

Shohl's solution (Na^+ citrate and citric acid)
- Sodium citrate 500 mg and citric acid 334 mg per 5 mL (480 mL)
- HCO_3^- equivalent 1 mEq/mL and Na^+ 1 mEq/mL

$NaHCO_3$ tablets
- 325 mg (3.8 mEq)
- 650 mg (7.6 mEq)

Baking soda (60 mEq/tsp)
K-Lyte (25 or 50 mEq/tablet)
Polycitra (K^+ Shohl's)
Granules, effervescent (Brioschi)

TYPE 3 RENAL TUBULAR ACIDOSIS: MIXED PROXIMAL AND DRTA

Carbonic anhydrase II deficiency is a very rare autosomal recessive abnormality characterized by osteopetrosis, RTA, and cerebral calcification.[26] Additional features that have been reported include severe mental retardation, stunted growth, microcephaly, dental malocclusion, high-arched palate, and broad thumbs. Reported patients with this syndrome have been predominantly from the Middle East and Mediterranean region. The evidence for both a proximal and distal cause of the RTA includes frank bicarbonate wasting consistent with proximal tubular dysfunction and an inability to acidify the urine during a sustained systemic acidosis, as well as a low urinary PCO_2 with bicarbonate loading—the latter two indicating DRTA.

RENAL TUBULAR ACIDOSIS ASSOCIATED WITH HYPERKALEMIA: GENERALIZED DISTAL NEPHRON DYSFUNCTION

The distinguishing features of generalized DRTA (type 4 RTA) are displayed in Table 18.12. This disorder occurs with hyperkalemia, whereas both proximal and classical DRTA occur with hypokalemia. A discussion of the unique pathophysiology, which localizes the nephron site of the abnormality, follows and is summarized in Table 18.13.

Pathophysiology of Voltage Defect

When a decrease in the negative transepithelial potential difference in the collecting tubule impairs proton secretion in

18.12 Distinguishing Features of Hyperkalemic (Generalized) Distal Renal Tubular Acidosis (Type 4)

1. Only variant associated with hyperkalemia
2. Collecting duct fails to secrete protons and potassium
3. Aldosterone is insufficient in quantity or activity or intrinsic (genetic) or acquired molecular defect in transport of Na^+, K^+, and H^+
4. Hyperkalemia contributes to acidosis by blunting NH_4^+ production and excretion

18.13 Pathophysiologic Basis of Hyperkalemic Acidosis and Generalized Distal Renal Tubular Acidosis (Type 4)

1. Abnormal CCD–MCD (intrinsic)
2. Primary decrease in mineralocorticoid (extrinsic)
3. Voltage defect—compromises H^+ and K^+ secretion
   a. Abnormal ENaC
   b. Chloride shunt

CCD, cortical collecting duct; MCD, medullary collecting duct.

this segment, it is referred to as a "voltage defect" (Fig. 18.7). Any process inhibiting sodium transport in the CCT would be expected to cause such a defect because translocation of the cation Na^+ from lumen to interstitium is the principal determinant of transepithelial voltage in the CCT. A decrease in negative voltage decreases the electrical gradient K^+ and H^+ secretion by the CCT. The traditional model of such a transport defect is that observed after amiloride administration. Amiloride occupies and interacts with the epithelial sodium channel (ENaC) on the apical membrane of the principal cell of the cortical collecting tubule inhibiting sodium transport, decreasing negative transepithelial voltage, and secondarily inhibiting both K^+ and H^+ secretion (the latter in Type A intercalated cells). Therefore, with a "voltage" lesion, both hyperkalemia and metabolic acidosis may develop. The mechanism for the generation of acidosis and hyperkalemia in patients receiving trimethoprim, pentamidine, and triamterene is similar in mechanism to that observed with amiloride.[55] Specific mutations in genes (SCNN1A, SCNN1B, SCNN1G) that encode the epithelial sodium channel (ENaC) have been shown to cause autosomal recessive pseudohypoaldosteronism type 1.[113] This defect in ENaC impairs Na^+ absorption, reduces transepithelial voltage, and subsequently impairs K^+ and H^+ transport. These mutations, by impairing ENaC and therefore Na^+ absorption, reduce the negative transepithelial voltage in the CCT, and result in a prototypical "voltage defect" in this segment. Therefore, autosomal recessive pseudohypoaldosteronism type 1 (PHA I) represents an example of an inherited "voltage" defect. This information is summarized in Figure 18.7.

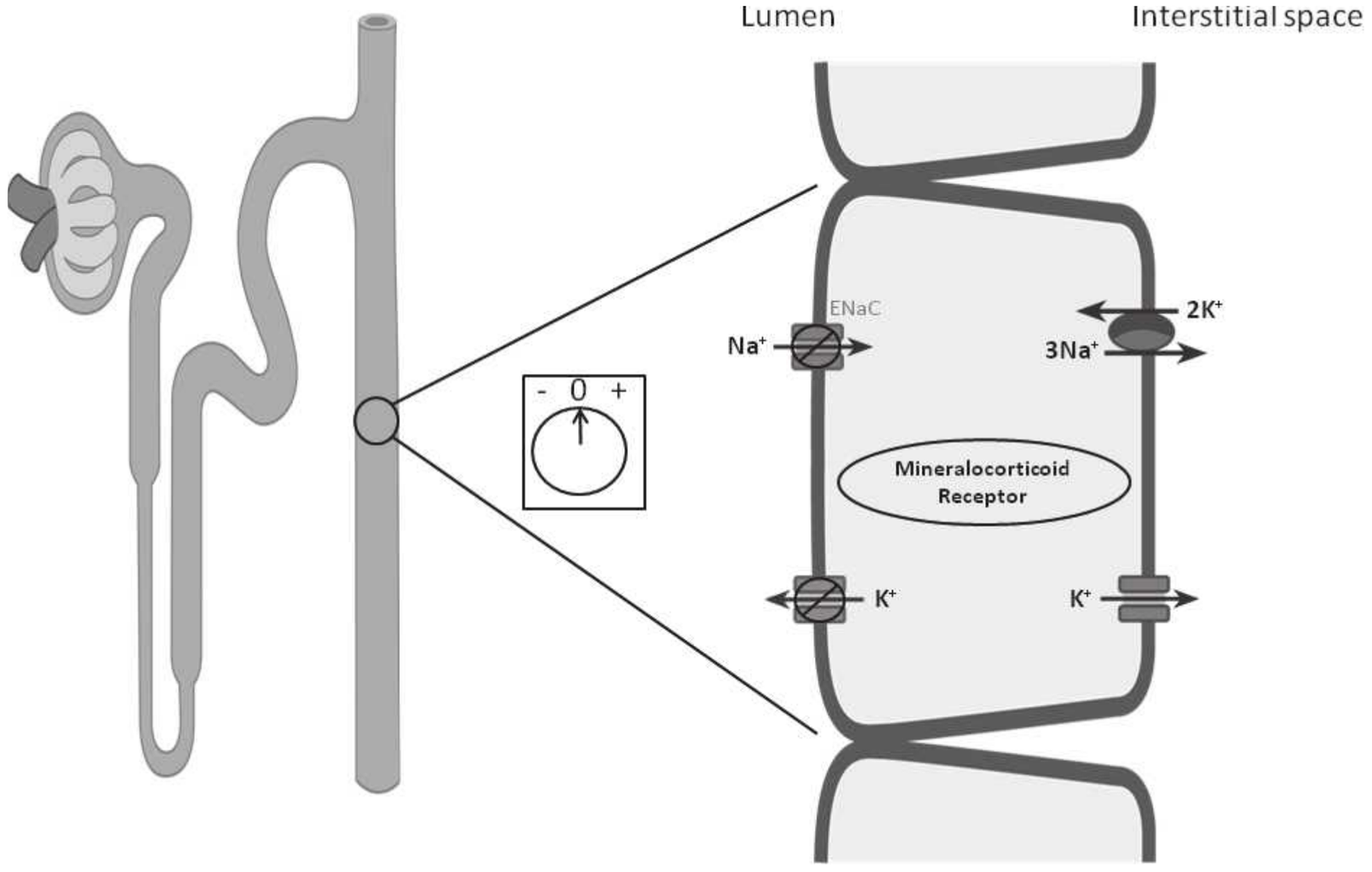

FIGURE 18.7 Model of principal cell in collecting tubule showing voltage defect. Example shows primary loss of function mutation of Na^+ channel (ENaC) or pseudohypoaldosteronism type 1 as example. Loss of function of ENaC causes a decrease in Na^+ absorption and loss of negative transepithelial voltage that secondarily causes decrease in K^+ secretion. Other examples of voltage defects are explained in the text.

Role of Aldosterone Deficiency in Generation of Hyperkalemic Acidosis

Aldosterone enhances the lumen negative transepithelial potential difference in the cortical collecting tubule through upregulation of Na^+ absorption. Initially, aldosterone upregulates ENaC, and subsequently the basolateral Na^+,K^+-ATPase, to stimulate Na^+ absorption, K^+ secretion, and H^+ secretion. Therefore, it is not surprising that aldosterone deficiency would result in the development of hyperkalemia and metabolic acidosis. In contrast to the CCT, aldosterone stimulates potassium and hydrogen ion secretion in the medullary collecting tubule, independent of sodium transport. Therefore, a relative decrease in the amount of aldosterone or decrease in responsiveness of the collecting tubule to aldosterone could result in a reduction in distal sodium absorption which would be expected to impair both potassium and hydrogen ion secretion. Aldosterone also plays a significant role in ammonium absorption and excretion by the inner medullary collecting duct.[57] Selective aldosterone deficiency in animal models has been reported to be associated with impaired ammonium excretion and reduced papillary PCO_2 during bicarbonate loading, verifying a defect in proton secretion. Both of these processes lead to a decrease in proton secretion, favoring the development of metabolic acidosis. The importance of mineralocorticoid in the regulation of net acid excretion has been documented in mineralocorticoid-deficient animals and man.[114] In patients who have undergone adrenalectomy, net acid excretion and plasma total CO_2 decline if mineralocorticoid is selectively discontinued, but increase with the reinitiation of mineralocorticoid.[115] The change in plasma total CO_2 correlated directly, in these studies, with changes in ammonium excretion and, inversely, with corresponding changes in potassium balance.

Role of Hyperkalemia in the Generation of Hyperchloremic Metabolic Acidosis

Hyperkalemia has an independent effect on net acid excretion through inhibition of renal ammoniagenesis. Hyperkalemia is associated, in addition, with a decrease in ammonium transport and, thereby, excretion. This contributes importantly to the development of metabolic acidosis.[58,116] DuBose et al. have shown that hyperkalemia decreases ammonium excretion and production in the proximal tubule.[52,57] Hyperkalemia markedly impairs ammonium absorption in the thick ascending limb, reducing inner medullary concentrations of total ammonia and decreasing secretion of NH_3 into the inner medullary collecting duct.[48,49,52,58] The mechanism for impaired absorption of NH_4^+ in the medullary thick ascending limb of Henle is competition between K^+ and NH_4^+ for the K^+ secretory site on the Na^+-$2Cl^-$-K^+ cotransporter. The importance of hyperkalemia as a cause of metabolic acidosis is underscored by the observation that correction of hyperkalemia is associated with a significant increase in net acid excretion and a parallel correction of the acidosis.

Clinical Examples of Mineralocorticoid Deficiency and Resistance

Hyperkalemic and hyperchloremic metabolic acidosis can occur as a result of one of three abnormalities: (1) deficiency of mineralocorticoid, (2) resistance to mineralocorticoid, or (3) renal tubular dysfunction. The causes of voltage defects (tubular dysfunction) and deficiency or resistance to mineralocorticoid are outlined in Table 18.14. Mineralocorticoid deficiency may occur in concert with general adrenal failure (Addison disease), where there is a decrease in elaboration of both glucocorticoid and mineralocorticoid. Addison disease can be caused by destruction of the adrenal cortex with hemorrhage, infection, invasion by tumors, or autoimmune disease. Patients have hypoglycemia, anorexia, weakness, hyperpigmentation, relative or frank hypotension, and a failure to respond to stress. Renal salt wasting, hyponatremia, hyperkalemia, and metabolic acidosis coexist.[117] Typically, serum aldosterone levels are low and the plasma renin high. Metabolic acidosis occurs because of a decrease in H^+ secretion in the collecting tubule from decreased H^+-ATPase number and function. Hyperkalemia further aggravates the acidosis by depression of ammonium production and excretion.

Selective or isolated hypoaldosteronism can occur in critically ill patients, particularly in the setting of sepsis or cardiogenic shock.[118] Heparin (either low molecular weight or unfractionated heparin), often administered in this setting, impairs aldosterone synthesis as a result of direct toxicity to the zona glomerulosa with direct inhibition of the enzyme aldosterone synthase.[119] Moreover, hypoxia and cytokines may contribute to aldosterone synthesis failure in the critically ill patient.[118,120]

One of the best examples of resistance to mineralocorticoid is autosomal dominant pseudohypoaldosteronism (PHA I). This disorder is clinically less severe than autosomal recessive PHA I (discussed later) and is associated with hyperkalemia, renal salt wasting, metabolic acidosis, elevated renin and aldosterone levels, and hypotension. The autosomal dominant disorder has been shown to be the result of a mutation in the intracellular mineralocorticoid receptor in the collecting tubule.[113] Unlike the autosomal recessive disorder, this defect is not expressed in organs other than the kidney, and becomes less severe with advancing age. Carbenoxolone raises the intracellular concentration of cortisol, overcoming the functional defect in the mutant receptor and improving mineralocorticoid resistance. Because the decrease in mineralocorticoid reduces apical Na^+ absorption and activity of ENaC, transepithelial potential difference declines and K^+ secretion is impaired.[121]

Clinical Examples of Voltage Defects in the Collecting Tubule (Table 18.14)

The prototype for a "voltage" defect is pseudohypoaldosteronism type 1 (PHA I). Most cases of autosomal dominant PHA I link to mutations in the mineralocorticoid receptor (MR). In general, patients with autosomal recessive PHA I

18.14 Generalized Abnormality of Distal Nephron with Hyperkalemia

MINERALOCORTICOID DEFICIENCY

Primary mineralocorticoid deficiency

Combined deficiency of aldosterone, desoxycorticosterone, and cortisol
- Addison disease
- Bilateral adrenalectomy
- Bilateral adrenal destruction
- Hemorrhage or carcinoma

Congenital enzymatic defects
- 21-Hydroxylase deficiency
- 3β-Hydroxy dehydrogenase deficiency
- Desmolase deficiency

Isolated (selective) aldosterone deficiency
- Chronic idiopathic hypoaldosteronism
- Heparin (unfractionated or LMW) in critically ill patient
- Familial hypoaldosteronism
  - Corticosterone methyl oxidase deficiency, types 1 and 2
- Primary zona glomerulosa defect
- Transient hypoaldosteronism of infancy
- Persistent hypotension and/or hypoxemia in critically ill patient

Angiotensin II–converting enzyme inhibition
- Endogenous
- ACE inhibitors and AT_1 receptor antagonists

Secondary mineralocorticoid deficiency

Hyporeninemic hypoaldosteronism
- Diabetic nephropathy
- Tubulointerstitial nephropathies
  - Nephrosclerosis
  - Nonsteroidal anti-inflammatory agents
  - Acquired immunodeficiency syndrome
  - Immunoglobulin M monoclonal gammopathy

MINERALOCORTICOID RESISTANCE

Pseudohypoaldosteronism type 1 (PHAI)—autosomal dominant

RENAL TUBULAR DYSFUNCTION (Voltage Defect)

Pseudohypoaldosteronism type 1 (PHAI)—autosomal recessive

Pseudohypoaldosteronism type 2 (PHAII)—autosomal recessive

Drugs that interfere with Na^+ channel function in CCT
- Amiloride
- Triamterene
- Trimethoprim
- Pentamidine

Calcineurin inhibitors (interfere with Na^+, K^+-ATPase in CCT)
- Cyclosporin A
- Tacrolimus

(continued)

18.14 Generalized Abnormality of Distal Nephron with Hyperkalemia (continued)

Drugs which inhibit aldosterone effect on CCT
- Spironolactone

Disorders associated with tubulointerstitial nephritis and renal insufficiency
- Lupus nephritis
- Methicillin nephrotoxicity
- Obstructive nephropathy
- Kidney transplant rejection
- Sickle cell disease
- Williams syndrome with uric acid nephrolithiasis

ACE, angiotensin-converting enzyme; CCT, cortical collecting tubule; LMW, low molecular weight.

as opposed to autosomal dominant PHA I have a more severe clinical phenotype and do not spontaneously improve during early childhood. Furthermore, given the generalized expression of ENaC in epithelial tissues, autosomal recessive PHA I associates with systemic manifestations, most notably recurrent pulmonary infections.[122–125] Children with autosomal recessive PHA I have severe hyperkalemia and renal salt wasting because of a functional block of sodium transport across the apical membrane through impairment of ENaC, which secondarily prevents potassium secretion and causes hyperkalemia. In addition, the hyperchloremic metabolic acidosis may be severe and is associated with hypotension and marked elevations of plasma renin and aldosterone. These children also present with vomiting, hyponatremia, failure to thrive, and respiratory distress. The latter is related to involvement of ENaC in the alveoli, preventing Na^+ and water absorption in the lungs, and increasing the frequency of infection. Patients with this disease respond to a high salt intake and correction of the hyperkalemia. Unlike the autosomal dominant form, autosomal recessive PHA I persists throughout life and is a more severe disease.

A "voltage defect" with a contrasting phenotype is pseudohypoaldosteronism type II (PHA II), or Gordon syndrome, first described in the 1960s (Table 18.14). This disorder occurs in adults and results in hyperkalemia, hyperchloremic metabolic acidosis, hypertension, mild volume expansion, and suppressed renin and aldosterone levels.[121,126] These patients typically respond to thiazide diuretics, suggesting a disorder of the distal tubule Na^+-Cl^- cotransporter. PHA II is transmitted in an autosomal dominant fashion and is caused by mutations in the WNK1 and WNK4 genes that interact with the Na-Cl cotransporter in the distal tubule, causing a gain of function (loss of degradation) of this transporter and resulting in salt retention and hypertension. The response of these patients to Na_2SO_4 infusion validates the presence of a voltage defect, perhaps as a result of preferential absorption ("shunting") of chloride in the collecting tubule beyond the site of the defect, the distal convoluted tubule.

In addition to inherited voltage defects, there are examples of acquired voltage defects due to drugs or tubulointerstitial disease (Table 18.14).[127,128] Examples of the former include amiloride and the structurally related compounds trimethoprim and pentamidine.[129–131] As discussed previously, this explains the occurrence of hyperkalemic hyperchloremic acidosis in patients receiving higher doses of these agents. Spironolactone and triamterene are more likely to cause hyperkalemia in patients with renal insufficiency or hyporeninemic hypoaldosteronism.[132,133] Those tubulointerstitial diseases, which may be associated with impairment or destruction of collecting duct function, include obstructive uropathy, lupus nephritis, sickle cell nephropathy, analgesic nephropathy, or multiple myeloma.[101,127,134,135]

Hyporeninemic-hypoaldosteronism

In hyporeninemic-hypoaldosteronism, both the metabolic acidosis and the hyperkalemia are out of proportion to the level of reduction in GFR. Many patients have congestive heart failure, diabetic nephropathy or tubulointerstitial renal disease, cardiac arrhythmias, and hypertension. Ammonium excretion is impaired because hyperkalemia inhibits both ammoniagenesis and ammonia/ammonium transport and excretion. Both ammoniagenesis and ammonia excretion improve with correction to normal plasma potassium. Many of these patients have progressive renal disease and at stage 4 or 5 chronic kidney disease develop the typical high anion gap acidosis of renal failure.

Identification and Treatment of Generalized Defect

The first priority in the treatment of hyperkalemic-hyperchloremic metabolic acidosis is identification of the underlying disorder. To this end obtaining a careful dietary and

drug history is critical and can identify unsuspected sources of exogenous K^+ intake. It is also important to identify contributing or precipitating factors that include low urine flow, decreased distal Na^+ delivery, superimposed acute renal failure, hyperglycemia, and hyperosmolality. The distinguishing diagnostic features are summarized in Table 18.12. The workup should consist of an evaluation of K^+ excretion by the (TTKG) or the fractional excretion of potassium, an estimate of renal ammonium excretion (urine anion gap), urine pH, the U-B PCO_2, and evaluation of PRA and aldosterone secretion. Aldosterone secretion, if evaluated, should be obtained under stimulated conditions with dietary salt restriction and/or furosemide-induced volume depletion. Drugs associated with hyperkalemia such as triamterene, spironolactone, amiloride, pentamidine, trimethoprim, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), or K^+ supplements should be discontinued. Salt substitutes and herbal preparations should be avoided and a diet low in potassium emphasized.

The severity of the hyperkalemia is the primary determinant in the decision to treat patients with this disorder and the therapy is summarized in Table 18.15. Patients with combined glucocorticoid and mineralocorticoid deficiency should receive both steroids in replacement dosages. Mineralocorticoids should be avoided in the face of hypertension or congestive heart failure. If supraphysiologic doses of mineralocorticoids are needed, patients should be monitored closely for volume overexpansion, hypertension, and hypokalemia. Effectively reducing the serum potassium with either cation-exchange resin (sodium polystyrene powder, 15 Gm dissolved in water not in sorbitol, daily) or loop diuretics will often improve the metabolic acidosis by increasing ammonium excretion. Volume depletion should be avoided unless the patient is volume overexpanded or hypertensive. Children with PHA I should receive NaCl supplement and adults with PHA II should receive thiazide diuretics. In summary, because hyperkalemia interferes with the kidney's response to metabolic acidosis, treatment consists of correction of hyperkalemia, restoration of euvolemia, alkali therapy, loop diuretics, and dietary potassium restriction. In severe hypoaldosteronism, the effect of loop diuretics can be enhanced by the addition of replacement mineralocorticoids.

18.15 Treatment of Generalized Dysfunction of the Nephron with Hyperkalemia

Alkali therapy
Loop diuretic (furosemide, bumetanide)
Sodium polystyrene sulfonate powder (Kayexalate 15 gm p.o. in water—not Sorbitol—three times a week)
Fludrocortisone (0.1–0.3 mg/day)
Avoid in hypertension, volume expansion, heart failure, chronic kidney disease
Combine with loop diuretic
Avoid drugs associated with hyperkalemia
In pseudohypoaldosteronism type I: add NaCl supplement

p.o., by mouth.

Summary

The diagnostic features and studies to distinguish the various types of RTA are displayed in Table 18.16.

BARTTER SYNDROME AND GITELMAN SYNDROME

Bartter and Gitelman syndromes are autosomal recessive inherited renal tubular transport disorders characterized by salt wasting and relative hypotension. Typical symptoms of both are severe hypokalemia, metabolic alkalosis with hyperaldosteronism, and hypertrophy and hyperplasia of the juxtaglomerular apparatus. Although the two syndromes are similar, Gitelman syndrome is uniquely associated with magnesium deficiency and hypocalciuria.

Both Bartter and Gitelman syndromes are caused by specific genetic mutations associated with salt transporters. Genetic abnormalities causing Bartter syndrome localize to transporters in the thick ascending limb of loop of Henle, whereas, in contrast, the defects in Gitelman syndrome reside in the distal convoluted tubule. There are three different specific types of Bartter syndrome. Type 1 is caused by a mutation of the Na^+-K^+-$2Cl^-$ cotransporter[136]; type 2 by mutations in the apical K^+ channel[137,138]; and type 3 (neonatal) by mutations in the basolateral Cl^- channel[139] (Fig. 18.8A). Gitelman syndrome is caused by mutations in the NaCl cotransporter in the distal tubule[140–142] (Fig. 18.8B). Gitelman syndrome is relatively common, affecting about 1% of the population.

The differential diagnoses for Bartter and Gitelman syndromes include chronic or surreptitious vomiting, laxative or diuretic abuse, or licorice ingestion. Daily treatment with 150 to 300 mEq of potassium along with amiloride, spironolactone, or triamterene is recommended. Although associated with relative hypotension, some patients respond when ACE inhibitors or ARBs are added, but with careful attention to blood pressure.

DISORDERS OF CARBOHYDRATE TRANSPORT

Mechanisms of Carbohydrate Reabsorption

The proximal tubule not only reabsorbs approximately 70% of the salt, bicarbonate, and water, but also all of the glucose and amino acids. The sodium-dependent glucose

18.16 Summary of Diagnostic Studies in Renal Tubular Acidosis

	Type of RTA		
Finding	**Proximal (II)**	**Classical Distal (I)**	**Generalized Distal Dysfunction (IV)**
Plasma [K^+]	Low	Low	High
Urine pH (with acidosis)	<5.5	>5.5	<5.5 or >5.5
Urine net charge	Positive	Positive	Positive
Fanconi lesion	Present	Absent	Absent
Fractional bicarbonate excretion	>10%–15%	<5%	<5%–10%
U-B PCO_2	Normal	Low[a]	Low
Response to therapy	Least readily	Readily	Less readily
Associated features	Fanconi syndrome	Nephrocalcinosis/ hyperglobulinemia	Renal insufficiency

[a]Except with Amphotericin B.
RTA, renal tubular acidosis; U-B, urine-minus-blood.

cotransporters (SGLT1 and SGLT2) are integral membrane proteins that mediate the transport of glucose across the plasma membrane by secondary active cotransport of glucose molecules and sodium ions. The lumen-to-cell sodium concentration gradient, maintained by the basolateral Na^+,K^+-ATPase, provides the energy for transport of sodium across the apical membrane, augmented by the lumen-negative potential difference in the early proximal tubule (−2 mV). The sodium-glucose transporters localize to the brush-border membranes of S_1 and S_3 segments. Most glucose is reabsorbed in the first 25% of the proximal tubule. Healthy kidneys reabsorb all filtered monosaccharides if their plasma concentrations are normal or low, but if plasma concentrations increase due to disease or a systemic infusion, sugar appears in the urine. A plot of the plasma concentration versus the urinary excretion rate allows the maximum rate of tubular reabsorption (T_{max}) to be obtained.[143]

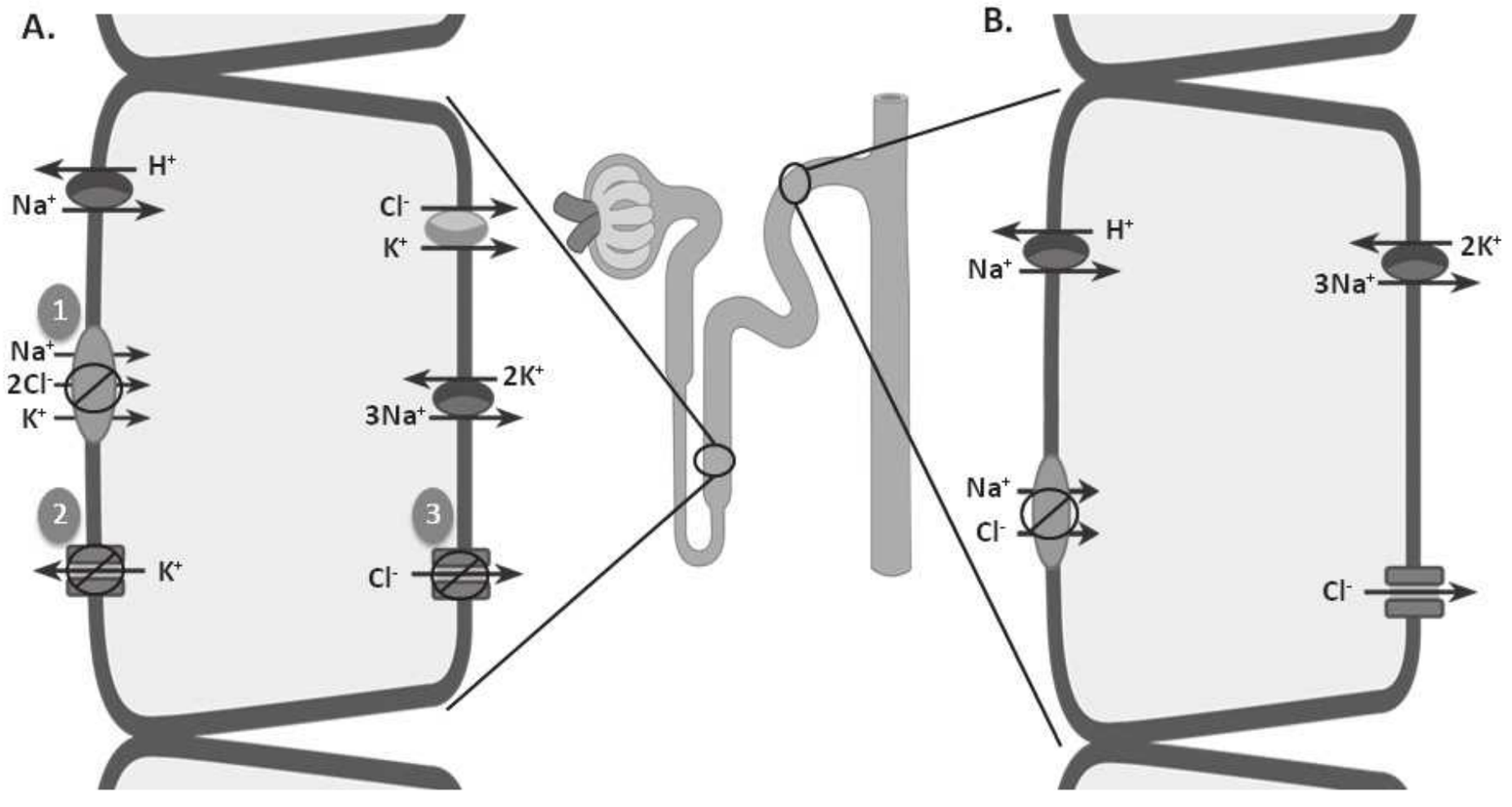

FIGURE 18.8 Inherited solute wasting disorders. **A:** Examples of three types of Bartter syndrome: type 1 Bartter, abnormality of apical Na^+-$2Cl^-$-K^+ tri-transporter; type 2 Bartter, abnormal apical K^+ conductance; and type 3 Bartter, abnormality of basolateral Cl^- channel. **B:** Distal convoluted tubule showing cause of Gitelman syndrome. See text for details.

Primary Renal Glycosuria

Primary renal glycosuria occurs when plasma glucose is normal but urinary glucose excretion is increased. GFR and intestinal absorption of carbohydrates are also normal. Although mechanisms underlying primary renal glucosuria are not fully appreciated, three types (A, B, and O) have been noted, based on glucose titration curves.[144] However, the genetic description of disorders of glucose transport have more recently elucidated the molecular basis for glycosuria.[145]

Congenital defects in SGLT2, located in the apical membrane of the S1 segment in proximal renal tubule cells, lead to a primary renal glucosuria. Patients with this disease have normal blood glucose levels, normal oral tolerance test results, and persistent glucosuria. In the most severe cases, a major portion of the filtered glucose is excreted.

The Fanconi-Bickel syndrome (FBS), a rare autosomal recessive inborn error of metabolism A, is the result of an inherited defect within the SLC2A2 gene and resembles type I glycogen storage disease. Thirty-four different GLUT2 mutations have been reported in 132 patients. Because GLUT2 is the predominant glucose transporter in hepatocytes, pancreatic beta cells, enterocytes, and renal tubular cells, a loss of GLUT2 leads to a typical combination of hepatorenal glycogen accumulation, glucose and galactose intolerance, fasting hypoglycemia, a proximal tubular nephropathy, and severely stunted growth.

The diagnostic criteria for the diagnosis of primary renal glycosuria includes glucosuria without hyperglycemia, which may increase during pregnancy; glucosuria independent of dietary intake, present after an overnight fast; a normal (or flat) glucose tolerance curve in the presence of normal plasma insulin, free fatty acids, and glycosylated hemoglobin; normal renal transport of other sugars; and normal carbohydrate utilization in affected subjects.

Glycosuria of Pregnancy

There are two reasons that glucose T_{max} could decrease during pregnancy: an increased GFR without parallel increases in glucose reabsorption, or a significant inhibition of reabsorption due to volume expansion. Glucose transport tends to normalize approximately 2 months after delivery. It does not appear that women with prenatal glucosuria are at higher risk for developing diabetes mellitus.

Disorders of Amino Acid Transport

Mechanisms of Amino Acid Transport. The kidney filters approximately 70% to 90% of the body's protein each day, mostly as small peptides and amino acids, with about 2% to 3% of amino acids being excreted in the urine and the remaining being reabsorbed by the proximal tubule for recirculation to the body. Sodium-dependent cotransport systems for many of the acidic and neutral amino acids have been identified,[146,147] which are energized by the basolateral Na^+,K^+-ATPase.[148–150] The nutritionally essential amino acids in adults are valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, arginine, and histidine (taurine appears to be an essential amino acid for infants). There are at least six separate transport systems for the different types of amino acids: dicarboxylic amino acids glutamate and aspartate; dibasic amino acids lysine, arginine, ornithine, and cystine[36,37]; neutral amino acids glycine, valine, leucine, and isoleucine (and 13 other amino acids[146,151,152]; imino amino acids proline, hydroxyproline, and sarcosine (and other N-methyl amino acids); β-amino acids β-alanine, β-aminoisobutyric acid, β-aminobutyric acid, and taurine[153,154]; and cystine–cysteine.[146,152]

Specific Clinical Diseases Associated with Aminoaciduria. Genetic studies in patients with specific aminoacidurias, and phenotypic observations in knockout mice, have resulted in major contributions to a better understanding of amino acid transport in the kidney. The recognition of changes in individual amino acid levels in urine and plasma of patients with aminoaciduria has been behind much of the recent progress in this field. However, these recent advances, along with changes in nomenclature, have caused some consideration among renal physiologists and nephrologists as to classification, now based on specific solute carrier transporter types. The major classes of disorders include cystinuria A, cystinuria B, cystinuria AB, lysinuria, and Hartnup disease. Urinary amino acid screening has allowed identification of additional asymptomatic aminoacidurias, iminoglycinuria, and dicarboxylic aminoaciduria.[155]

Cystinurias. The overall incidence of cystinuria is approximately 1:7,000 births. Although the most evident consequence of this disease, cystine bladder stones, was first described almost two centuries ago, the proteins responsible were identified only recently. Alexander Garrod placed the clinical findings within the context of disordered amino acid metabolism, but it was not until 50 years ago that cystinuria was proposed to be an inborn error of dibasic amino acid transport. Charles Dent was the first to recognize that not only urinary cystine but also lysine and arginine were constantly elevated in patients with cystinuria.

SLC3A1 (rBAT) was the first protein related to epithelial amino acid transport to be identified. Interacting proteins have been identified as SLC7A9 ($b^{0,+}$AT) and SLC3A1 (rBAT). Both mediate dibasic amino acid and cystine transport across the apical membrane of renal tubule cells. These proteins can be localized to the brush border membranes of proximal tubules. By overexpression of SLC3A1 (rBAT) and SLC7A9 ($b^{0,+}$AT) in MDCK cells, both proteins were shown to colocalize in the apical membrane, whereas without expression of the heavy subunit SLC3A1 (rBAT), the light subunit SLC7A9 ($b^{0,+}$AT) was retained within the cell. Therefore, SLC3A1 (rBAT) is essential for proper cell surface expression of SLC7A9 ($b^{0,+}$AT). Together they exchange dibasic amino acids and cystine from the tubular lumen for intracellular neutral amino acids.

Mutations in either interacting subunit, SLC3A1 (rBAT) or SLC7A9 ($b^{0,+}AT$), cause cystinuria. Cystinuria due to mutations in SLC3A1 is an autosomal recessive trait, whereas mutations in SLC7A9 are autosomal dominant and lead to an abnormal pattern of urinary amino acid excretion in heterozygotes. The initial nomenclature, based on the excretion status of obligate heterozygotes (cystinuria types I, II [non-I], III [non-I]), has been replaced by a classification based on genotype. Accordingly, mutations in SLC3A1 cause cystinuria type A, mutations in SLC7A9 cause cystinuria type B, and mutations in both genes (compound heterozygotes) cause cystinuria type AB. The severity of disease appears similar in cystinuria types A and B. A mouse model develops stones and the overall phenotype resembles type B cystinuria. Animals develop massive hyperexcretion of cystine and dibasic amino acids, and cystine crystalluria, with 40% having cystine calculi in the urinary system. As in humans, oral treatment of this mouse model with D-penicillamine has been shown to reduce the size and number of calculi.[155]

Clinically, cystinuria presents during the second or third decade of life as renal colic and stone disease. Urinary tract obstruction, recurrent infection, and hypertension occur. The disease may progress and may require kidney transplantation. Commonly associated diseases include hyperuricemia, hemophilia, Down syndrome, hypotonia, retinitis pigmentosa, and pancreatitis. The intestinal absorption defects in cystinuria, however, do not appear to impair growth.

The diagnosis may be made with the urinary cyanide-nitroprusside test, in which the urine turns magenta red; characteristic cystine crystals may also be seen on microscopic urinalysis. Ion exchange chromatography reveals excretion of excess cystine and cationic amino acids with normal plasma levels.

Dietary control with low methionine intake and restriction of salt intake are only slightly effective.[156] Alkalinizing the urine to pH 7.5 is of no benefit because urinary cystine concentration is always greater than 0.8 mmol per L in affected subjects. Nevertheless, penicillamine (1 to 2 g per 24 hours) is the treatment of choice for dissolving stones and preventing further formation. The drug, however, may have serious side effects, including nephrotic syndrome, membranous nephropathy, serum sickness, fever, rash, and pancytopenia. As a result, its long-term use is quite limited. The compound α-mercaptoproprionyl glycine has also been used. This drug has a higher redox potential than penicillamine and is said to be less toxic. Even so, proteinuria, membranous glomerulonephritis, fever, rash, and positive antinuclear antibodies have been reported.[157] ACE inhibitors have been used in a few cases with beneficial results.[158] The mechanism of action appears to be related to the increased solubility of a cysteine–captopril complex excreted in the urine. Bladder irrigation using N-acetyl penicillamine, penicillamine, mercaptoproprionyl glycine, or trimethylamine has been attempted with some success. Cases in which the urinary tract is obstructed have been treated with lithotomy. Lithotripsy is of no benefit in this disorder because cystine stones are particularly refractory to extracorporeal shock waves. Use of this approach may result in substantial problems, including hematuria, infection, and renal parenchymal scarring. Fortunately if kidney transplantation is required, the disease does not appear to recur in the allograft.[159]

Lysinuria. The most widely appreciated disorder, lysinuria, is inherited as an autosomal recessive disorder more prevalent in Finland, Italy, and Spain.[159] Homozygotes are intolerant of a protein-rich diet. The mutations causing this autosomal recessive disorder have, in contrast to cystinuria, been identified in SLC7A7, which encodes a protein involved in transport of dibasic amino acids. Prenatal diagnosis is now possible. Patients show signs of failure to thrive, vomiting, hypotonia, and episodes of stupor or coma after consuming high amounts of dietary protein. Additional features include hepatomegaly, hyperammonemia, low blood urea nitrogen levels, and orotic aciduria. Osteoporosis is commonly seen and a severe interstitial lung disease may occur at any time during life. Intestinal absorption of three amino acids (lysine, ornithine, and arginine) is abnormal. Elevations of urinary dibasic amino acids lysine, arginine, and ornithine are diagnostic and more pronounced than in cystinuria; urinary cystine levels, in contrast to those of cystinuria, are almost normal.

Therapy consists of l-citrulline (0.5 mg per g) with dietary protein restriction (1.5 g per kg per day).[160] l-citrulline supplements improve tolerance for protein intake.

Type I hyperdibasic aminoaciduria is also an autosomal recessive disorder. Those affected appear to have an incomplete phenotype and modest aminoaciduria (lysine, ornithine, and arginine). It has a different ethnic distribution from that of the type II disorder in that cases are not clustered in one area. Although homozygotes have no hyperammonemia, mental retardation occasionally is observed.[161]

Iminoglycinuria. Iminoglycinuria is a benign autosomal recessive disorder that presents with elevated urinary glycine, proline, and hydroxyproline. It is important to analyze the fractional excretion of all amino acids to exclude urinary losses due to elevated plasma levels, as occurs in hyperprolinemia, where urine findings can mimic iminoglycinuria. Heterozygotes have glycinuria alone. The defect is thought to reside in the apical membrane amino acid transporter. It has been hypothesized that mutations in multiple genes, such as SLC36A1, SLC6A20, and SLC6A19, could be defective in iminoglycinuria. However, preliminary genetic studies[155] exclude SLC36A and SLC6A20.

Neutral Aminoaciduria (Hartnup Disease). Hartnup disease is one of the best described disorders of amino acid transport.[151] The neutral monocarboxylic amino acids—including tryptophan, phenylalanine, valine, leucine, isoleucine, alanine, serine, histidine, glutamine, asparagine, and threonine—are not absorbed normally and, subsequently, are excreted into the urine and stool in massive quantities. Elevated urinary neutral amino acids are the first means of

diagnosing the disorder, and must be differentiated from generalized aminoaciduria, the hallmark feature of the Fanconi syndrome.

The disorder occurs with an estimated incidence of 1 in 20,000 live births, but is multifactorial and depends on both diet and environment. Patients can present with signs and symptoms of pellagra (including light-sensitive dermatitis), intermittent cerebellar ataxia, and psychosislike symptoms. These symptoms are assumed to be the result of niacin deficiency, because tryptophan is a precursor of niacin and serotonin.

Mutations in SLC6A19, encoding the neutral amino acid transporter B^0AT1, which mediates neutral amino acid transport from the luminal compartment into the cells, are causative for autosomal recessive Hartnup disease. Therefore, a defect exists in the transporter(s) for the $^{\alpha}$-amino acids and the neutral amino acids in both the brush border of the renal tubule and the jejunum.

Treatment includes increasing protein intake and providing niacin (40 to 100 mg per day) to prevent or treat pellagralike symptoms.

Additional resources are available for the reader interested in a more in depth review of the genetic disorders of amino acid transporters in mammalian cells.[155,159,162–164]

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CHAPTER

19

Urinary Tract Obstruction and Reflux Nephropathy

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URINARY TRACT OBSTRUCTION

Introduction

Urinary tract obstruction is a common problem encountered by nephrologists, urologists, primary care physicians, hospitalists, and emergency medicine physicians. Obstruction can occur at any point in the urinary tract from the kidneys to the urethral meatus. It may develop secondary to calculi, tumors, strictures, anatomic abnormalities, or functional abnormalities.

Functionally, urinary tract obstruction can be divided into upper and lower urinary tract etiologies. The upper tract consists of the kidneys, the ureteropelvic junction (UPJ), ureters, and the ureterovesical junction (UVJ). The lower urinary tract consists of the bladder, the bladder neck, the urethra, the urethral meatus, and, in male patients, the prostatic urethra. Each entity has its own causes of urinary tract obstruction along with its own set of symptoms and treatments to relieve obstruction.

Obstructive uropathy can result in pain, urinary tract infection (UTI), a loss of renal function, sepsis, or death. Thus, suspected cases of urinary tract obstruction merit a consultation with a specialist for an evaluation. Because long-term obstruction can lead to irreversible damage to the function of the nephron, early detection is essential to preserving renal function. The principles of treatment are identification and a relief of the obstruction.

Incidence

The incidence of hydronephrosis reported by Bell[1] in a series of 32,360 autopsies was 3.8% (3.9% in males, 3.6% in females). The incidence of clinical manifestations of obstructive uropathy prior to death was not reported, and it is likely that hydronephrosis was an incidental autopsy finding in many of these patients. The incidence of hydronephrosis at autopsy is somewhat lower in children than in adults, at 2% in one series of 16,000 autopsies.[2] Over 80% of children with hydronephrosis at autopsy were less than 1 year old with the balance of childhood cases being distributed uniformly through the childhood years. In women, hydronephrosis is more likely to occur between the ages of 30 to 70 years secondary to pregnancy and gynecologic malignancies. In men, hydronephrosis is most likely to occur after the age of 60 secondary to prostatic obstruction from benign prostatic hyperplasia.[3]

Etiology

Urinary tract obstruction impedes the flow of urine from the kidney to the urethral meatus. This obstruction causes distention of the urinary tract proximal to the point of obstruction. This distention is caused by the increase in intraluminal pressure and can result in pain, which may be the first sign of obstruction. Distortion of the urinary tract and renal failure can develop from obstruction. The severity of damage depends on the degree and duration of obstruction. When the urinary tract is obstructed, urinary stasis develops predisposing the patient to acute or chronic renal failure, UTI, sepsis, or death.

Upper Urinary Tract Obstruction

Obstruction of the upper urinary tract can occur anywhere from the kidney down to the ureterovesical junction. Certain points along this path are more susceptible to obstruction. There are three main points of anatomic narrowing that urine must pass to get from the upper urinary tract to the lower urinary tract. These points are the ureteropelvic junction, the crossing of the ureter over the area of the pelvic brim as it runs anterior to the common iliac vessels, and the ureterovesical junction. The narrowest point among these three is the ureterovesical junction. The bladder itself can also cause a functional obstruction to the upper tract in cases of neurogenic bladder or severe bladder outlet obstruction.

The most common causes of upper tract obstruction are listed in Table 19.1. Intraluminal obstruction is most commonly caused by calculi, blood clots, tumors, or sloughed papilla. These obstructions tend to be acute in nature, leading to symptoms of severe renal colic with flank pain, hematuria, nausea, vomiting, and fever. Ureteral strictures tend to develop over time, causing chronic obstruction and renal atrophy. They can be caused by stone disease, cancer, maldevelopment, and iatrogenic injuries from ureteroscopy.

19.1 Urinary Tract Obstruction

Types of Upper Urinary Tract Obstruction	Types of Lower Urinary Tract Obstruction
UPJ obstruction	BPH
Renal/ureteral calculi	Urethral stricture
Renal or urothelial cancer	Posterior urethral valves
Ureteral stricture	Prostate cancer
UVJ obstruction	Bladder cancer
Fibroepithelial polyp	Meatal stenosis
Infindibular stenosis	Prolapsing ureterocele
Retroperitoneal fibrosis	Bladder/urethral calculi
Pelvic lipotomasis	Foreign body
Retrocaval ureter	Bladder neck contracture
Retro-ovarian vein syndrome	Pelvic organ prolapse
Lower pole crossing vessel	
Retroperitoneal/pelvic mass	
Ureterocele/ectopic ureter	
Abdominal aorta aneurysm	

UPJ, ureteropelvic junction; BPH, benign prostatic hyperplasia; UVJ, ureterovesical junction.

In female patients, an additional area of narrowing can occur as the distal ureter crosses posterior to the pelvic blood vessels and the broad ligament in the posterior pelvis. Women may also experience urinary tract obstruction when the ureters become externally compressed by pelvic tumors or by locally advanced gynecologic malignancies. In older women, a severe prolapse of the pelvic organs, such as the bladder (cystocele) or uterus (procidentia), can lead to urinary tract obstruction. In younger women, pregnancy can cause urinary tract obstruction secondary to ureteral obstruction from the gravid uterus. Gynecologic malignancies must always be considered when upper tract obstruction is present.

Extrinsic causes of upper urinary tract obstruction are less common but can still cause significant obstruction by applying pressure to the urinary tract or by impairing ureteral peristalsis. A lower pole vessel arising from the aorta, known as a crossing vessel, can cause an obstruction to the UPJ or the proximal ureter, which can lead to significant renal atrophy or failure of that kidney. Abdominal aortic aneurysms and common iliac artery aneurysms have also been implicated in causing ureteral obstruction. Vascular graft placement has been shown to cause hydronephrosis in up to 10% to 20% of patients from a mechanical obstruction of the ureter, which may or may not resolve spontaneously.[3]

A retrocaval ureter caused by the persistence of the posterior subcardinal vein in utero causes obstruction by coursing the ureter behind the inferior vena cava, which in turn causes an obstruction. A majority of these cases happen on the right side with a male predominance. Most patients become symptomatic in the third or fourth decade of life.[3]

Retroperitoneal fibrosis can cause unilateral or bilateral ureteral obstruction by trapping the ureters in the fibrotic tissue. This trapping inhibits ureteral peristalsis, thus impeding the flow of urine. Grossly, retroperitoneal fibrosis can appear as fibrosis, a whitish plaque surrounding the retroperitoneal structures, including the ureters. An underlying malignancy can be the cause in 8% to 10% of such cases.[3]

In children, an obstruction may be more commonly due to a ureteropelvic junction or a ureterovesical junction

obstruction, a ureterocele, an ectopic ureter, or an obstructed megaureter. Prenatal screening with ultrasonography is important and vital for the early detection of an obstruction. In addition, children with new onset incontinence or UTI need a workup because they may also have some type of urinary tract obstruction.

Lower Urinary Tract Obstruction

A lower urinary tract obstruction can occur anywhere from the bladder to the urethral meatus. A good history and physical examination can help delineate an upper from a lower urinary tract obstruction in a majority of patients. In older men, the most common cause of obstruction is benign prostatic hyperplasia (BPH), which causes growth of prostatic tissue into the prostatic urethra. This causes the mechanical obstruction of urine flow from the bladder to the distal urethra. Symptoms of BPH can include the frequency of urination, an urgency of urination, increasing nocturia, slow stream, postvoid dribbling, incontinence, or an inability to void. In younger boys, posterior urethral valves can cause significant obstruction.

Urethral or bladder neck strictures can cause a mechanical obstruction to the flow of urine from the bladder to the meatus, which usually results in severe lower urinary tract symptoms or an inability to void. These strictures can be developmental in nature as well as infectious or iatrogenic. Prior prostate surgery can also lead to bladder neck contractures or the regrowth of prostate tissue, which can lead to obstructive symptoms or an inability to void.

Clinical Syndromes of Urinary Tract Obstruction

The clinical presentation of urinary tract obstruction varies with the location, duration, degree of obstruction, and the patients themselves. A thorough history and physical examination is key in the patient evaluation. An upper urinary tract obstruction (kidney, ureter) can manifest as flank pain, ipsilateral back pain, ipsilateral groin pain, or no pain at all. Nausea and vomiting are also common and usually occur in acute obstruction. Chronic obstruction is usually indolent and may be asymptomatic. When infection is present, the patient may experience fever, chills, and/or dysuria. Hematuria may or may not be present. When a bilateral obstruction or a unilateral obstruction in a solitary kidney is severe and renal failure exists, a uremia can be present in the patient, as well as anuria. Symptoms of uremia can include weakness, peripheral edema, mental status changes, and pallor. If hydronephrosis is severe, the kidney may be palpable on a physical examination, especially in children. In cases that involve an infectious process, costovertebral angle tenderness may indicate pyelonephritis. Pain from an obstruction of a stone in the ureter will manifest as waxing and waning severe pain. During these pain episodes, the patient will often be found rolling around in bed unable to get comfortable. In contrast, a patient with peritonitis would lie very still and not move.

Lower urinary tract obstruction (bladder, urethra) can manifest as voiding dysfunction such as urgency, frequency, nocturia, incontinence, decrease in the force of stream, hesitancy, postvoid dribbling, and a sensation of incomplete emptying. Suprapubic pain or a palpable bladder indicates urinary retention in some cases. An infection may be present, and patients may experience dysuria. Hematuria may be present with or without infection. Gross hematuria may indicate the presence of a tumor; stone; infection; blood clot; bleeding vessel in the kidney, ureter, bladder, prostate, or urethra; or a foreign body present within the lower or upper urinary tract.

A digital rectal examination can reveal prostate enlargement, a normal-sized prostate, or inflammation suggestive of prostatitis. A urethral stricture often requires a cystoscopy for diagnosis. A urethral meatal stenosis is usually apparent on a physical examination. Patients with a urethral stricture may report a history of trauma, instrumentation, radiation to the pelvis, or a sexually transmitted disease. They may also experience a split urinary stream. In women, the presence of a uterine or bladder prolapse can be visualized on a pelvic examination. A urethral diverticulum can also be palpated on a physical examination.

Indications to treat a patient with urinary obstruction include a patient with bilateral complete obstruction; any type of obstruction in a solitary kidney; an obstruction causing fever, infection, or both; or a renal failure needs immediate attention by a trained physician. Patients with pain that is uncontrolled with oral pain medications or persistent nausea and/or vomiting, which causes dehydration, also need immediate medical attention as well as hospital admission.

In addition, patients may also present with sequelae of urinary tract obstruction such as renal cortical atrophy, hydronephrosis, kidney failure, polycythemia, hypertension, bladder or renal calculi, UTI or sepsis, or urinary ascites.

Hydronephrosis, Renal Cortical Atrophy, and Renal Failure

The volume of the unobstructed collecting system of a kidney is between 5 to 10 mL. Once a kidney or ureter becomes obstructed, the proximal collecting system will become dilated. Chronic urinary obstruction can lead to massive dilation. With this dilation, an increase in intraluminal pressure can occur proximal to the obstruction. This pressure and dilation will eventually affect the medulla of the kidney, with increasing pressure in the tubules of the cortex of the kidney causing cortical thinning and atrophy. In children, this can happen at an accelerated rate with minimal dilation or symptoms, so prompt management is required.

Although hydronephrosis is a strong sign of urinary tract obstruction, a renal cortical atrophy of the kidney is what leads to acute and chronic renal failure. Three mechanisms

have been proposed as to why hydronephrosis leads to renal failure. The first is pressure atrophy due to the rise of intraluminal pressure proximal to the obstruction, leading to damage to the cortex of the kidney. The second is an intrarenal reflux, most commonly seen in children with vesicoureteral reflux, which is the reflux of urine into the ureters and kidneys from the bladder during micturition due to insufficient tunnel length of the intravesical portion of the ureters, causing the ureters to be open during a bladder contraction. This reflux can cause damage to both the medulla and cortex of the kidney, especially if the urine is infected.[4,5] The third cause is ischemia due to a lack of renal blood flow. This can be caused by a massive renal pelvis impeding blood flow through the renal hilum. A chronic obstruction also can lead to decreased renal blood flow, which can lead to ischemic atrophy of the kidney.

Urinary Tract Infection Associated with Obstruction

A UTI can be a serious or even a fatal complication of urinary tract obstruction. The stasis of urine behind the obstruction provides a perfect medium for bacteria to proliferate. The symptoms of a UTI depend on the location and severity of the infection. Acute pyelonephritis will usually present with fever and ipsilateral flank or back pain and costovertebral angle tenderness, whereas acute cystitis or urethritis will present with suprapubic tenderness, dysuria, and increased frequency or urgency.

The main goal of treating a UTI with urinary tract obstruction is to relieve the obstruction if possible and place the patient on empiric antibiotics while the culture is pending. Upper urinary tract obstruction often requires ureteral stenting and, less frequently, nephrostomy tube placement. Lower urinary tract obstruction typically requires Foley catheter placement. The urine culture can be negative if the sample is taken distal to the obstruction. If the patient continues to have fevers 48 hours after appropriate antibiotics are started, the patient should undergo repeat imaging.

Urolithiasis

Urinary tract calculi are a common cause of obstruction in the upper urinary tract (Fig. 19.1). Ureteral calculi can obstruct the ureter and may require surgical management if medical expulsive therapy is unsuccessful or not advisable. In the absence of infection, conservative management for a patient with a ureteral stone smaller than 5 mm is appropriate as there is a high likelihood of stone passage.[3] Ureteral stones larger than 5 mm are unlikely to pass spontaneously and may require earlier surgical intervention.

Complicated upper urinary tract calculi are often struvite (magnesium ammonium phosphate–calcium carbonate) and result from the association of urinary infection with urea-splitting bacteria. The current management of these stones is an aggressive surgical approach. The American Urological Association (AUA) guidelines strongly recommend endoscopic approaches for these stones to remove all stone burden within the collecting system.[3] These stones have a natural tendency to reoccur if all stone burden is not removed or if the infection causing the stones is not eradicated.[3]

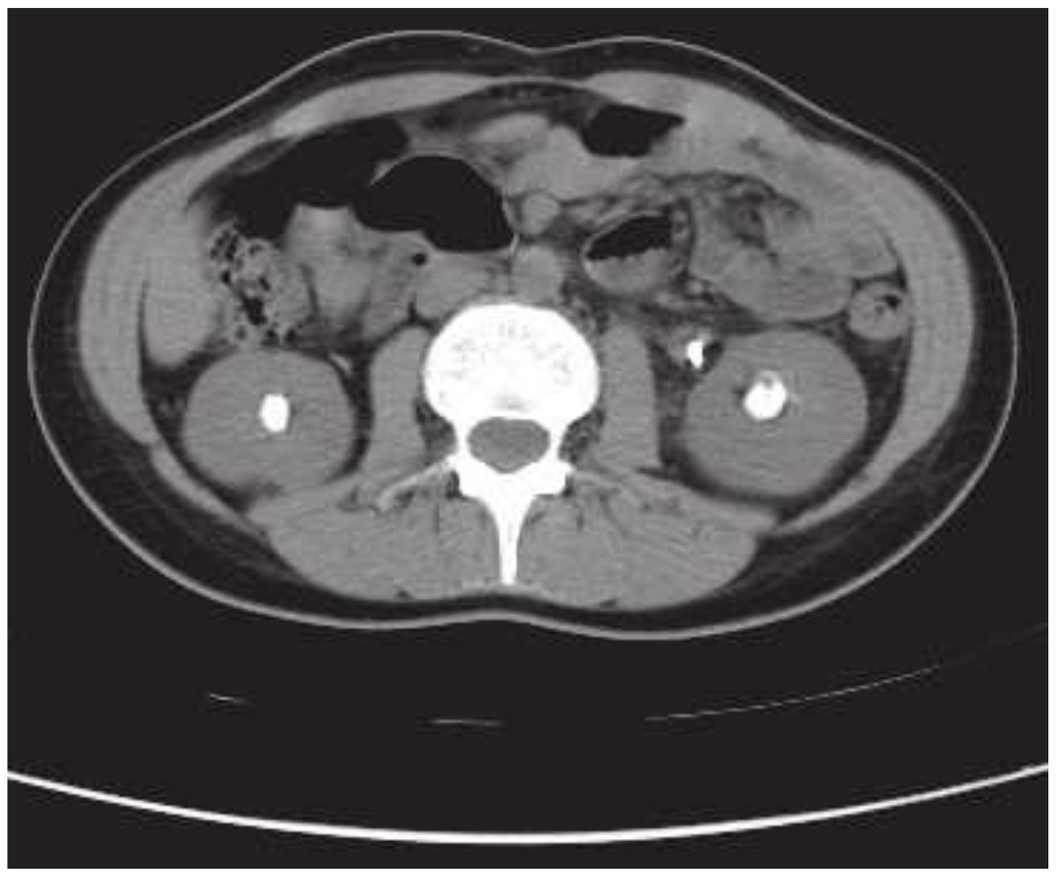


FIGURE 19.1 An axial computed tomography image of a known cystine stone former showing bilateral lower pole stones and a stone within the patient's left proximal ureter.

Hypertension Associated with Urinary Tract Obstruction

In urinary tract obstruction, hypertension may develop for many reasons, including extracellular fluid (ECF) volume expansion, increased renin secretion, or decreased synthesis of prostaglandins. After the relief of bilateral obstruction, volume overload from fluid resuscitation may result in temporary hypertension.

A unilateral urinary tract obstruction (UUO) has been proven as a cause of temporary hypertension. The relief of temporary hypertension and the return of normal renal vein renin levels has been shown with the removal of upper tract unilateral obstruction compared to the obstructed kidney.[5–7] This sequence resembles the hypertension associated with unilateral renal artery stenosis both in rats and humans. Indeed, ureteral occlusion does cause an acute increase in renin release from the obstructed kidney.[8,9]

PATHOPHYSIOLOGY OF URINARY TRACT OBSTRUCTION

The aforementioned clinical syndromes also influence the pathophysiology of obstructive uropathy. Specifically, the age of the patient and level of obstruction are important, as well as the severity and duration of obstruction and the presence or absence of complications such as infection or sepsis.

The effects of obstruction on renal function may be discussed by considering the functions of the kidney, including renal blood flow (RBF), glomerular filtration rate (GFR), and tubular function. The endocrine–metabolic aspects of renal function must be considered, particularly the renin–angiotensin and prostaglandin systems.

Hydrodynamics in the Urinary Tract

Under normal conditions, the propulsion of urine from the kidney to the bladder under normal conditions is the result of three factors: (1) hydrostatic pressure, (2) ureteral and pelvic peristalsis, and (3) the rate of urine flow.[10,11] The urinary collecting system is lined by a transitional epithelium and surrounded by circular and longitudinal layers of smooth muscle. Action potentials that originate in smooth muscle cells of the minor calyx in the renal pelvis are conducted along the pelvis and down the ureter. The urine enters the pelvis and the proximal ureter in a passive state, but the presence of urine in the ureter causes peristalsis of the ureteral wall. Dilation of the ureter clearly interferes with this process. The nature of normal ureteral peristalsis also prevents retrograde transmission of the pressures generated during coaptation (10 to 25 mm Hg) to the renal pelvis, and renal pelvic pressures seldom rise above 4 mm Hg.[10]

After acute obstruction, smooth muscle fibers increase in tension within the urinary tract in response to the increase in pressure by contracting. With persistent obstruction, tension may decrease as the smooth muscle of the ureteric wall contracts with less force, and dilation of the wall continues. With a superimposed urinary infection, as often occurs in chronic obstruction, the loss of muscle tone is even more dramatic and progressive dilation occurs with no further increase or decrease in wall tension.[12]

Evidence shows that even with a completely obstructed kidney, filtration at the level of the glomeruli does not stop. Urine may escape through the walls of the collecting system, known as forniceal extravasation, and can relieve pressure within the collecting system. In addition to renal tubular reabsorption, urine may be reabsorbed directly across the walls of the renal pelvis through the lymphatics (pyelolymphatic reflux) or the renal venous system (pyelovenous reflux). The lymphatic flow from the kidneys is increased markedly during acute and chronic obstruction. This is most likely due to increased pressure within the venous system of the kidney rather than to urine reabsorbed from the renal pelvis. [13]

Changes in Intrarenal Pressure, Glomerular Filtration, and Renal Hemodynamics

The factors determining the fall in GFR during obstructive uropathy have been clarified by micropuncture studies of glomerular dynamics in experimental animals. Changes in intratubular pressure including stop-flow pressure, which represents glomerular filtration pressure, have provided important insight on the pathophysiology of obstructive nephropathy after unilateral (UUO) and bilateral ureteral obstruction (BUO).

Glomerular filtration may be expressed by the formula: $GFR = K_f(P_{GC} - [P_T + \pi_{GS}])$, where K_f is the glomerular ultrafiltration coefficient, P_{GC} is the glomerular capillary pressure, P_T is the intratubular pressure, and π_{GC} is the mean oncotic pressure along the glomerular capillary. δP is the difference between P_{GC} and P_T and represents the pressure gradient across the glomerular capillary wall. An increase in P_T without a concomitant increase in P_{GC} will result in a decrease in δP, the driving force for filtration.[3] Glomerular filtration also depends on the rate of blood flow entering the glomerular capillary. A decrease in glomerular blood flow during obstructive uropathy will decrease GFR because the rate at which capillary oncotic pressure rises is accelerated when a given volume of filtrate is removed from a smaller volume of blood. Both glomerular blood flow and hydrostatic pressure depend on renal vascular resistance, which is largely divided between two resistance segments—the preglomerular segment (afferent arteriole) and the postglomerular segment (efferent glomerular arteriole). Peritubular capillaries may also provide a postglomerular vascular resistance in urinary tract obstruction.

During Obstruction

After either a unilateral or bilateral ureteral obstruction, renal blood flow increases significantly (15% to 25%) in the first 1 to 2 hours and is accompanied by an increase in P_T. This decrease in renal vascular resistance immediately after a complete ureteral obstruction is probably secondary to the synthesis and release of vasodilator prostaglandin (PG; see the following paragraphs). With persisting unilateral or bilateral ureteral obstruction, renal blood flow progressively decreases to 40% to 50% of normal levels by 24 hours.[14,15] GFR is more markedly reduced than renal blood flow; that is, filtration fraction is low, and GFR is 20% to 30% of normal levels in both UUO and BUO obstruction after 24 hours.[16,17] However, the site of changes in intrarenal vascular resistance and, therefore, the mechanisms responsible for the decrease in GFR differ between UUO and BUO (Table 19.2).

After an acute UUO, there is an immediate increase in intrapelvic and proximal tubular hydrostatic pressure, the severity of which depends on the diuretic state of the animal.[18] Despite this increase in intratubular pressure, the GFR in surface nephrons is about 80% of normal because of an increase in glomerular capillary hydrostatic pressure and glomerular plasma flow secondary to afferent arteriolar dilation and decreased renal vascular resistance.[19] As unilateral obstruction persists, progressive vasoconstriction and a decrease in nephron filtration rate develop within about 4 hours. By 24 hours, surface nephron GFR is 30% of normal because of a decrease in glomerular capillary pressure and plasma flow associated with an increase in renal vascular resistance, presumably at the level of the afferent arteriole.[16] Proximal intratubular pressure is now normal rather than increased as during the first few hours of obstruction.[14,16,20–22]

During acute BUO, proximal tubular hydrostatic pressure increases to a higher level than after unilateral obstruction

19.2 Comparison of Changes in Hemodynamics and Filtration Dynamics in Complete Unilateral and Bilateral Ureteral Obstruction

	Unilateral Ureteral Obstruction			Bilateral Ureteral Obstruction		
	During			During		
	1–2 hr	18–24 hr	After	1–2 hr	18–24 hr	After
RBF	↑	↓	↓	↑	↓	↓
P_T	↑	N	N	↑↑	↑	N
GFR	↓	↓↓	↓↓	↓	↓↓	↓↓
GPF	↑	↓	↓	↑	N	↓
P_{GC}	↑	↓	↓	↑	N	↓

RBF, renal blood flow; P_T, proximal tubular pressure; GFR, glomerular filtration rate; GPF, glomerular plasma flow; P_{GC}, glomerular capillary pressure; ↑, increase; ↓, decrease; N, normal.

and, in sharp contrast to unilateral obstruction, intrarenal pressure remains twice the normal level after 24 hours. Renal blood flow changes are similar to unilateral obstruction.[15,23,24] The surface nephron GFR after 24 hours is reduced to about 30% of normal levels in BUO as in UUO. However, the decrease in GFR in BUO is because of a persistent increase in proximal tubular hydrostatic pressure, whereas glomerular capillary pressure and plasma flow are normal.[17] The predominant site of increased vascular resistance thus appears to be the efferent arteriole during a bilateral obstruction, compared to the afferent arteriole with a unilateral obstruction.

In addition to increasing renal blood flow for several hours and increasing pressures in the ureter and renal tubules, an acute ureteral obstruction has hemodynamic effects.[25] Several effects have been observed and include blunted vasoconstrictor responses and decreased autoregulation of renal blood flow when ureteral pressure exceeds 75 mm Hg. RBF is directly related to arterial pressure, and vasodilator effects may be shifted due to increased levels of renin being released from the obstructed kidney.[26–30]

During a chronic complete ureteral obstruction, renal blood flow progressively decreases. After 1 day of a complete obstruction, there is a 40% to 50% decrease in renal blood flow to the kidneys. Prolonged UUO is associated with a further decrease in blood flow to 30% at 6 days, 20% at 2 weeks, and 12% at 8 weeks.[31] The glomerular filtration rate depends on the fluid status of the patient and may not change during a chronic partial obstruction.

After Relief of Obstruction

At 1 day postrelief of a unilateral obstruction, GFR remains reduced and renal vascular resistance is increased. However, they soon normalize after 1 week.[16,21,22] Following the relief of bilateral obstruction for 24 hours, intratubular pressure decreases from elevated levels to normal, but glomerular capillary pressure and plasma flow also decrease because of afferent arteriolar vasoconstriction, resulting in a persistent decrease in GFR.[17,24,27,32,33] Afferent arteriolar pressures appear to keep renal blood flow and glomerular filtration similar the first day after relief of the obstruction.

GFR recovery is dependent on both the duration and the severity of the obstruction. Studies in dogs have looked at reversible factors in the recovery of GFR.[34,35] The maximum GFR retained after obstruction of 7 days' duration was about two-thirds of the GFR before obstruction. When the duration of obstruction was 1 month, the GFR returned to only 20% of its original function. In general, the maximal degree of recovery was observed within 2 to 4 weeks after release of the obstruction.[34,35]

Nephron function after recovery from an obstruction is not uniform throughout the kidney. There is a decrease in the functioning juxtamedullary nephrons in the superficial cortex of rats after 1 day of UUO.[21] One study showed an 85% recovery of the whole obstructed kidney and 100% function in the contralateral nonobstructed kidney after 2 weeks with a return to normal GFR.[36] The single-nephron GFR of functioning superficial and juxtamedullary nephrons was higher postobstruction than in the contralateral normal kidney.

The findings of function recovery after an obstruction have also been observed in humans, but the number of supporting clinical studies is limited to children with congenital lesions. These studies have shown that the earlier the relief of obstruction, the greater return in GFR during follow-up.[37,38]

Mechanisms of Changes in Glomerular Filtration Rates and Renal Hemodynamics

Changes in renal hemodynamics and GFR in obstructive nephropathy are of interest because of their clinical significance. Nishikawa et al.[39] demonstrated increased PG synthesis in the hydronephrotic isolated perfused kidney, which leads to changes within the kidney on a metabolic and hemodynamic level. Vasodilator and vasoconstrictor PGs have been studied in relation to the functional changes of urinary tract obstruction with respect to renal hemodynamics.

The early hemodynamic consequences of acute ureteral obstruction are blunted or prevented by the inhibition of PG synthesis after indomethacin. This was studied by giving it prior to obstruction, then measuring intraureteral pressure of the affected kidney.[40] The studies also found decreases in glomerular capillary pressures and proximal tubular pressure with its administration.[40–44] The increase in RBF beginning immediately after UUO is prevented by indomethacin or meclofenamate and a similar effect is seen on the vasodilatation that follows BUO.[40–44,46,47] The impairment of autoregulation that is seen in the kidney with ureteral obstruction is prevented by indomethacin, and normal autoregulation of renal blood flow with changes in arterial pressure is restored in the obstructed kidney.[44] A recent study in 2010 showed that Cox-2 inhibition led to decreased intrarenal levels of prostaglandins in bilateral ureteral obstruction with concomitant ureteral relaxation and decreased contractility in rats.[48]

Two vasoconstrictors, TXA_2 and Ang II, play a major role in the decrease in renal plasma flow per nephron and the decline in single-nephron GFR seen following ureteral obstruction.[47] Both TXA_2[49] and Ang II[50] are able to contract mesangial cells in culture and reduce the glomerular capillary area available for filtration, which leads to a decrease in GFR. Rats pretreated with angiotensin-converting enzyme (ACE) inhibitors and thromboxane synthesis inhibitors before ureteral obstruction were observed not to show a decline in renal function.[51]

The Renin–Angiotensin System

Maximal renin secretion into the renal veins has been observed shortly after ureteral obstruction due to afferent arteriole dilatation associated with the aforementioned postobstructive renal hemodynamics.[52,53] This secretion has been shown to be completely halted by the preobstruction administration of cyclooxygenase inhibitors, leading to the conclusion that renal cortical prostaglandins may act as a strong stimulus for renin secretion.[54,55]

Angiotensin II plays a central role in the modulation of hemodynamic changes following a ureteral obstruction. The preobstruction administration of captopril[56,57] and enalapril[57] appear to be highly effective in ameliorating the decline in GFR and renal plasma flow in response to a ureteral obstruction.[56] Rising levels of angiotensin II also can lead to tubulointerstitial fibrosis during an obstruction due to increased levels of tumor necrosis factor alpha (TNF-α) within the first few hours of an obstruction. This cascade of events can lead to tubular cell apoptosis.[3]

Postobstructive Diuresis

During acute partial UUO, there is a significant decrease in sodium, potassium, and solute excretion with a decrease in urine sodium concentration and an increase in urine osmolality.[58] This is due to the increase in both sodium and water reabsorption within the tubules during a partial obstruction,[58] which had first been thought to be due to a decline in GFR seen with obstruction.[49,59] This reverse phenomenon seen with increased blood flow and increased reabsorption of sodium with the juxtaglomerular cortical nephrons is not expected and appears not to be due to renal nerve activity.[60,61]

During chronic partial obstruction, the gradual decrease in GFR is accompanied by an increase in the fractional excretion of filtered sodium, thus indicating decreased tubular reabsorption. A micropuncture of surface nephrons in chronic partial UUO or of a solitary kidney with a partial obstruction in the rat indicates that the increased fractional excretion of sodium is due to decreased reabsorption in the distal tubule or the collecting duct of the nephron.[62]

After the relief of chronic partial UUO in humans, there is no increase in absolute sodium and water excretion from the hydronephrotic kidney, although a decreased concentrating ability and an increased fractional excretion of sodium are observed.[63] Other factors, such as volume expansion or a further reduction in functioning nephron mass with uremia, are necessary to cause an increase in salt and water excretion (postobstructive diuresis) following the relief of an obstruction.

After the relief of a complete obstruction of both kidneys or a solitary kidney, there is a prolonged diuresis due to massive losses of water, sodium, and other solutes. A single study showed urine output can equal one-half of the GFR, indicating a dramatic decrease in the reabsorptive ability of the nephron.[64] If not replaced, such losses can lead to severe hypovolemia and life-threatening electrolyte imbalance due to significant losses in sodium and water from an inability to reabsorb them in the nephron.[65,67–70] However, a brisk diuresis following the relief of a urinary tract obstruction may also be physiologically appropriate or even iatrogenic rather than an indicator of tubular malfunction. The degree of fluid replacement in a given case will depend on the mechanism involved. Salt diuresis due to ECF expansion can lead to hypervolemia and an increase in atrial natriuretic peptide (ANP) levels, stimulating a diuresis after the relief of the obstruction. This can be continued after the relief of an obstruction iatrogenically due to physicians keeping up with urine output during the diuresis with intravenous fluids that maintain a high ECF volume. Another factor could be the amount of retained urea within the bloodstream and nephrons that act as an osmotic diureticlike mannitol,[65,67,71] leading to natriuresis. Recovery of GFR is dependent on the duration of obstruction and will dictate the length of postobstructive diuresis.[72]

Impaired Urinary-Concentrating Ability

A well documented feature of obstructive uropathy is the loss of the ability to concentrate urine. One exception is during an acute partial obstruction due to the increase in renal tubular absorption.[73] A recent relief of an obstruction or chronic obstruction has been well documented to show a decrease in renal concentration ability.[74,75] Patients with a marked impairment may present with nephrogenic diabetes insipidus and may demonstrate polyuria and persistently hypotonic urine.[76–79] Hypernatremia and severe dehydration can develop if fluid intake is not adequate.[80] The distal portions of the nephrons, particularly the juxtamedullary nephrons, are the major sites affected.[81,82] Aquaporins are a family of membrane water channels. Aquaporin-2 (AQP2) is predominantly found in the cytoplasm apical domain of the collecting duct principal cells. Berlyne and colleagues[83,84] report that AQP2 expression is decreased in the setting of bilateral or unilateral obstruction. The reduction in aquaporin expression may explain the postobstructive polyuria and the impairment in urinary-concentrating capacity found in bilateral ureteral obstruction due to a decrease in water reabsorption.

Recovery of Tubular Function after Obstruction

Although the acute effects of relief of a ureteral obstruction are well described, the long-term effects are less known. Studies after the release of a UUO after 24 hours in rats revealed that abnormalities of tubular function persist beyond a time (14 days) when the whole kidney GFR had returned to normal.[36]

DIAGNOSIS OF URINARY TRACT OBSTRUCTION

Symptoms and Signs

The diagnosis of urinary tract obstruction is not always straightforward and is sometimes found incidentally with routine imaging or lab work. A working diagnosis of a urinary tract obstruction should always be included in the differential when presented with a patient with acute or chronic renal failure. Early diagnosis is important to resolve the obstruction with a greater probability of reversing damage.

Symptoms of urinary tract obstruction more than likely result from the origin of the obstruction and the related effects on the urinary tract. A lower tract obstruction usually results in urinary urgency, frequency, hesitancy, slow stream, retention, or dysuria. An upper tract obstruction can result in abdominal or flank pain, nausea, or vomiting, or may be asymptomatic. Both may have gross hematuria as a symptom. A past medical history may suggest another probable etiology, such as a history of stones or malignancy.

Signs of a urinary tract obstruction can also be found on a physical examination. Chronic urinary tract obstruction can lead to severe renal failure that presents as peripheral edema, pulmonary congestion, hypertension, or congestive heart failure. Patients may also present with pallor due to anemia of chronic renal failure. On a physical exam, the kidney may be palpated with severe hydronephrosis from an obstruction or from a large mass on the kidney such as a malignancy. A palpable bladder may result from chronic or acute urinary retention in the bladder from an outlet obstruction. A prostate exam should be performed in all men and a formal pelvic examination should be performed in all women presenting with signs of a urinary tract obstruction.

Urinalysis and Laboratory Findings

A urinalysis should be one of the first tests performed when a urinary tract obstruction is suspected. White blood cells (WBCs) in the urine can help identify infection or inflammation. WBCs may be present in the urine for reasons such as ureteral stent placement; stones in the kidney, ureter, or bladder; a malignancy; or recent instrumentation of the urinary tract. Leukocyte esterase or nitrate-positive urine usually indicates an infection or a chronic colonization of the urinary tract. All urine that is positive for WBCs, leukocyte esterase, or nitrates should be sent for urine culture and antibiotic sensitivity. Red blood cells (RBCs) in the urine can be present in an infection, stones, clots, trauma, nephrologic causes, or a tumor within the urinary tract. A few RBCs in the urine can be present without significant pathology. Significant hematuria in a patient should be evaluated by a urologist with a urine culture, a urine cytology, an upper tract imaging, and a cystoscopy.

A serum blood urea nitrogen (BUN) and a serum creatinine can assess the degree of renal insufficiency associated with the obstruction. A renal insufficiency can result from bilateral renal obstruction, solitary kidney obstruction, or a complete bladder obstruction. It is rare to see renal insufficiency in a unilateral obstruction with a normal contralateral kidney unless the patient is severely dehydrated. There are many electrolyte abnormalities that can be associated with a urinary tract obstruction, as discussed in earlier sections of this chapter. A basic metabolic panel will help identify these abnormalities. Hyperkalemia and acidosis may be present and, if severe, should be corrected as soon as possible.

A complete blood count (CBC) should be drawn during the workup. An elevated count can be seen in the presence of an infection or sepsis. Anemia can be detected in the face of chronic renal insufficiency, malignancy, or in acute processes such as blood loss.

Imaging of the Urinary System

Ultrasound

Ultrasonography of the kidneys and bladder is a useful initial imaging modality. This noninvasive and inexpensive test is especially helpful when an allergy to intravenous contrast or an elevated creatinine is present. In children and in pregnant patients, it is most often the initial imaging test. Ultrasonography is sensitive for the detection of renal

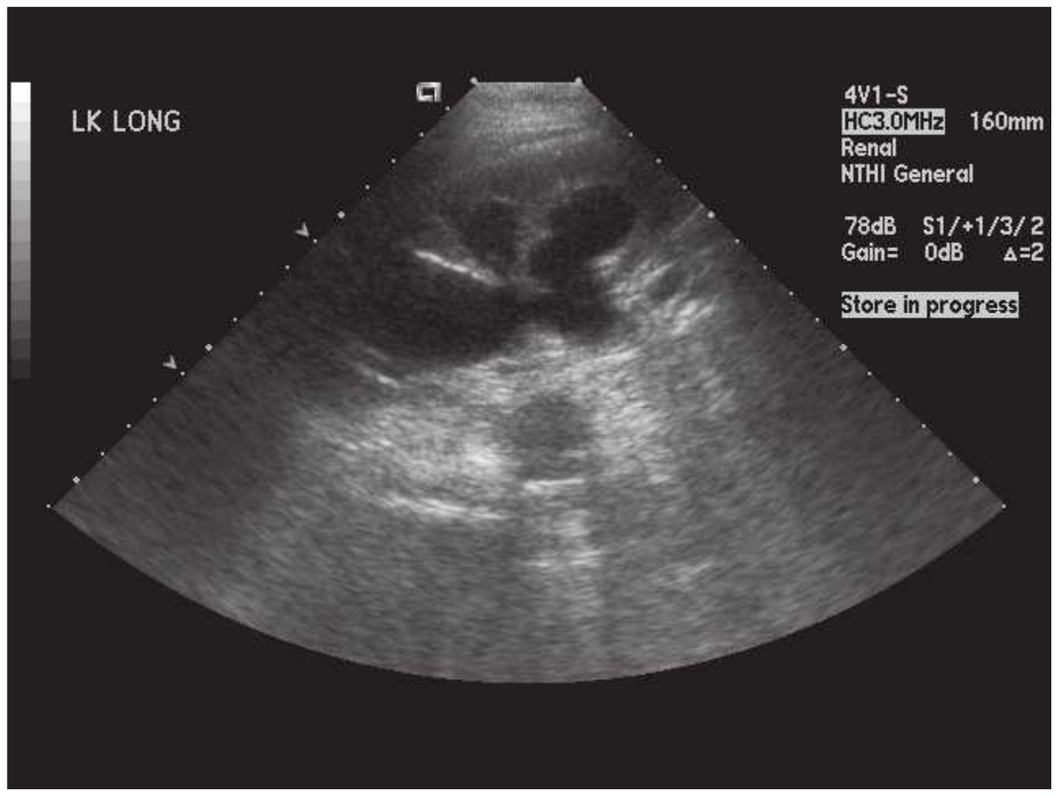


FIGURE 19.2 A renal ultrasound photo showing hydroureteronephrosis of a left kidney due to a bladder outlet obstruction in a patient with known prostate cancer.

masses, hydroureteronephrosis (Fig. 19.2), or a distended bladder (Fig. 19.3). One pitfall of this imaging modality is that the quality and accuracy of the images are dependent on the ultrasonographer. In adults, if the ultrasound findings are abnormal, follow-up imaging is recommended. The combination of renal ultrasonography with flat-plate radiography of the kidneys, ureters, and bladder (KUB) is an inexpensive initial combination.

Intravenous Pyelography

Intravenous pyelography (IVP) involves contrast injection and a series of KUB images over 15 to 30 minutes (Fig. 19.4). It can be performed in patients with a creatinine ≤ 1.5 mg

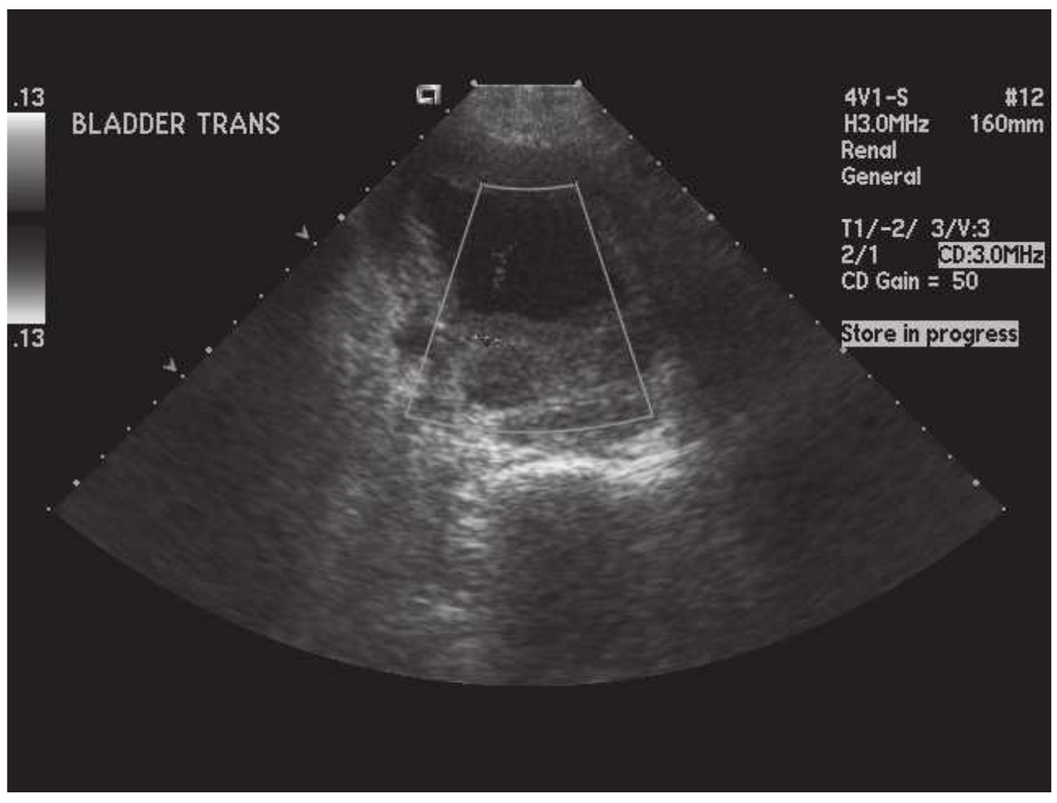


FIGURE 19.3 A fully distended bladder is shown here on ultrasound from a bladder outlet obstruction. Notice the thickened bladder wall circumferentially, which is often seen from a prolonged outlet obstruction from the prostate.

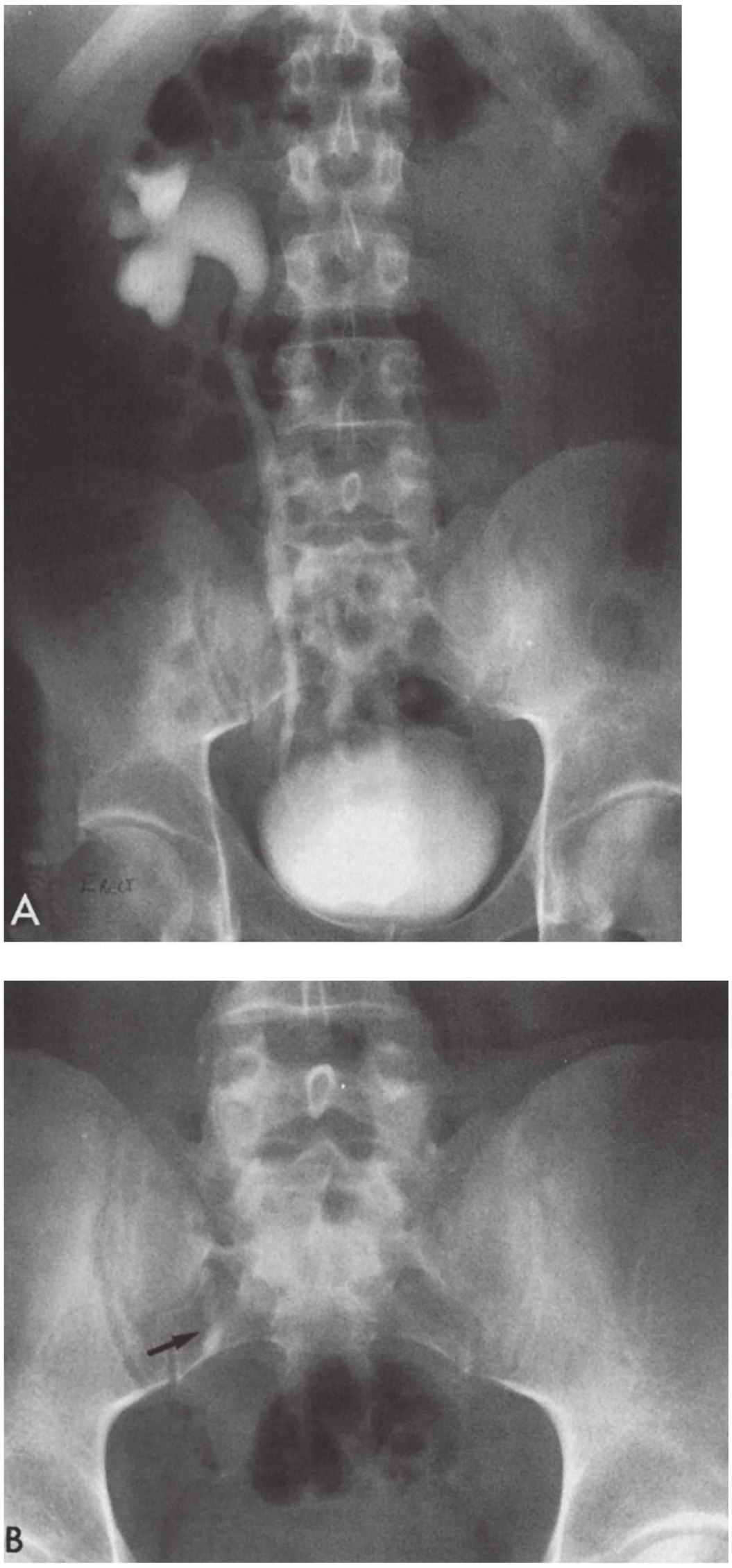


FIGURE 19.4 **A:** An intravenous pyelogram from a patient with right flank pain and hematuria intermittently for several weeks. The radiograph, taken 1 hour after the injection of contrast media, shows a markedly dilated right renal pelvis and a dilated completely filled right ureter. **B:** An examination of the plain radiograph before the dye injection shows several radiopaque stones overlying the sacrum. (From Flamenbaum W, Hamburger RJ. *Nephrology*. Philadelphia: Lippincott; 1982.)

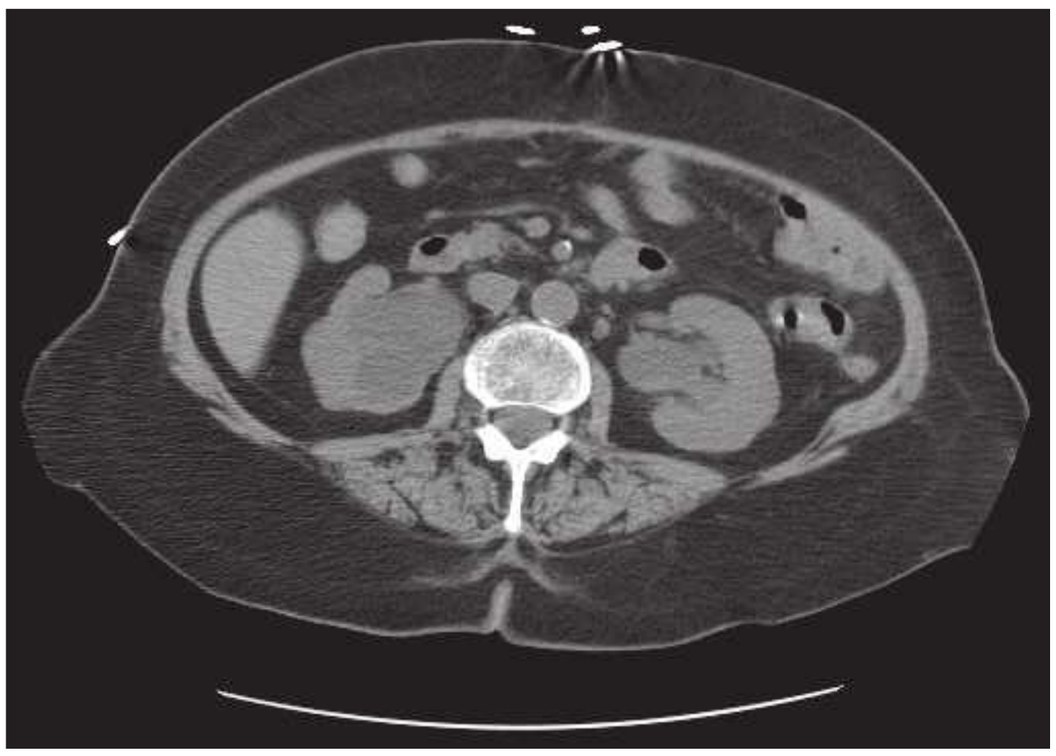


FIGURE 19.5 An axial computed tomography image showing severe hydronephrosis of the patient's right kidney and mild hydronephrosis of the left kidney.

per deciliter for visualization of the upper urinary tracts. It may not be performed in patients with an allergy to IV contrast. This test is unique in that it provides the physician with both anatomic and functional information. Delayed calyceal filling, delayed contrast excretion, prolonged nephrography results, and dilatation of the proximal tract above the obstruction characterize a urinary tract obstruction.

Computerized Tomography Scan

Computerized tomography (CT) imaging is very useful for the identification of urinary tract obstruction, often as a first-line radiographic test (Figs. 19.5 and 19.6). A CT scan will provide information regarding the urinary tract, as well as any possible retroperitoneal or pelvic conditions that can affect the urinary tract via a direct extension or an external compression. A noncontrast CT scan should be obtained first to assess for the presence of calculi within the urinary tract. A contrast-enhanced CT scan is very useful for evaluating renal and ureteral sources of obstruction such as urothelial neoplasms, strictures, and sloughed papilla. Delayed images can be used to monitor the collecting system of the kidney to search for any filling defects and to provide excellent reconstructions of the entire upper urinary tracts. Delayed nephrographic images of the kidney can be seen in pyelonephritis as well as in an obstruction of the kidney. A CT scan can also evaluate for other intra-abdominal processes that can cause similar presenting symptoms (e.g., appendicitis, cholecystitis, diverticulitis, ovarian cysts, abdominal aneurysms, small and large bowel masses with or without obstruction).

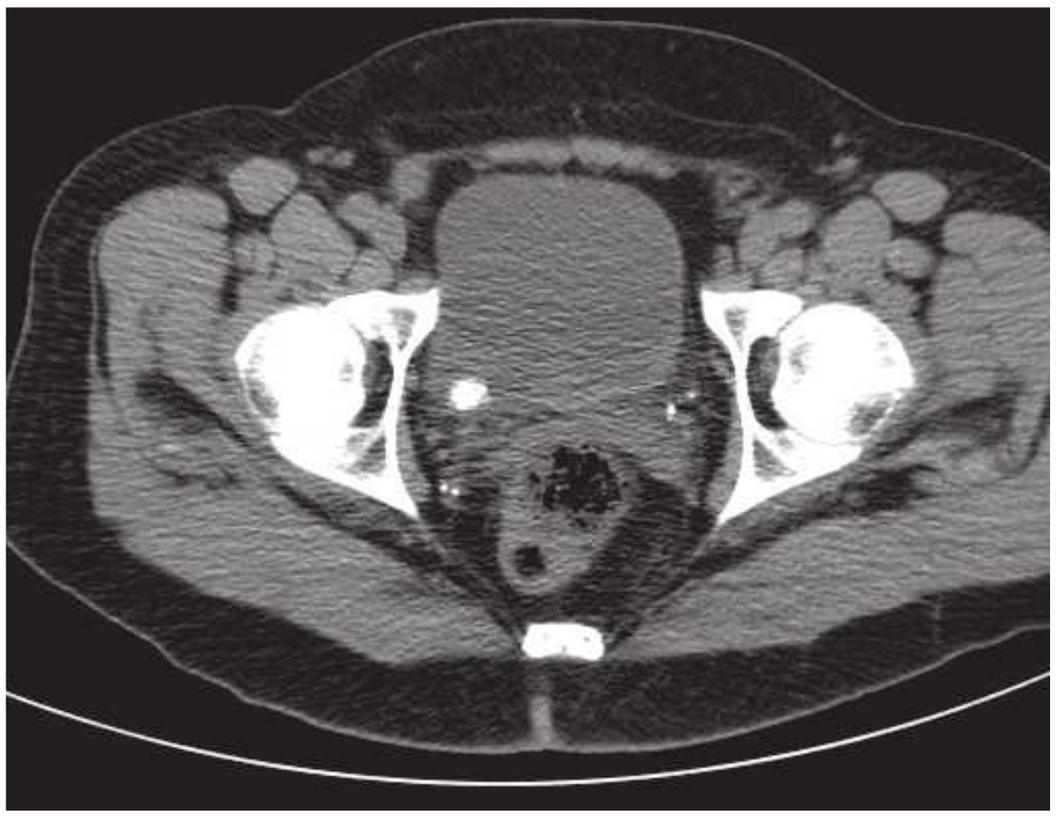


FIGURE 19.6 A computed tomography image of a pelvis showing a large bladder stone in the right side of the patient's bladder.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is not used as a first-line modality to evaluate the urinary tract. However, it may be useful in select patients for whom CT imaging is nondiagnostic. An MRI is also useful in delineating specific tissue planes for surgical planning of complex cases as well as in evaluating the presence or the extent of a renal vein or inferior vena cava thrombus associated with a renal cell carcinoma. One drawback to the MRI in imaging the kidney is that it is not very sensitive for urinary stones.

Radionucleotide Studies

A mercaptoacetyltriglycine (MAG)-3 renal scan can be performed to determine the differential function of the kidneys as well as to demonstrate the concentrating ability, excretion, and drainage of the urinary tract (Fig. 19.7). Furosemide can be administered with the renal scan to verify delayed excretion and the presence of obstruction. This imaging is commonly used to evaluate for a UPJ obstruction. An obstructed kidney has a $t_{1/2}$ greater than 20 minutes, normal is less than 10 minutes, and a $t_{1/2}$ between 10–20 minutes is indeterminate.

Retrograde Urethrogram

During a retrograde urethrogram, a catheter tip is placed into the urethral meatus and contrast dye is injected. Fluoroscopic imaging is then performed. This test is mostly performed in the setting of trauma where there is blood at the urethral meatus and a membranous urethral disruption is suspected. It is also helpful in defining the extent of urethral strictures prior to repair.

Retrograde Pyelogram

A retrograde pyelogram, also referred to as a pyeloureterogram, is performed during a cystoscopy in the operating room (Fig. 19.8). A 5- or 6-Fr cone-tip or open-ended ureteral catheter is placed through the cystoscope into the ureteral orifice. Contrast is then flushed through the catheter into the ureter under fluoroscopy. These images will show the anatomic outline of the ureters and the renal pelvis up to the level of obstruction or

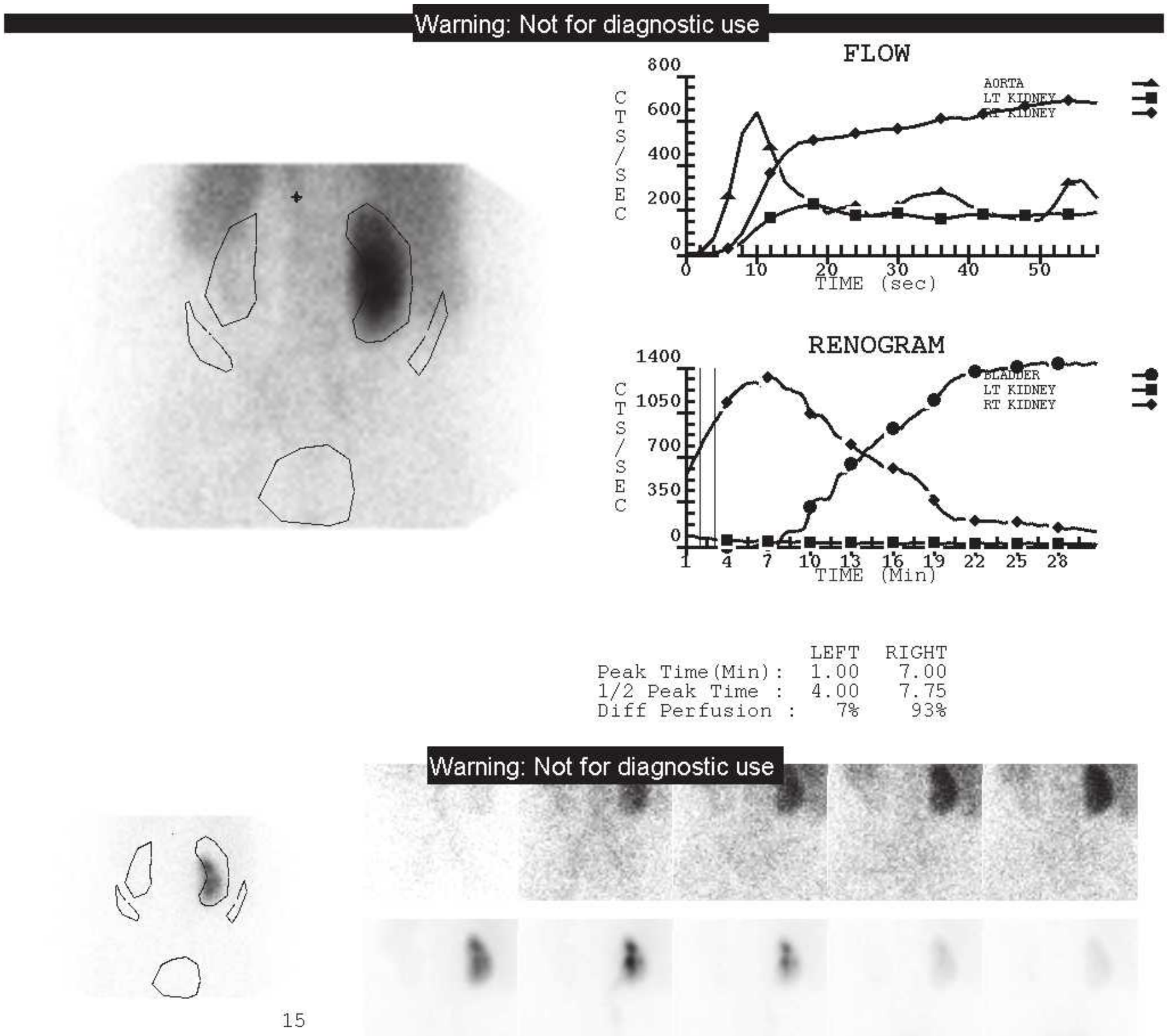


FIGURE 19.7 MAG3 scan images showing the perfusion and drainage studies of the test. This patient was found to have 93% function on the right and 7% function on the left with severe hydronephrosis from obstruction on the left side.

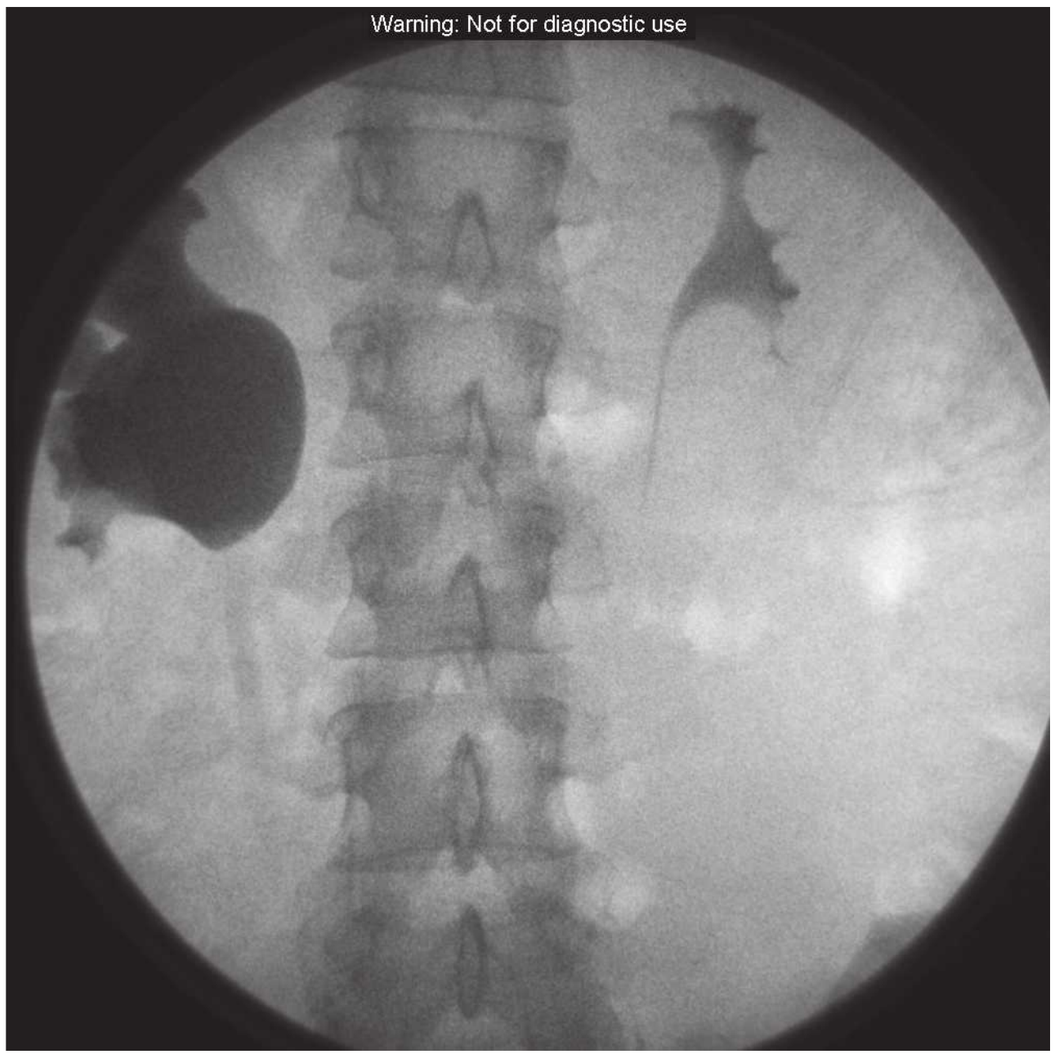


FIGURE 19.8 Bilateral retrograde pyelograms shown here with a normal pyelogram on the right and evidence of a ureteropelvic junction obstruction on the left with a large box-shaped renal pelvis and blunted calyces.

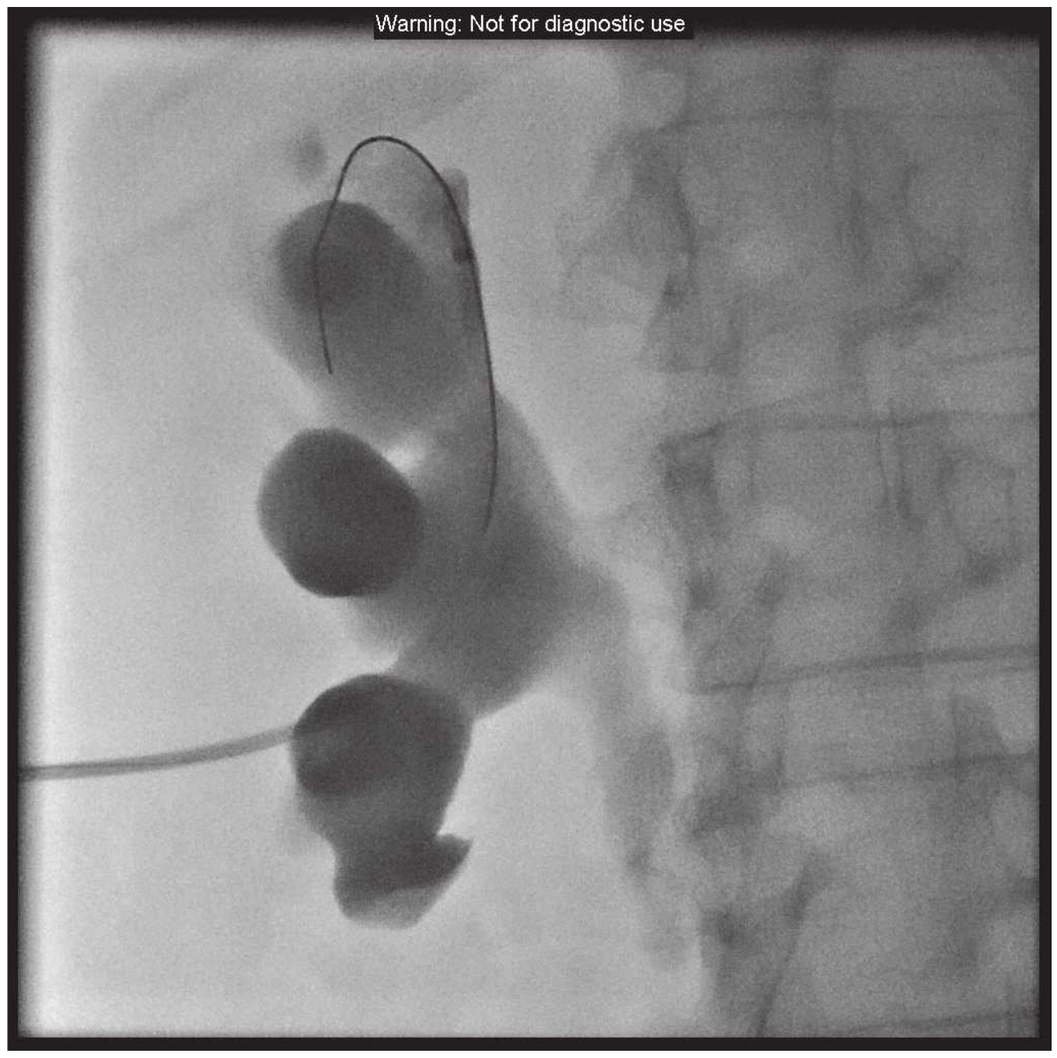


FIGURE 19.9 An antegrade nephrostogram showing the filling of the entire renal pelvis and calyces during percutaneous access of the right kidney.

will show filling defects in the presence of a mass, stone, or clot. Because the contrast load is not significantly absorbed, it may be used in patients with renal insufficiency. It may be also used in patients with an IV contrast allergy.

Nephrostogram

A nephrostogram can be used if the patient has a nephrostomy tube (Fig. 19.9). Contrast dye is flushed through the catheter under fluoroscopy in a similar fashion to the retrograde pyelogram. This imaging is helpful for the evaluation of renal and ureteral pathology when retrograde access is contraindicated or difficult, and it is often combined with interventional intent. This test can also be performed on patients with renal insufficiency or IV dye allergy.

Treatment of Urinary Tract Obstruction

Medical Therapy

The mainstay of treatment for urinary tract obstruction is to eliminate any life-threatening disorder, relieve any complete obstruction of the urinary tract to preserve renal function, and determine the cause of the obstruction (Table 19.3).

Septicemia with pyelonephritis is a possible sequelae of severe partial or complete obstruction of the urinary tract. A fever, an elevated white cell count, hypotension, or severe flank pain may alert the physician to this impending problem. Relief of the obstruction while starting empirical wide-spectrum antibiotics is the best form of treatment for this condition. This should be performed on an emergent basis as these patients may develop urosepsis. Antibiotics can be tailored to blood and urine culture results once they become available. A patient with complete urinary tract obstruction, any type of obstruction in a solitary kidney, obstruction with severe dehydration or uncontrolled pain, or with associated renal failure needs immediate attention.

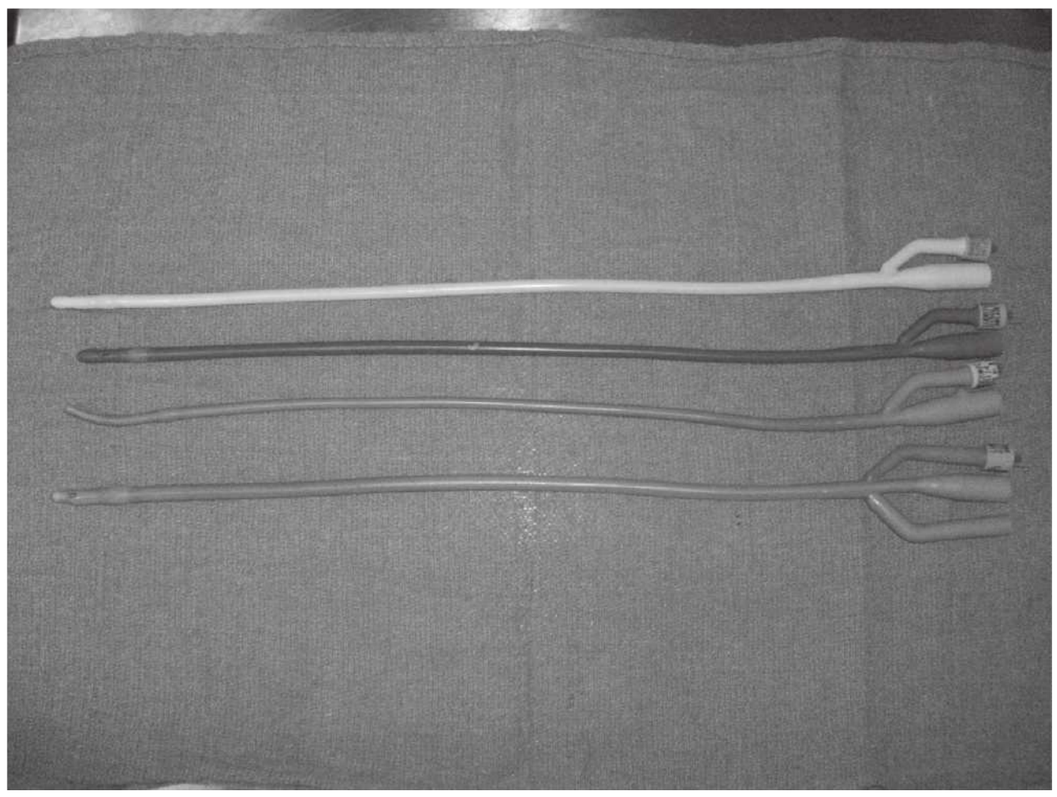
A partial urinary tract obstruction in the absence of infection or renal failure can be managed on an outpatient

19.3 Indications to Relieve Obstruction

Indications to Relieve Obstruction

Unilateral Obstruction (UUO)	Bilateral Obstruction (BUO)
Pain unrelieved by oral analgesics	Same as UUO
Signs and symptoms of sepsis	Elevated BUN or creatinine
Unable to tolerate food or water by mouth	Uremia
High grade obstruction	Anuria
	Hyperkalemia

BUN, blood urea nitrogen.



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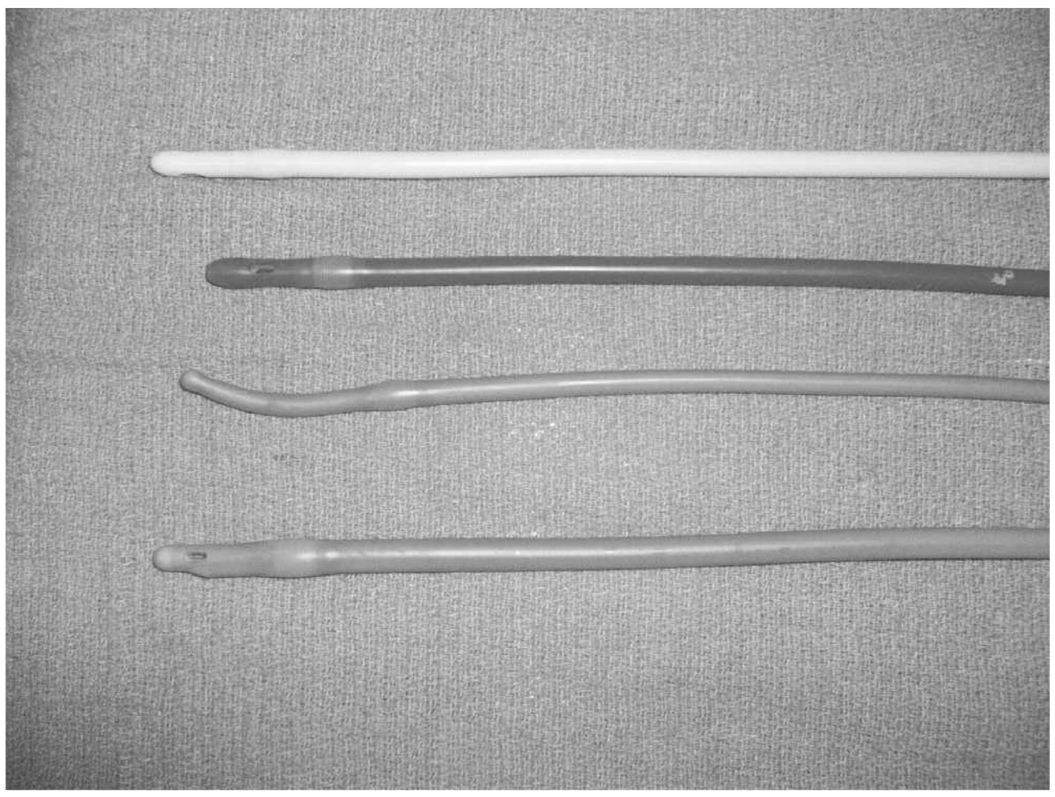

B

FIGURE 19.10 **A:** Various types of urethral catheters pictured here. Top to bottom: Regular Foley catheter, council-tip catheter, coude catheter, and three-way Foley catheter. **B:** A close up of the tips of each catheter.

basis with analgesics and antibiotics until a complete urologic evaluation can be performed. Antibiotics are often given for prophylaxis and should cover common urologic pathogens. Commonly used antibiotics include trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin, and cephalosporins. Pain secondary to urinary tract obstruction is often managed with opioid analgesics and nonsteroidal anti-inflammatory medications.

Acute or chronic renal failure with hyperkalemia, acidosis, convulsions, coma, or pericarditis is another life-threatening clinical presentation of obstructive uropathy that may require an immediate treatment by dialysis until definitive measures can be taken to correct the obstruction.

Identifying the cause of the obstruction is important for determining how and when treatment is to be performed. Urologic and nephrology evaluations are important components of the assessment.

Surgical Therapy

The goal of surgical intervention is to completely relieve the urinary tract obstruction. The specific type of surgical intervention depends on the level of obstruction and the underlying pathology.

Lower Urinary Tract Obstruction

Temporary forms of relief.

1. Urethral (Foley) catheter: A urethral catheter (size: 8 to 24 Fr) is a flexible catheter that extends from the bladder to the urethral meatus to allow urine to flow unimpeded from the bladder (Fig. 19.10). With a difficult catheter insertion, a urologist may need to use a coude catheter, cystoscopy, or urethral dilators for placement.
2. Suprapubic catheter: A catheter is placed through the anterior abdominal wall just above the pubic symphysis directly into the bladder. Performance can be at the bedside percutaneously or in the operating room as an open procedure. Ultrasound guidance should be used to locate the bladder if done at the bedside. An open approach in the operating room should be performed if the patient has had prior lower abdominal surgery.

Temporary forms of relief for an upper urinary tract obstruction include the following:

1. Ureteral stent: A ureteral stent is a flexible tube that extends from the renal pelvis down the ureter and into the bladder (Fig. 19.11). The stent curls at both ends

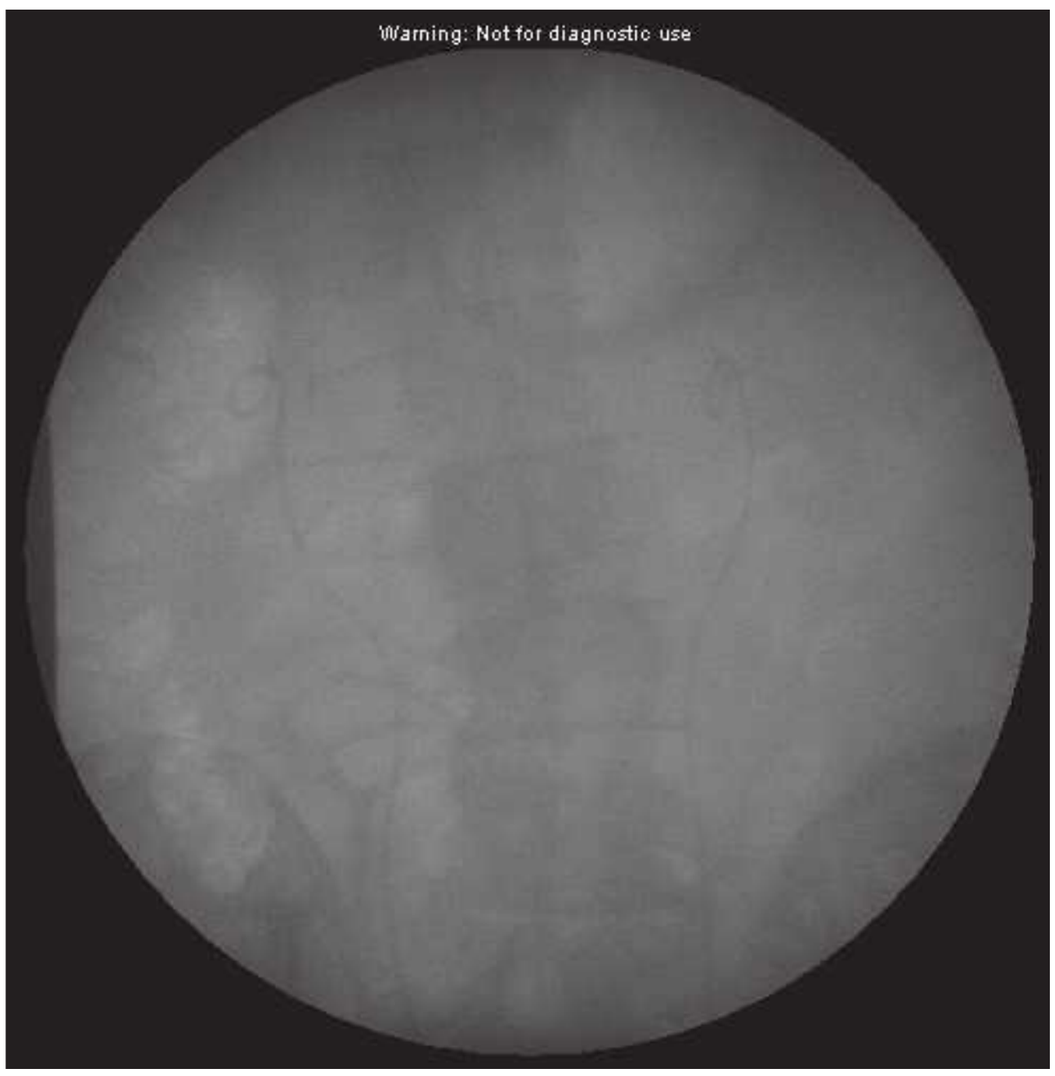

FIGURE 19.11 A fluoroscopic image showing bilateral double J stents in the correct position within both renal pelvises.

in the shape of a "J" to maintain its position. Common sizes range from 4.5 to 8 Fr in diameter. It can be placed during cystoscopy over a guide wire to relieve the obstruction along any point in the ureter. A ureteral stent generally needs to be replaced or removed after 3 to 6 months due to calcification or obstruction.

2. Nephrostomy tube: A nephrostomy tube is a flexible tube that is placed percutaneously through the flank/back directly into the renal pelvis. If a ureteral stent cannot be placed from the bladder, a nephrostomy tube can be placed into the kidney to allow the obstruction to be relieved. A stent may be placed through the nephrostomy tube tract over a guide wire in an antegrade fashion if a stent cannot be placed in a retrograde fashion.

Definitive Therapies

A meatal stenosis can be dilated with urethral sounds. Urethral strictures can be dilated with filiforms and followers, which are a series of dilators (Fig. 19.12). They may also be endoscopically incised at the 12 o'clock position with a cystoscopic knife under anesthesia.

The prostate and bladder neck are very common causes of a lower urinary tract obstruction in men of increasing age. The gold standard of relieving an obstruction secondary to benign prostatic hyperplasia (BPH) has been the transurethral resection of prostate (TURP) (Fig. 19.13). Traditionally, this has been performed with a resectoscope loop. Currently, most TURPs are performed endoscopically using a variety of lasers resulting in less bleeding and greater potential for the procedure being considered as an outpatient procedure. Bladder neck contractures may be seen after a TURP.

Upper Urinary Tract Obstruction

The most common causes of upper urinary tract obstruction are renal and ureteral calculi (Fig. 19.14). Urinary tract

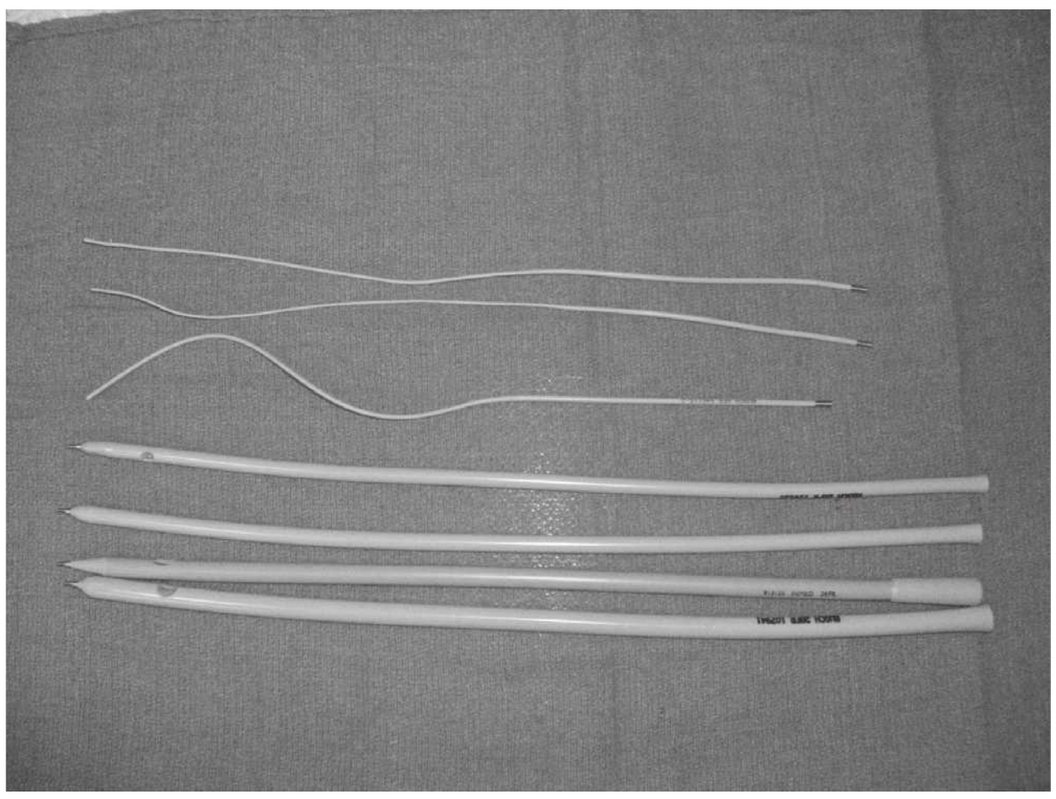

FIGURE 19.12 A picture of various filiforms (*top*) and followers (*bottom*) used for urethral dilatation.

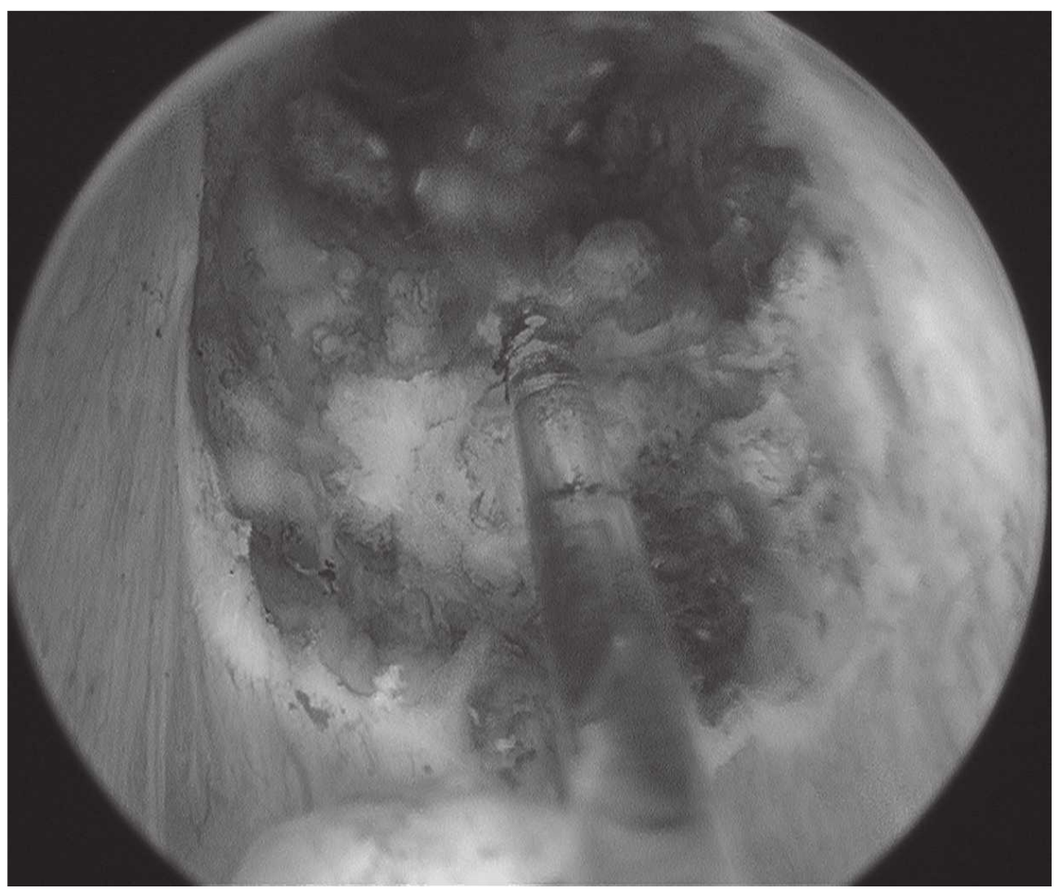

FIGURE 19.13 A single-shot photo of a laser transurethral resection of the prostate. This laser ablates the prostate tissue to widen the urethral channel through the prostate.

calculi can be removed surgically or monitored for spontaneous passage. In one study, ureteral calculi less than 4 mm, 4 to 6 mm, and greater than 6 mm have a chance of passing on their own in 80%, 59%, and 21% of patients, respectively.[85,86] Patients may have 4 weeks to pass a partially

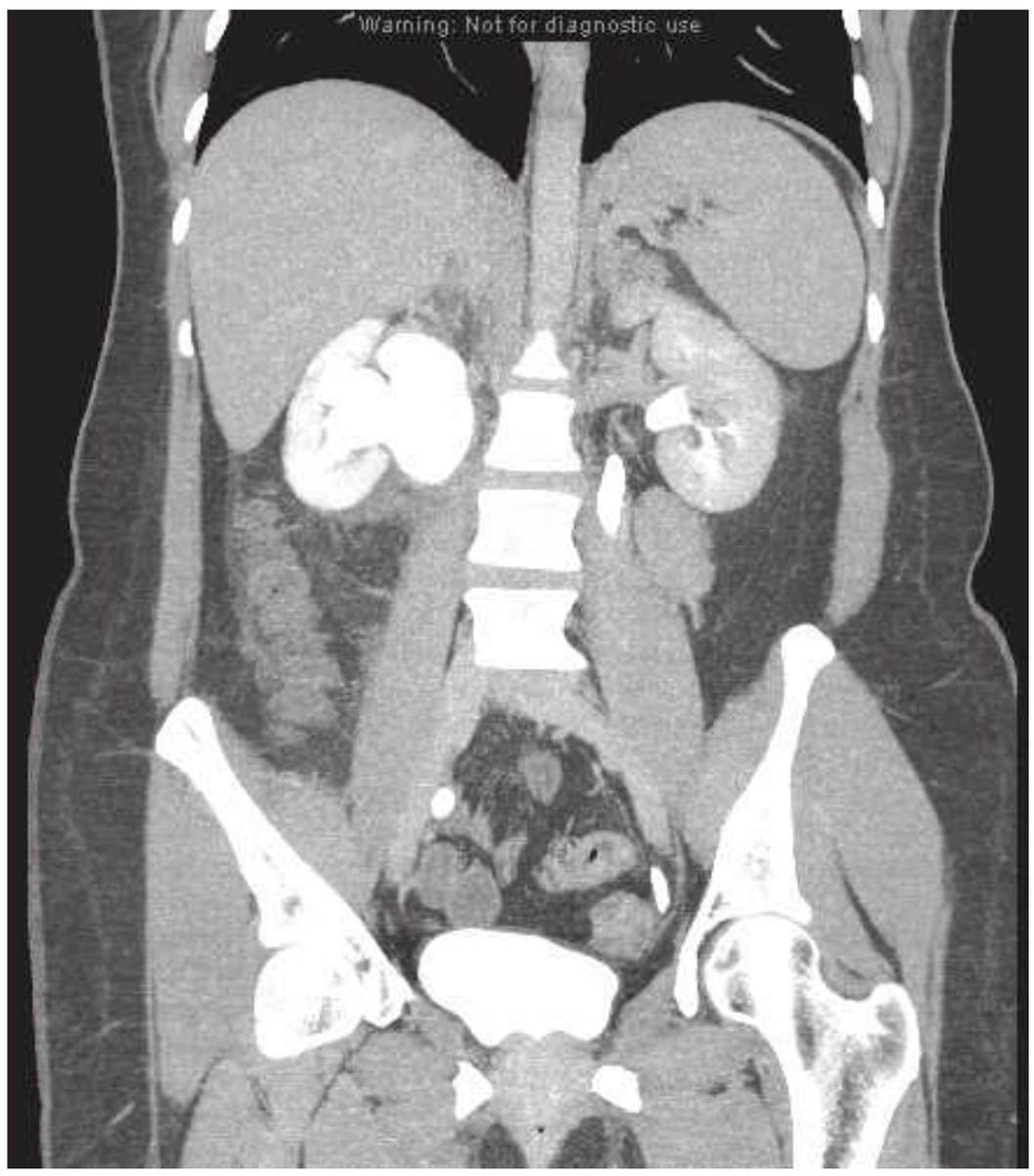

FIGURE 19.14 A coronal computed tomography image of the abdomen showing a ureteropelvic junction obstruction with hydronephrosis of the patient's right kidney on delayed contrast images with a normal left-sided collecting system.

obstructing stone before renal damage starts to occur. If the pain is intractable, then they have failed medical management and need operative intervention. Distal ureteral stones are commonly removed using a ureteroscopy. Proximal and midureteral stones are removed either by a ureteroscopy or an extracorporeal shockwave lithotripsy (ESWL) depending on their size and location. Open surgery for stones is usually reserved for ureteral calculi greater than 2 cm or an anatomic abnormality, which would preclude other options. Renal calculi surgery is dependent on the size of the stone and surgeon preference. Options include flexible ureteroscopy, ESWL, or percutaneous removal through a nephrostomy tract.

Ureteroscopic, laparoscopic, or open surgery may be required depending on the pathology of upper tract obstruction. Ureteropelvic junction obstructions are most commonly treated with a pyeloplasty, using an open or laparoscopic technique to remove the obstructed ureteral segment. A balloon dilation and an incision of the UPJ obstruction are less frequently used alternatives.

Ureteral strictures can be treated using a variety of methods. Small strictures can be dilated endoscopically with a balloon dilator. Larger or treatment-refractory strictures require a resection and reanastomosis to the nondiseased segment. Distal ureteral strictures may be treated with ureteral reimplantation directly into the bladder, a psoas hitch, or Boari flap.

Obstruction due to urothelial neoplasms are treated with either a segmental resection of the mass or a complete removal of the kidney and ureter if the mass is within either entity. Endoscopic management is now being used in superficial lesions of the ureter or kidney if oncologic principles can be maintained to spare as many nephrons as possible (Fig. 19.15).

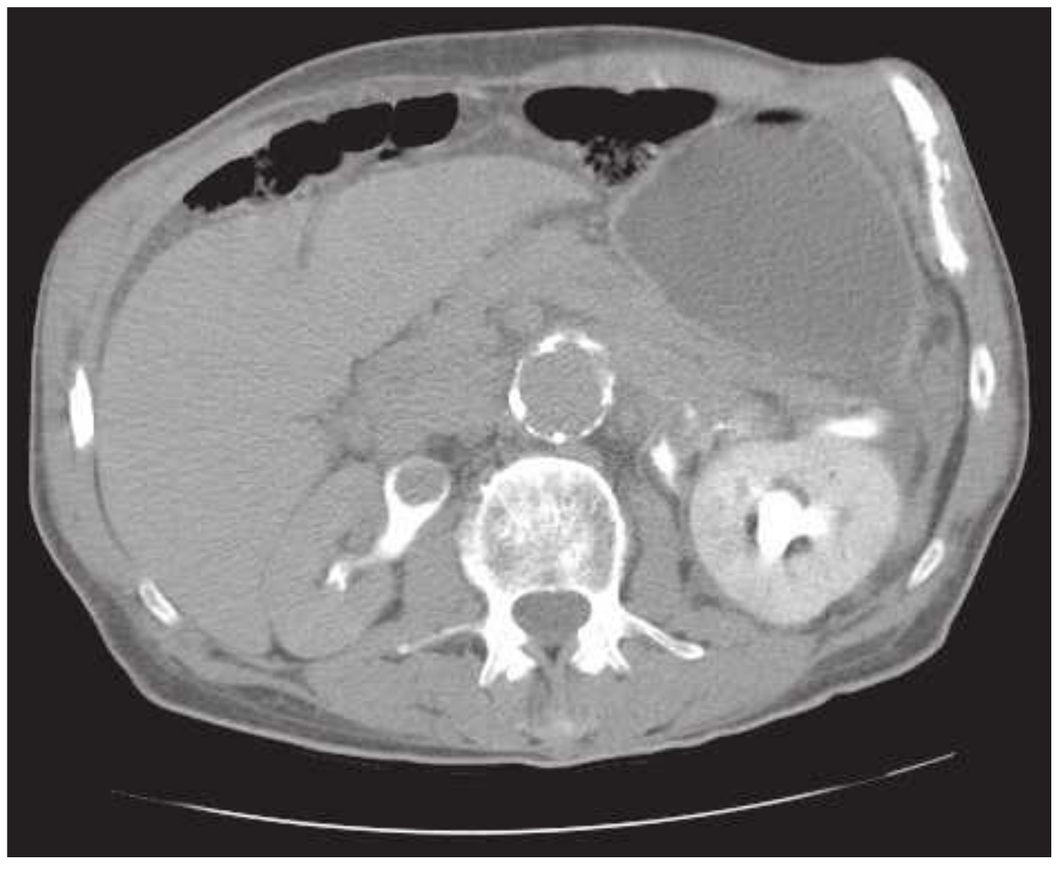

FIGURE 19.15 An axial computed tomography image showing a filling defect of the patient's right proximal ureter during delayed contrast imaging.

Conclusions

The prognosis of urinary tract obstruction depends on the cause, location, degree, and duration of obstruction as well as the presence of urinary tract infection. The longer the duration of obstruction, the greater the severity of obstruction and the presence of a concomitant infection, which can lead to a poor prognosis. The prognosis is favorable if the renal function is normal, the infection is cleared, and the obstruction is relieved in a timely manner.

VESICOURETERIC REFLUX AND REFLUX NEPHROPATHY

Introduction

Vesicoureteral reflux (VUR) is the retrograde flow of urine from the bladder to the upper urinary tract. Because VUR can be relatively asymptomatic, diagnosis may be difficult. When symptomatic, it can lead to pyelonephritis, scarring of the kidney, and even end-stage renal failure. The diagnosis and optimal management of VUR is controversial.

History of Vesicoureteral Reflux

In 150 A.D., Galen first described the UVJ as a mediator of unidirectional flow of urine from the kidney to the bladder.[87] da Vinci described the free flow of urine from the bladder to the kidneys most likely after dissecting the body of a patient with VUR.[88] It was not until 1893 that a gynecologist first described the backflow of urine from the bladder after having cut a distal ureter during a hysterectomy.[89] In 1907, Sampson suggested that the backflow of urine may lead to pyelonephritis after studying the UVJ.[90]

In the 1950s, there were two important breakthroughs on the impact of VUR on the upper urinary tracts. In 1952, Hutch[91] reported that VUR may lead to recurrent pyelonephritis in paraplegic patients. In 1959, Hodson[92,93] made the correlation between UTIs, renal scarring, and VUR. In 1979, Ransley and Ridson[94] defined the pathophysiology of VUR by showing the relationship between infection, scarring, and reflux. These discoveries and subsequent studies eventually led to a consensus system for grading reflux in 1985 by Lebowitz and colleagues.[94a]

For the past several decades, the treatment of VUR has evolved and has been widely debated. Newer, less invasive surgical treatments for VUR have broadened the treatment armamentarium and algorithm. However, the debate will continue until a true consensus can be reached.

Demographics

Because VUR can resolve spontaneously or can be asymptomatic, the true prevalence of this disease can be difficult to define. In 2000, a meta-analysis of children undergoing a cystography for various indications identified that those with UTIs had a 30% incidence of VUR, whereas 17% without a history of UTIs had VUR.[95] In contrast, an earlier study found that up to 70% of infants with UTIs may have some

element of reflux.[96] Using prenatal ultrasonography for screening, Gunn et al.[97] estimated that the incidence of VUR was 0.36:100 births in 1995.

VUR is more common in females. In 1992, the number of boys entered into the International Reflux Study in Children was 10% in the United States and 24% in Europe. This finding demonstrated an increased prevalence in the female population.[98] Several confounding factors play into finding the true incidence of VUR between the two groups because not all children are screened. For instance, uncircumcised boys are at a 12-fold increased risk of developing a UTI and are more likely to be screened for VUR.[99] The true incidence and difference between males and females with respect to VUR may never be made unless screening of the general population becomes available.

Because VUR tends to resolve spontaneously, its incidence decreases with age. A study by Baker[100] showed that the incidence of reflux in patients diagnosed with a UTI decreases with age. The incidence of VUR in children <1 year old was 70%, 25% in 4 year olds, 15% in 12 year olds, and 5% in adults who were screened for VUR after being diagnosed with a UTI.

Whether race predisposes patients to VUR is still not known because reflux has been studied primarily in Western countries. A 10-fold decrease in the frequency of reflux in female children of African American descent has been reported, as well as a quicker resolution of their reflux.[101]

Primary Vesicoureteral Reflux

Primary VUR is the regurgitation of urine through a vesicoureteric junction that has been rendered incompetent by a congenital defect of the length, diameter, muscle, or innervation of the submucosal segment of the ureter (Fig. 19.16). The vesicoureteric junction works normally as a flap-valve, which permits the flow of urine from the ureter into the bladder but prevents flow in the opposite direction. The submucosal segment of the ureter holds the key to ureteric continence and is dependent on factors such as the length of the intramural portion of ureter, the nature of the ureteric orifice, and the integrity of the bladder wall musculature.

The most common explanation for the defective valve mechanism of the vesicoureteric junction is the shortness of the submucosal segment secondary to the congenital lateral ectopia of the ureteric orifice.[105] Paquin described the principle that the ureteral tunnel must be five times as long as the ureteral diameter at the UVJ for reflux not to occur. When the bladder fills, a further shortening of the submucosal segment of the ureter may take place, accounting for the observation that reflux may only occur if the bladder is either full or in the act of voiding. The complex anatomy of the vesicoureteric junction and its relationship to the bladder trigone and the urethra is best approached from a developmental point of view.[103] Oswald et al.[104] proposed that a congenital muscular structural insufficiency of the distal ureter is important in the pathogenesis of the vesicoureteral reflux.

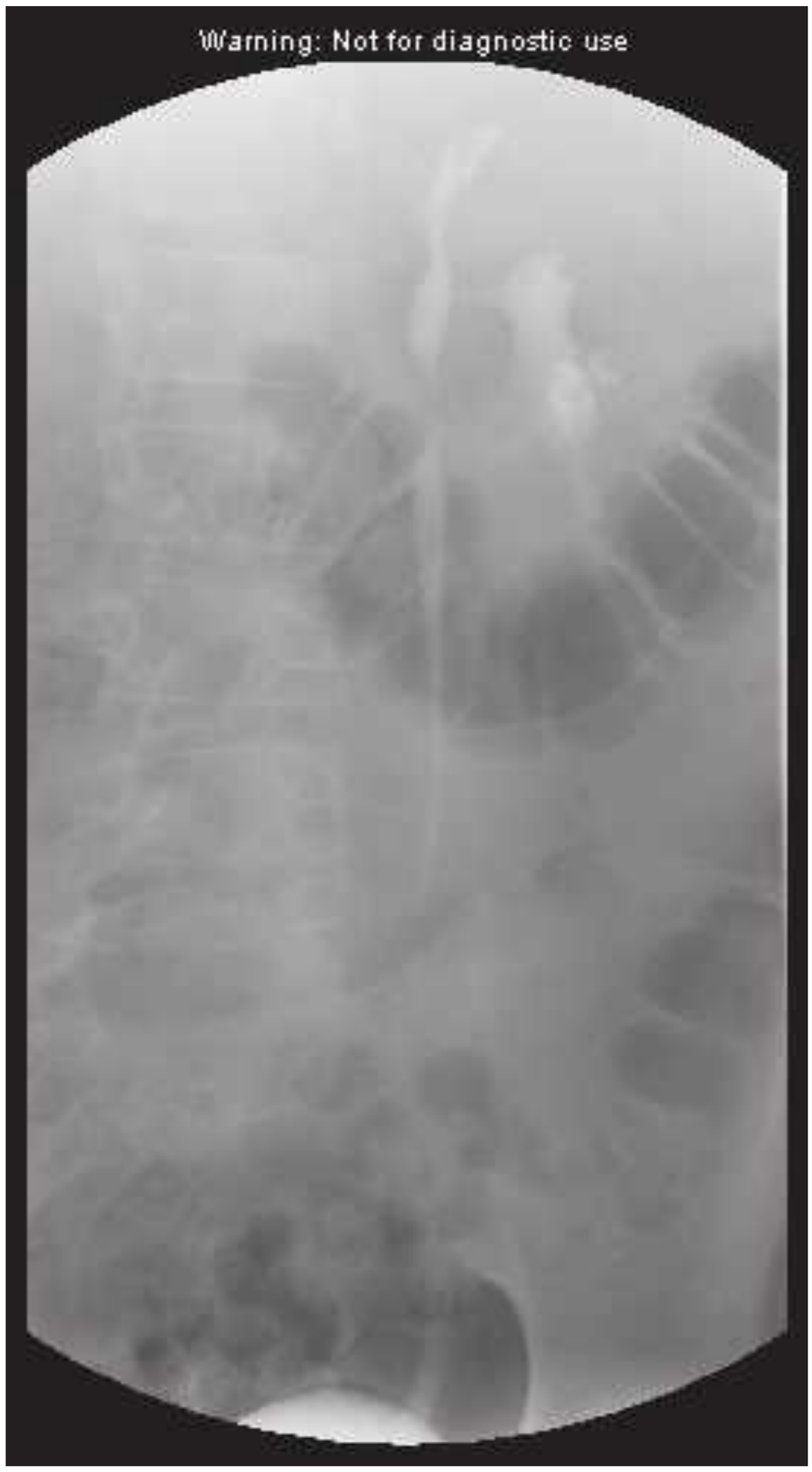

FIGURE 19.16 A single-shot fluoroscopic image of a patient's abdomen showing a contrast within the bladder as well as a reflux of contrast up the left collecting system.

The ureter, both extravesically and intravesically, must remain fixed with adequate ureteral tunnel length for the ureter not to reflux. Another important principle is that the intramural ureter must remain compressed during bladder filling so urine will not reflux up during this time. Opening of the UVJ is achieved by contraction of the longitudinal muscle of the ureter within the intramural tunnel. This causes the extravesical and intravesical points to decrease in length while opening the ureter to allow the bolus of urine into the bladder from the ureter. If the tunnel length is not long enough, urine can reflux back into the upper tracts at the end of this process. This can occur even in the presence of a low-pressure urine storage profile in the bladder.

Secondary Vesicoureteral Reflux

Increased bladder pressure, which may overcome the antirefluxing mechanism in a normal UVJ, can lead to VUR. The backflow of urine can lead to the dilation of the upper tracts and potential scarring of the kidney or renal failure. The most common pediatric cause in boys is posterior urethral valves (PUVs). Reflux, oftentimes high grade, can be associated with 70% of these boys due to an obstruction of the urethra. Valve ablation successfully treats the obstruction.[105,106] The most common cause in girls is a prolapsing ureterocele across the bladder neck, causing urinary obstruction.[107]

A neurogenic bladder without an anatomic obstruction is also a very common cause of VUR. In pediatric patients, a host of neurologic diseases that affect the lower and upper motor neurons can have damaging effects on the bladder. A physiologic obstruction can lead to high pressure systems within the bladder that lead to reflux into the upper tracts as a pop-off mechanism. Subsequent dilatation of the upper tracts and, eventually, scarring of the kidney may ensue. During the first years of potty training, dysfunctional voiding can lead to high-pressure bladders.

McGovern et al.[108] observed that myelodysplasia can be associated with high-pressure bladders and reflux. In a study of 42 children with myelodysplasia, 35 had detrusor areflexia. Of the latter, 5 had flat detrusor pressures during bladder filling and the remaining 30 had steeply rising, low-compliance patterns. When the voiding pressure to produce leakage was higher than 40 cm of water, 15 showed reflux.

Urinary Tract Infections and Reflux

Reflux does not cause a UTI. However, if a uropathogen is introduced into the urinary system, reflux can help maintain the pathogen within the system by allowing the bacteria to enter the upper tracts from the bladder, stay within the upper tracts due to dilatation of the upper tracts from reflux, or not be effectively cleared by voiding. Cystitis can cause irritability at the UVJ, resulting in a decreased threshold for reflux. Thus, reflux with a UTI can potentiate pyelonephritis by allowing uropathogen entry into the upper tracts. These pathogens can cause scarring of the renal parenchyma.

Pathophysiology of Reflux

The association with VUR and reflux nephropathy has been studied extensively in porcine models. Some studies have shown that the intrarenal reflux of sterile urine can cause focal scarring. This has yet to be proven in humans.[109] Most pathologic findings in humans have been taken from nephrectomy specimens due to chronic pyelonephritis or hypertension. These specimens have a tendency to show more scarring at the poles of the kidneys, which is most likely due to the fact that compound papillae are found at these poles and are more susceptible to damage from reflux.

Renal scarring or reflux nephropathy depends on a multitude of factors. The grade of reflux has been shown to be directly proportional to the development of scarring. A study of 735 children with primary VUR showed that 43.5% of patients had evidence of renal scarring, especially when high-grade reflux was present.[110] This association was also reported by Smellie[111] using intravenous urography. Renal scarring was detected in 69% of children with moderate to severe reflux and in 24% of those with mild reflux. In a separate study, 41 male patients with primary VUR were screened with a dimercaptosuccinic acid (DMSA) scan to assess for congenital renal scarring. Thirteen patients had low-grade reflux and none of these patients had scarring on DMSA. Of the 28 patients with high-grade reflux, 10 had slight scarring of the kidneys, whereas 8 had severe scarring of the kidneys.[112]

The age of the child at the time of exposure to infected urine is also important. Children less than 4 years old were more susceptible to renal damage, most likely due to an immature system that was unable to fight off the infection.[113] The "big bang" theory observed that most renal scarring took place after the first bout of pyelonephritis and further scarring was unlikely if no further episodes of pyelonephritis occurred.[114]

Ransley and Ridson[115] performed a landmark study in pigs in which they unroofed the intravesical portion of the UVJ, allowing for reflux. When an infection was introduced into the urine, the pigs subsequently developed pyelonephritis and renal scarring. In the absence of infected urine, scarring did not occur. This study influenced the investigation into the principle that sterile urine did not lead to scarring of the kidney, which paved the way for nonsurgical management for VUR.

The long-term effects of VUR can be devastating, leading to chronic renal insufficncy and end-stage renal disease (ESRD). Although older studies identified that 15% to 30% of ESRD in children was due to VUR, newer studies show that only 2.2% of VUR children develop ESRD.[116,117]

Although hypertension is a well-known sequalae of VUR, the pathophysiology is not well described. Derangements in the renin-angiotensin-aldosterone (RAA) system as well as the intrarenal sodium-potassium pump dysfunction have been investigated. A 2004 study[118] showed that 20% of 157 adult patients with hypertension and no other renal parameters on cystography had VUR. It remains unclear whether it is nephropathy due to the scarring or congenital dysplasia associated with the reflux that causes or predisposes the patient to renovascular hypertension.

Associated Anomalies with Vesicoureteral Reflux

Several anatomic anomalies are associated with VUR in the pediatric patient. The presence of VUR should prompt an evaluation for these conditions. UPJ obstruction can be seen in 9% to 18% of children with VUR.[119] The UPJ obstruction may be secondary to the VUR or may represent two separate entities. High-grade reflux can cause kinking of the upper ureter that leads to a secondary or

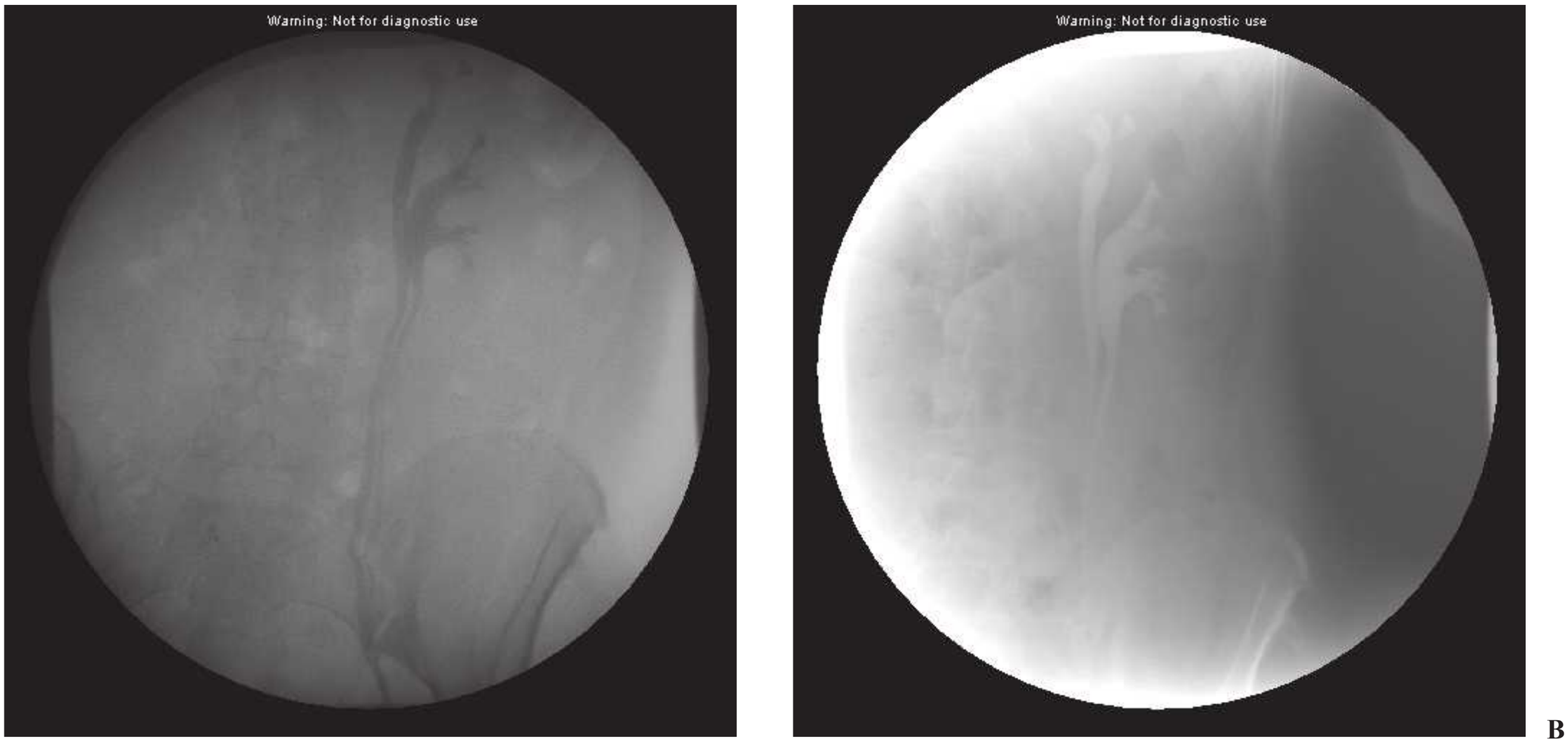

FIGURE 19.17 A fluoroscopic image from a voiding cystourethrogram showing contrast refluxing up into an incomplete duplicated system on the patient's left side.

"incipient" UPJ obstruction. High-grade VUR is five times more likely to be associated with UPJ obstruction than low-grade VUR.[120]

VUR is the most common abnormality associated with ureteral duplication (Fig. 19.17). The embryology of the duplication and the ureteral insertion into the trigone during development explains the association of these conditions. The lower pole ureter inserts into the trigone early in development. As the trigone expands, the ureteral orifice from the lower pole moves cranially and laterally. The resultant decrease in length of the intramural tunnel leads to VUR of the lower pole segment. The upper ureter enters the trigone later in development, is located more caudally and medially, and is more prone to obstruct than reflux. This is known as the Weigert-Meyer law.

Renal anomalies are also very common with VUR. With multicystic dysplastic kidneys, the incidence of VUR on the contralateral side is as high as 25%.[120] Renal agenesis is associated with a higher incidence of contralateral VUR with a prevalence of 28% in one study.[121]

Pregnancy and Vesicoureteral Reflux

Pregnant women are predisposed to bacteriuria because of decreased bladder tone. In a study of 321 patients evaluated with cystography during their third trimester, the incidence of pyelonephritis in pregnant women with VUR was 33% compared to <5% in women without VUR.[122] Women with a history of reflux have an increased risk of infection-related morbidity during pregnancy regardless of prior correction. The consensus is that the reflux should be corrected before pregnancy.[123]

Natural History of Vesicoureteral Reflux

VUR can spontaneously resolve as a result of remodeling of the UVJ with growth. The age of the child and the degree of reflux at the time of diagnosis influence the likelihood of spontaneous resolution. Lower grades of reflux have a higher likelihood of spontaneous resolution. Several studies have shown that low-grade reflux (I and II) will resolve on their own. A study of 500 children with known VUR in Brazil who were observed from birth reported a spontaneous resolution rate of 87.5% for grade I, 77.6% for grade II, 52.8% for grade III, 12.2% for grade IV, and 4% for grade V reflux.[124]

The age at which VUR is diagnosed can also be a factor in the likelihood of spontaneous resolution. For years, many studies had shown that the earlier the reflux is detected, the greater the likelihood of spontaneous resolution. Newer studies have shown that diagnosis at age 5 or at infancy had the same rate (20%) of resolution over a 5-year period regardless of age.[125] The accepted period of observation for resolution of reflux has been 5 years, but the patient's risk of UTI or pyelonephritis is the same throughout the observation period and should be discussed with the patient and his or her family.

Management of Vesicoureteral Reflux

The main goals of management of a patient with VUR are to prevent any or recurrent febrile UTIs, to prevent a loss of renal function or renal scarring, and to minimize the morbidity and mortality of the treatment option. The two primary options in treating VUR are watchful waiting with single

low-dose antibiotic suppression therapy or surgical correction. The traditional approach is medical therapy first, then surgical correction if the reflux does not resolve or if medical management fails. In 2010, based on a meta-analysis of the literature, the AUA published guidelines for the management and screening of primary VUR in children.[123]

The long-debated question is how long to wait to determine if the reflux will resolve. A landmark study by Olbing[126] in 2003 showed that renal scarring does not seem to develop after age 5 in severe reflux patients. It is common practice now to allow the watching of newborns with VUR until age 5 if they have no breakthrough infections.[123] Boys with low-grade reflux are generally not followed after the age of 5 and taken off suppression if they continue with good elimination habits while girls are offered surgical correction due to the dangers with pregnancy in the future. A study in 2000 showed that taking these patients off low-dose antibiotic suppression would lead to only 10% requiring surgical correction in the future for a febrile UTI.[127]

Diagnosis of Vesicoureteral Reflux

The two gold standard tests for diagnosing VUR are the voiding cystourethrogram (VCUG) and radionucleotide cystography (RNC). Both of these methods involve the administration of contrast into the bladder through the urethra with subsequent imaging.

The VCUG is a fluoroscopic study in which images are taken while the bladder fills with contrast through a catheter when the bladder is full, when the patient is voiding, and after the contrast has drained (Figs. 19.18 and 19.19). Anterior, posterior, and oblique images are obtained.

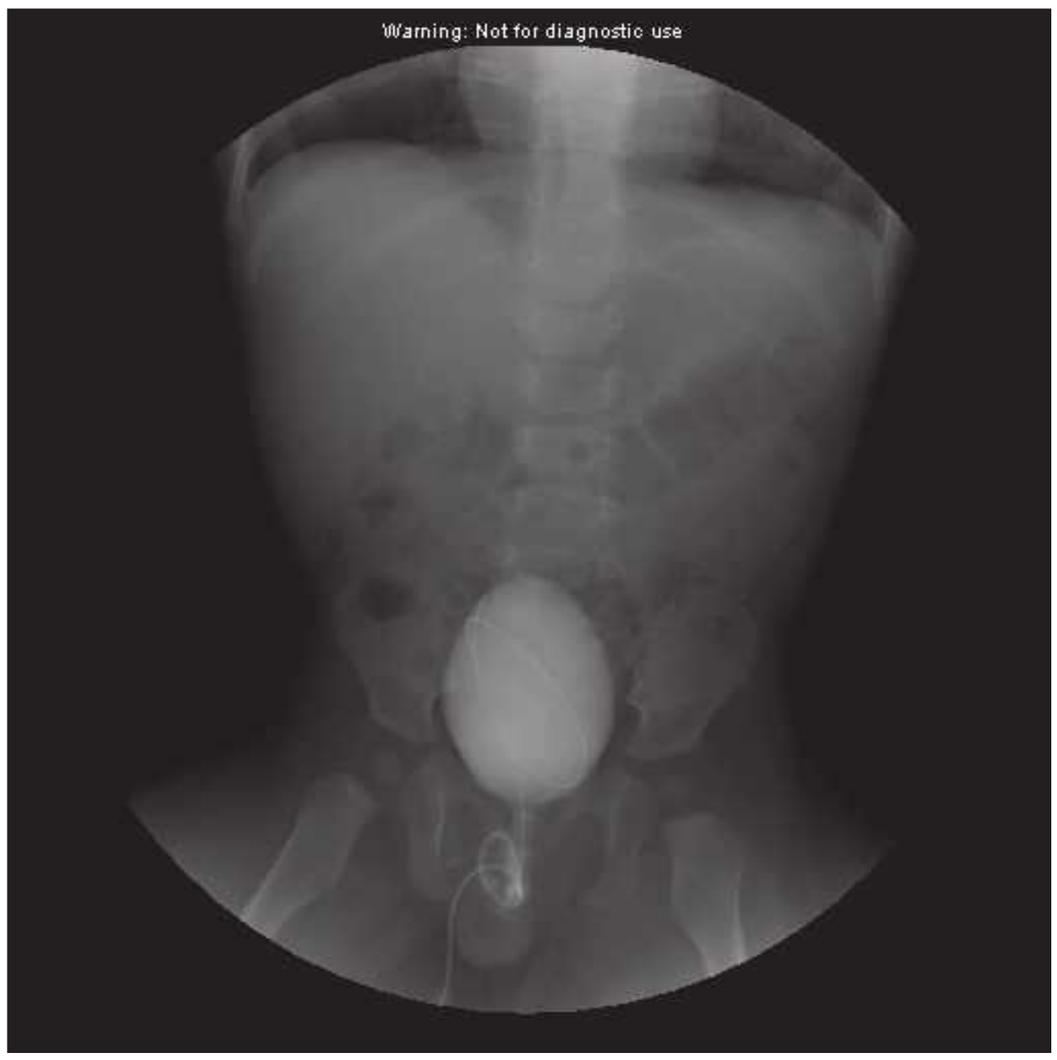

FIGURE 19.18 A voiding cystourethrogram showing a normal bladder without vesicoureteral reflux.

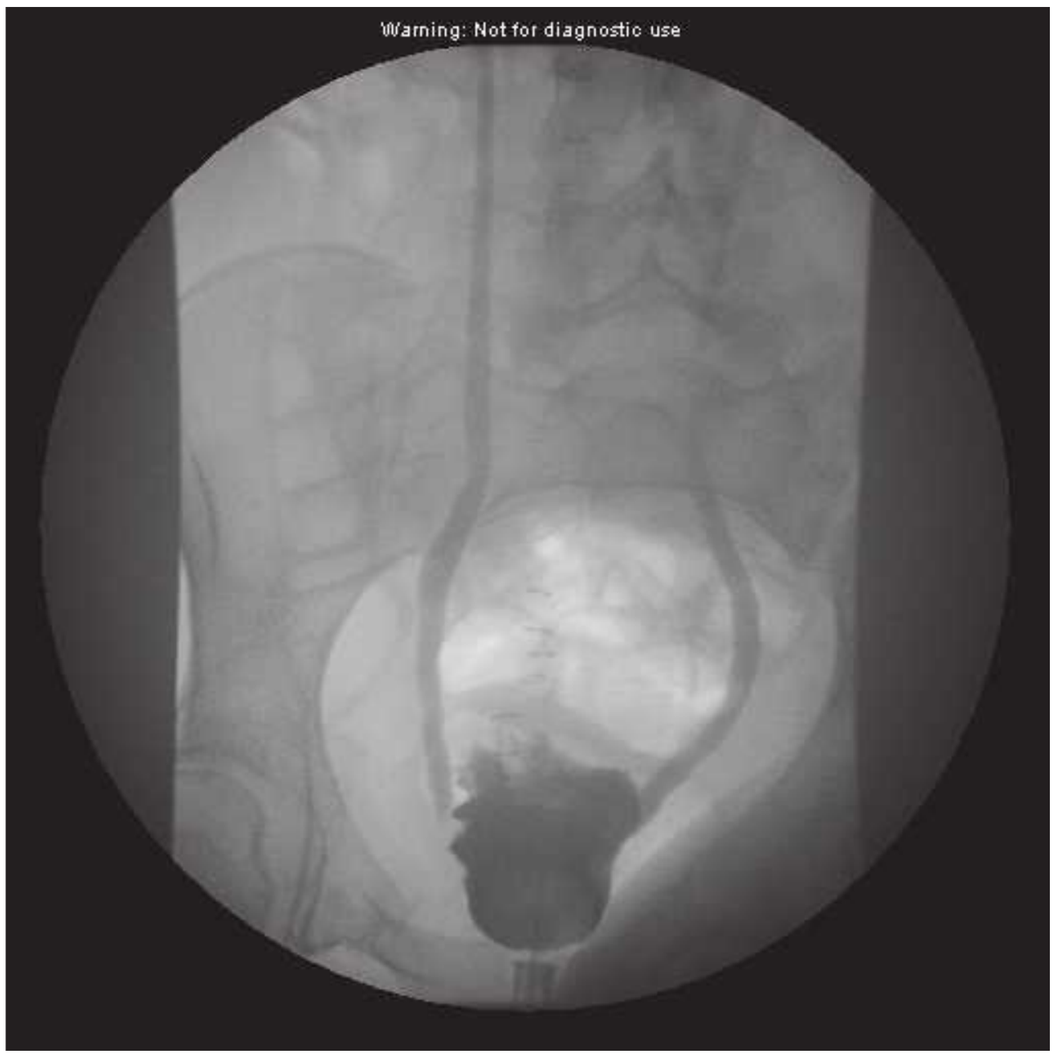

FIGURE 19.19 A voiding cystourethrogram fluoroscopic image showing bilateral vesicoureteral reflux.

Contrast can reflux into the upper tracts during all phases of the VCUG or only during the voiding phase of the test. This test provides functional and anatomic information of the bladder and the urethra. It can also show how the patient voids and if any dysfunction is present.

The RNC uses a radionucleotide tracer, usually technetium-99m (99mTc), in the bladder. Reflux is detected with a scintigraphic gamma camera and provides less radiation exposure (1%) compared to the VCUG.[128] The RNC is a functional test but gives little information on an anatomic level. The test is more sensitive for grades II through V reflux but can miss grade I reflux due to the overlying exposure of radionucleotides in the bladder.

Another option, DMSA (technetium-99m-labeled dimercaptosuccinic acid) renal imaging, can provide information regarding the degree of existing renal cortical abnormalities and can serve as a baseline for future comparison (Fig. 19.20). Children with VUR grades III through V, younger children, those with an abnormal renal ultrasound, and those with recurrent febrile UTIs are most likely to have renal scarring that would show up on a DMSA scan. The scan works by an IV injection of the radionucleotide, which enters the venous system and eventually is filtered by the kidney. The radionucleotides are taken up by functional proximal tubules where they bind for several hours. Images of the kidneys are taken and defects of the cortex can be seen if renal scarring or nonfunctional parenchyma are present. In a study of 79 children, DMSA scanning had a 98% sensitivity and a 92% specificity with regard to renal scar detection in children who were followed for 1 to 4 years.[112]

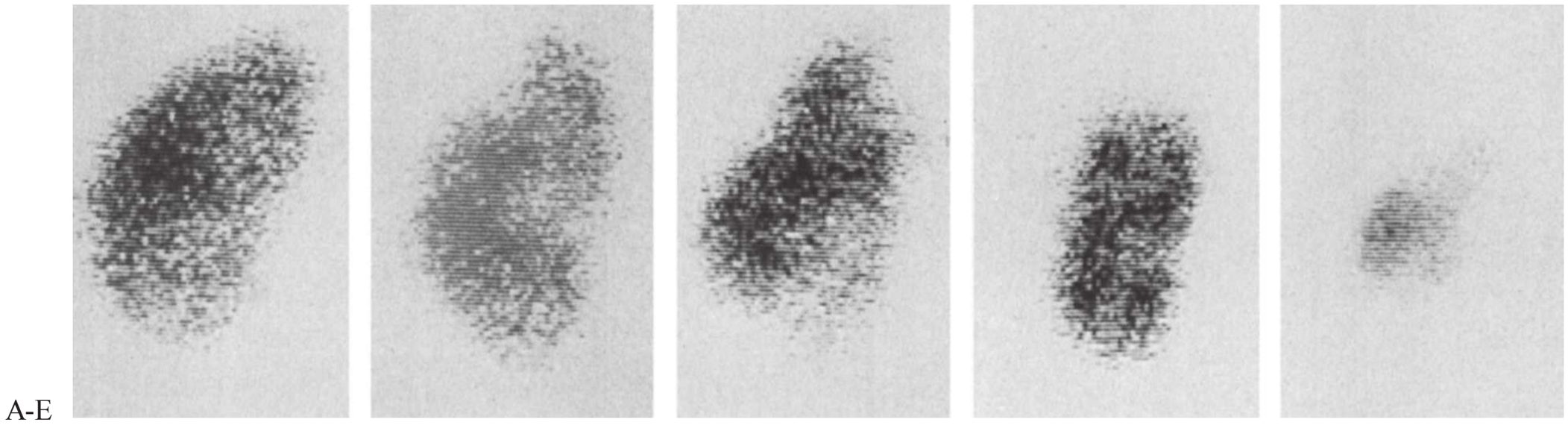

FIGURE 19.20 The classification of kidneys with reflux nephropathy (RN) on 99mTc-DMSA scanning. **A:** Normal. **B:** Type 1 (no more than two scarred areas). **C:** Type 2 (more than two scarred areas, with some areas of normal parenchyma). **D:** Type 3 (generalized damage to whole kidney, similar to obstructive nephropathy [i.e., contraction of entire kidney with or without focal scars]). **E:** Type 4 ("end-stage" kidney with little or no uptake of radionuclide [i.e., less than 10% of overall function].) (Courtesy of Dr. Noemia P. Goldraich, Porto Alegre, Brazil.)

Grading of Vesicoureteral Reflux

The current grading of VUR is based on the 1981 International Reflux Study Committee recommendations for five different grades of reflux (Fig. 19.21). These grades are based on VCUG imaging.[129]

Grade I shows contrast refluxing into a nondilated ureter.

Grade II shows contrast refluxing into the pelvis and calyces without dilatation.

Grade III shows mild or moderate dilatation and/or tortuosity of the ureter and mild or moderate dilatation of the pelvis. There is no or only slight blunting of the fornices.

Grade IV shows moderate dilatation and/or tortuosity of the ureter and moderate dilatation of the pelvis and calyces. There is complete obliteration of the sharp angles of the fornices. However, there is maintenance of papillary impressions in the majority of calyces.

Grade V shows gross dilatation and tortuosity of the ureter, pelvis, and calyces. Papillary impressions are no longer visible in the majority of the calyces.

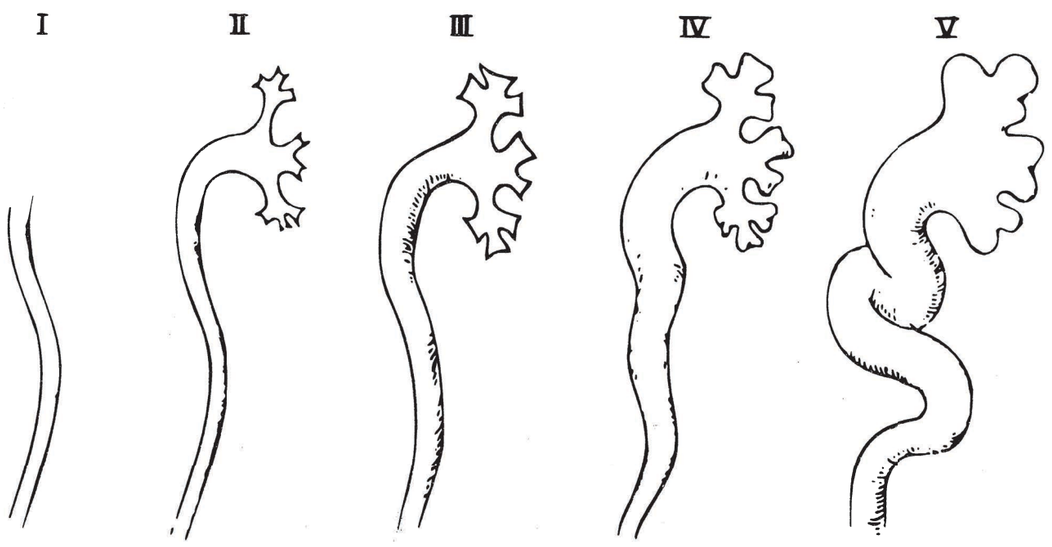

FIGURE 19.21 The classification of grades of vesicoureteral reflux used by the International Reflux Study Committee. (From Report of the International Reflux Study Committee. Medical versus surgical treatment of primary vesicoureteral reflux. *Pediatrics* 1981;67:392, with permission.)

There is no current grading system for reflux done with RNC. Grades II and III are lumped together as low-grade reflux and grades IV and V are combined as high-grade reflux.

Initial Management of Vesicoureteral Reflux

At the initial presentation of a patient with VUR, the first step is to treat any underlying infection if present. After a history and physical, a baseline renal ultrasound should be obtained. If bilateral renal abnormalities are identified, a serum creatinine is indicated. A baseline serum creatinine is obtained to establish an estimate of GFR for future reference. Height, weight, and blood pressure should be documented. An assessment of voiding patterns is recommended to detect the underlying voiding dysfunction. A urinalysis for proteinuria and bacteriuria is also recommended. If the urinalysis indicates an infection, a urine culture and sensitivity is recommended.[123]

Once VUR has been diagnosed, factors including age, race, degree of reflux, and compliance should be considered. Children under 1 year of age should be treated with surgery or antibiotic prophylaxis and should not be observed because of the increased risk of renal scarring in this age group. Treatment of children over the age of 1 year or adults should be based on clinical judgment, taking into account these factors including renal scarring.[123]

Medical Management of Vesicoureteral Reflux

Medical management, or "watchful waiting," has been the standard approach over the past 20 years (Tables 19.4 and 19.5). Sterile urine is maintained with low-dose antibiotic suppression therapy and is usually given at nighttime to achieve peak antibiotic concentrations during the longest period between voids when infection is most likely to develop. Children less than 2 months old are given either trimethoprim or amoxicillin at one-third to one-fourth the normal dose every day. After 2 months of age through adulthood,

19.4 Treatment of Vesicoureteral Reflux in Children

Clinical Presentation		Treatment					
		Initial (Antibiotic Prophylaxis or Open Surgical Repair)			Follow-up (Continued Antibiotic Prophylaxis, Cystography, or Open Surgical Repair)[a]		
Reflux Grade / Laterality	Pt. Age (yr)	Guideline	Preferred Option	Reasonable Alternative	Guideline[b]	Preferred Option[b]	No Consensus[c]
I–II/Unilat. orbilat.	Younger than 1	Antibiotic prophylaxis					Boys and girls
	1–5	Antibiotic prophylaxis					Boys and girls
	6–10	Antibiotic prophylaxis					Boys and girls
III–IV/Unilat. orbilat.	Younger than 1	Antibiotic prophylaxis			Bilat: Surgery if persistent	Unilat: Surgery if persistent	
	1–5	Unilat: Antibiotic prophylaxis	Bilat: Antibiotic prophylaxis			Surgery if persistent	
	6–10		Unilat: Antibiotic prophylaxis Bilat: Surgery	Bilat: Antibiotic prophylaxis		Surgery if persistent	
V/Unilat. or bilat.	Younger than 1		Antibiotic prophylaxis		Surgery if persistent		
	1–5		Bilat:Surgery Unilat: Antibiotic prophylaxis	Bilat: Antibiotic prophylaxis Unilat: Antibiotic prophylaxis	Surgery if persistent		
	6–10	Surgery		Surgery			

[a]For patients with persistent uncomplicated reflux after extended treatment with continuous antibiotic therapy.
[b]See duration of reflux regarding the time that clinicians should wait before recommending surgery.
[c]No consensus was reached regarding the role of continued antibiotic prophylaxsis, cystography, or surgery.
From Elder JS, Peters CA, Avant BS Jr, et al. Pediatric vesicoureteral reflux guidelines panel summary report on the management of primary vesicoureteral reflux in children. J Urol 1997;157:1846.

the drugs of choice become trimethoprim-sulfamethoxazole or nitrofurantoin.

The basis for medical management is based on several important studies from the 1990s. The hypothesis was that antibiotic prophylaxis could prevent infection and halt renal scarring and deterioration, whereas reflux resolved over time in children.

The International Reflux Study in Children[130] randomized children younger than 9 years old with high-grade reflux to either antibiotic prophylaxis or corrective surgery. Surgery reduced the incidence of pyelonephritis, but the incidence of UTI (38%) and new renal scarring was the same in both groups.

The Birmingham Reflux Study[131] prospectively randomized children to surgery versus medical management over a 5-year period. This study once again showed the incidence of new renal scarring to be similar in both groups, but more scarring was found in children within the first 2 years of observation. There was no significant difference in the incidence of breakthrough UTI between the two groups. However, there were two patients that did progress to ESRD in the observation group and four who developed extensive scarring and hypertension.

Recently, pediatricians question if antibiotic prophylaxis is needed in patients with reflux if good elimination habits are present. Two randomized studies[132,133] from 2008 demonstrated that patients with reflux followed over a period of 1 to 2 years experienced no difference in the incidence of pyelonephritis or renal scarring with respect to antibiotic prophylaxis or observation.

Unfortunately, infections on antibiotic suppression do occur. Breakthrough infections can represent noncompliance or even overdosing of the antibiotic. In this case, a urinalysis and urine culture must be obtained. If the urine culture grows bacteria that are sensitive to the antibiotic, noncompliance is suspected. If the bacteria are resistant to

19.5 Treatment of Vesicoureteral Reflux in Children

Clinical Presentation		Treatment					
		Initial (Antibiotic Prophylaxis or Open Surgical Repair)			Follow-up (Continued Antibiotic Prophylaxis, Cystography, or Open Surgical Repair)[a]		
Reflux Grade/ Laterality	Pt. Age (yr)	Guideline	Preferred Option	Reasonable Alternative	Guideline[b]	Preferred Option[b]	No Consensus[c]
I–II/Unilat. orbilat.	Younger than 1	Antibiotic prophylaxis					Boys and girls
	1–5	Antibiotic prophylaxis					Boys and girls
	6–10	Antibiotic prophylaxis					Boys and girls
III–IV/Unilat.	Younger than 1	Antibiotic prophylaxis			Girls: surgery if persistent	Boys: surgery if persistent	
	1–5	Antibiotic prophylaxis			Girls: surgery if persistent	Boys: surgery if persistent	
	6–10		Antibiotic prophylaxis		Surgery if persistent		
III–IV/Bilat.	Younger than 1	Antibiotic prophylaxis			Surgery if persistent		
	1–5		Antibiotic prophylaxis	Surgery	Surgery if persistent		
	6–10	Surgery					
V/Unilat. orbilat.	Younger than 1		Antibiotic prophylaxis	Surgery	Surgery if persistent		
	1–5	Bilat:surgery	Unilat:surgery			Surgery if persistent	
	6–10	Surgery					

[a]For patients with persistent uncomplicated reflux after extended treatment with continuous antibiotic therapy.
[b]See duration of reflux regarding the time that clinicians should wait before recommending surgery.
[c]No consensus was reached regarding the role of continued antibiotic prophylaxis, cystography, or surgery.
From Elder JS, Peters CA, Avant BS Jr, et al. Pediatric vesicoureteral reflux guidelines panel summary report on the management of primary vesicoureteral reflux in children. J Urol 1997;157:1846.

the medication, too high a dose of the suppressive antibiotic may lead to the killing of normal intestinal flora and the growth of resistant bacteria. Rates of infection can be as high as 12% to 33% on antibiotic suppression in a compliant patient.[134,135]

Surgical Management of Vesicoureteral Reflux

Surgical management has been the definitive treatment for VUR since the 1950s when Hutch[136] first described his technique on nine paraplegic patients. Open ureteral reimplantation into the bladder has been a standard of care. In the early 1980s, endoscopic management for the treatment of VUR emerged and has since become an alternative to open surgery. Surgical therapy is indicated for females with unresolved reflux who may become pregnant, for noncompliant patients, and for failed medical management patients who have had breakthrough infections or who are unable to tolerate the antibiotics.

Surgical techniques all have the same principles, which include mobilizing the distal ureter without compromising its blood supply, creating a submucosal tunnel that provides the 5:1 ratio previously described, gentle handling of the bladder, and adequate muscle backing to the ureter within the submucosal tunnel. Cystoscopy at the time of the procedure can be performed, but is not mandatory.

Open Ureteral Reimplantation

Open ureteral reimplantation can be classified as either intravesical, extravesical, or combined. Using a Pfannenstiel incision, intravesical repairs require opening of the bladder from the dome of the bladder anteriorly to just proximal to the bladder neck. The ureteral orifices are then identified and a 3- or 5-Fr feeding tube is placed into the ureter to aid in dissection. The distal ureter is then dissected away from the bladder to the level of the peritoneum, keeping the blood supply and the ureteral sheath intact. Once an adequate length is obtained, the surgeon's preference determines how the ureter will be reimplanted.

The Politano-Leadbetter technique requires moving the ureter into a new submucosal tunnel with the neo-orifice located in a more medial and inferior position on the trigone (Table 19.6). The Paquin technique is similar to this except the dissection of the distal ureter and placement of the proximal end of the submucosal tunnel is done extravesically. The Glenn-Anderson technique uses the same hiatus as the old orifice but moves the orifice closer toward the bladder neck to increase the length of the submucosal tunnel. The Cohen cross-trigonal technique moves the right orifice to the left side of the bladder under a submucosal tunnel while the left ureteral orifice is moved to the right side of the bladder. This should not be done on patients with a known history of stones or a family history of nephrolithiasis.

The Lich-Gregoir repair is the most common extravesical ureteral reimplant technique used. The ureter is dissected after ligation of the obliterated hypogastric artery off the bladder muscle and trigone. The bladder serosa and muscle are dissected off the bladder mucosa 4 to 5 cm superior and lateral from the ureteral orifice. The ureter is placed between the detrusor flaps, and the flaps are sewn over the distal ureter, thus creating a submucosal tunnel.

An open surgical correction of VUR, regardless of which technique is used, has a very high success rate with an overall cure rate of 98.1%.[123] Postoperative complications include recurrent reflux, contralateral reflux, UTI, and obstruction.

Endoscopic Treatment of Vesicoureteral Reflux

The first case of cystoscopic treatment for VUR was described in 1981 by Matouschek[137] when he injected polytetrafluoroethylene (PTFE) in a subureteric fashion. It did not become a popular mode of treatment until O'Donnell and Puri[138] published their series of 103 ureters injected with Teflon leading to a 75% success rate after one injection. One year later, they treated ureters with grades IV to V reflux with an 84% success rate at a 6-month follow-up.[139] Resolution rates

19.6 Types of Ureteral Reimplants into the Bladder

Author	Technique	Advantages	Disadvantages
Politano–Leadbetter	Intravesical ureteroneocystotomy	Potential for a long submucosal tunnel; anatomic alignment of the ureters for retrograde catheterization	Potential complications as a result of neocystotomy (obstruction, transperitoneal placement, bowel injury)
Glenn–Anderson	Intravesical trigonal advancement	No neocystotomy and anatomic alignment of ureters; simple to perform	Not suitable for dilated ureters as a result of limitation of submucosal tunnel length
Cohen	Intravesical cross-trigonal advancement	No neocystotomy and potential for long tunnel length; simple to perform	Ureters not in anatomic alignment could prevent retrograde catheterization
Gil–Vernet	Intravesical medial advancement	No neocystotomy; simple and rapid procedure because of limited dissection of ureters	Less successful than other intravesical techniques and only suitable if ureters are highly mobile within the bladder
Lich–Gregior and Zaontz and colleagues	Extravesical neocystotomy with or without advancement	Ureters in anatomic alignment; less postoperative bladder symptoms and hematuria	Difficult dissection; potential temporary urinary retention when performed bilaterally

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are lower in children with bowel and bladder dysfunction (50%) compared to those without dysfunction (89%).[123]

Under a general anesthesia, a cystoscope is placed into the bladder and the refluxing ureteral orifice is identified. A cystoscopic needle is placed at the 6 o'clock position and the material is injected into the submucosal space, causing the submucosal tunnel to lengthen and allowing for easier compression of the ureter at rest and during voiding. The injection should be done slowly and the surgeon should see a mound forming at the ureteral orifice and the orifice itself rising into a "volcanolike" orifice. There are several types of injectable material used for this procedure including PTFE, bovine collagen, polymethysiloxane, and Deflux as well as autologous material such as fat, collagen, muscle, and chondrocytes (Table 19.7). The overall success rate of endoscopic treatment for VUR varies from 50% to 92%.[123]

Follow-up for Medical Management

Patients on antibiotic prophylaxis should have an ultrasound at least every 12 months and a VCUG every 12 to 24 months.[123] In patients with grades I to II VUR, follow-up imaging is not considered mandatory, and there is no evidence to support continuous follow-up imaging with a VCUG unless to prove a resolution of the VUR.[123] In the presence of an abnormal ultrasound, a great concern of scarring due to a breakthrough UTI, or a decrease in the patient's overall kidney function, DMSA scanning of a child can be done.[123]

Follow-up for Surgical Therapy

The standard of care after a patient has a corrective surgery for VUR is a renal ultrasound to rule out or diagnose an obstruction of the upper tract. A postoperative VCUG may be done if the patient had an open surgery, but it is recommended when endoscopic correction was performed because the success rate is lower.[123] The rate of obstruction after open or endoscopic surgery is less than 1%. There is no current recommendation on the duration of prophylactic antibiotics after surgery.[123]

Screening of Family Members

VUR can be familial, and the mode of transmission is autosomal dominant. Kaefer[140] reported a 100% prevalence in identical twins. The current recommendation is that a noninvasive test be done on siblings. If scarring is present, then invasive testing may be performed, especially if the sibling is less than 3 years of age.[112]

19.7 Success Rates of Endoscopic Management of Vesicoureteral Reflux

Implant	% Reflux Cured with Implant One Injection[a]	Biodegradable	Comments
Autologous chondrocytes	55	Autologous	Requires two procedures: cartilage harvest and the subsequent subureteric injection
Bovine cross-linked collagen	72	Yes	Skin allergy testing prior to treatment; high relapse rate after 1 year
Dextranomer/hyaluronic acid copolymer	72	Yes	Nonimmunogenic; easy to inject with syringe and needle; only implant FDA approved for the treatment of reflux in children in the United States
Polydimethylsiloxane	82[b]	No	Solid silicone elastomere soft tissue-bulking agent suspended in bioexcretable carrier gel
Polytetrafluoroethylene	73	No	Teflon particles suspended in glycerine; small particle size makes migration a concern; most studied of all implants with largest series

[a]Overall for all refluxgrades.
[b]Grades I–III only.
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Conclusion

Clinical consequences of vesicoureteral reflux can range from asymptomatic to end-stage renal disease. A prompt diagnosis of the condition in a patient with a UTI is mandatory to salvage renal function, especially in children less than 1 year of age. Surgical or medical therapies are accepted management options and should be discussed after the diagnosis. The main goal in the treatment of VUR is to prevent damage to the upper tracts and to preserve renal function.

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CHAPTER

20

Nephrolithiasis

Keith A. Hruska • Anne M. Beck

In the United States, the prevalence of kidney stones has risen over the past 30 years.[1] By 70 years of age, 11% of men and 5.6% of women will have a symptomatic stone.[1] Nephrolithiasis is a costly malady to society. Estimates in the 1970s exceeded $5 billion annually in the United States.[2] More current estimates exceed $30 billion. The incidence of nephrolithiasis in the United States is highest in the Southeast,[3,4] with peak incidence occurring in the late summer months. In addition, sedentary, white collar workers are more likely to form stones than are active, blue collar laborers.[5,6] The rising prevalence of renal calculi highlights the importance of environmental factors, such as diet, in their formation. Fructose consumption, which increased since the introduction of high fructose corn syrup, is associated with stone formation.[7] Increased rates of hypertension and obesity are linked to nephrolithiasis.[8] Stones are distinctly less common in African Americans, American Indians, and people of Asian descent. The age and sex distribution of patients referred for evaluation of nephrolithiasis shows a 2:1 ratio of male to female patients and a maximum incidence in the 30- to 50-year-old group (excluding patients with cystinuria and infection stones).

The natural history of stone disease is characterized by recurrence. Although many studies of the natural history and incidence of stones have been biased by referral practices and differences in definition of recurrence, there is clear evidence in the literature of the recurrent nature of stone disease.

A common dilemma faced by the clinician is whether or not to evaluate and treat the first-time stone former. The risk of recurrence after an initial episode has been estimated to be about 50% by 5 years, with almost two thirds of patients having a recurrence by 9 years.[6,9] Even when nephrolithiasis is evaluated and treated appropriately, the incidence of recurrence in first-time stone formers is not significantly different from that in treated patients with a history of multiple stone episodes.[10] In fact, these patients most likely represent recurrent stone formers in the initial stage of their disease. These and other data demonstrating Randall's plaque[11,12] suggest that such patients should be evaluated and treated in the same manner as patients with recurrent nephrolithiasis.

Patients who have undergone shockwave lithotripsy represent another group at risk for stone recurrence. Stone fragmentation often leads to regrowth of stone material, because residual fragments may act as a nidus for ongoing crystal deposition and stone formation. Medical therapy aimed at correcting underlying urinary abnormalities in these patients may prevent or limit stone growth and recurrence.[13]

Despite the almost inevitable risk of nephrolithiasis recurring if it is left untreated, diagnostic evaluation and selective treatment of metabolic abnormalities that decrease the incidence of new stone formation and can induce complete remission are not routinely practiced.[14] Calcium, uric acid, cystine, and struvite stones differ from one another in terms of pathogenesis and treatment. Therefore, each stone type is described separately. The clinical manifestations by which the stones present are not related to the composition of the stone, and this should be kept in mind.

CLINICAL MANIFESTATIONS

The classic presentation of nephrolithiasis is acute renal colic manifesting as colicky flank pain radiating to the groin. As stones descend in the ureter, the pain may localize to the abdominal area overlying the stone and radiate to the gonad. Peritoneal signs are generally absent. Stones at the ureterovesical junction may cause lower quadrant pain that radiates to the urethral tip, urinary urgency, frequency, and dysuria resembling bacterial cystitis. Exam often reveals patients in distress, unable to find a comfortable position, with tenderness in the costovertebral angle or lower quadrant. Gross or microscopic hematuria is present 90% of the time, but the absence of hematuria does not preclude the diagnosis of nephrolithiasis. Owing to the shared splanchnic innervation of the renal capsule and intestines, hydronephrosis and distension of the renal capsule may produce nausea and vomiting. Thus, renal colic may mimic acute abdominal or pelvic conditions.

The best means of confirming the diagnosis of a urinary stone is unenhanced computed tomography (noncontrast helical CT scan) of the abdomen and pelvis. The sensitivity approaches 96% with a specificity of 100%.[15] Positive and

negative predictive values are 100% and 91%, respectively. Negative CT scans often detect other abnormalities including appendicitis, pelvic inflammatory disease, diverticulitis, abdominal aortic aneurysm, and bladder cancer. Plain abdominal radiography assesses whether the stone is radiopaque, which is the case 75% to 90% of the time. Ultrasonography has a high specificity but a much lower sensitivity than CT. Ultrasonography is appropriate as the initial imaging test in pregnancy and in pediatrics and in patients who should avoid radiation. Intravenous pyelography has been replaced by helical CT as the preferred imaging test.[16] Secondary signs of urinary tract obstruction such as ureteral dilatation, hydronephrosis, and perinephric stranding, are variably seen depending on the duration of pain prior to imaging and the sign itself.[17]

Management of acute renal colic involves a decision whether urgent intervention is required or not. The presence of an obstructed infected upper urinary tract, renal deterioration, intractable pain or vomiting, anuria, or obstruction of a solitary or transplanted kidney are all indications for urgent intervention. Intervention is carried out by urology, and is beyond the scope of this chapter. Pain management has traditionally included narcotics, but stimulation of dependency and long-term effects make nonsteroidal anti-inflammatory drugs (NSAIDs) attractive alternatives. When urgent intervention is not selected, the interval between acute colic and elective intervention for failure of stone passage is an important topic. Observation for up to 4 weeks is considered generally reasonable.[18]

CALCIUM STONES

Classification of Calcium Nephrolithiasis by Urinary Chemistries

Biochemical and physical disturbances that contribute to the formation of calcium stones are quite varied, based on two surveys from the mid-1990s.[19,20] Several disturbances have the potential to create the environment conducive to renal stone formation. Several investigators utilize the presence of such disturbances as the basis for diagnostic categorization of nephrolithiasis.[19–21] Earlier studies based on ambulatory evaluations of patients with nephrolithiasis reported 10 metabolic etiologies composing four types of hypercalciuria, hyperuricosuria, hyperoxaluria, renal tubular acidosis (RTA), uric acid stones, and infection stones, and an 11% incidence of finding no metabolic abnormalities.[22] Now more than 15 etiologic categories of nephrolithiasis have been described (Table 20.1). A single diagnosis is found in the minority of patients whereas approximately 60% have more than one diagnosis. The finding of no metabolic abnormality can be reduced to the range of 2% to 4% of patients with care and repeated measures. Hypercalciuric nephrolithiasis accounts for about 60% of the patients. Hyperuricosuria associated with calcium nephrolithiasis can be subdivided into hyperuricosuric calcium nephrolithiasis and patients with gouty diathesis. Hyperoxaluric calcium nephrolithiasis, which occurs in about 8% of patients with recurrent stones, has been subdivided into enteric, primary, and dietary variants. Hypocitraturic calcium nephrolithiasis, which affects about 30% of patients in its idiopathic variant, is also associated with incomplete RTA and the chronic diarrheal syndrome. Hypomagnesiuric calcium nephrolithiasis, infection stones, and cystinuria are uncommon, accounting for 7%, 6%, and 1% of patients, respectively. The acquired problem of low urinary volume, less than 1 L per day according to Levy and colleagues[19] and less than 1.5 L per day according to Seltzer and Hruska,[20] is the single most common abnormality.

The descriptions of clinical subtypes that follow represent the minimal diagnostic criteria used to establish the presence of the entities listed in Table 20.1, according to Hruska and Seltzer.

Absorptive Hypercalciuria Type I

Diagnostic criteria include: calcium nephrolithiasis, normocalcemia, normophosphatemia, hypercalciuria (>200 mg per day) on a calcium-restricted diet, normal fasting urinary calcium (<0.11 mg per dL glomerular filtrate [dL GF]), exaggerated calciuric response to an oral calcium load (>0.20 mg urinary calcium per mg urinary creatinine), and normal to suppressed serum parathyroid hormone (PTH) function.[23–30]

Absorptive Hypercalciuria Type II

Criteria are the same as for type I, except for normal urinary calcium (<200 mg per day) on the restricted diet.[23–29,31]

Absorptive Hypercalciuria Type III (Renal Phosphaturia)

Diagnostic criteria are characterized as similar to type I, except for persistent hypophosphatemia (2.5 mg per dL or less).[32]

Sodium-Linked Phosphate Transporter

Low serum phosphate concentrations due to a decrease in renal phosphate reabsorption occur in some patients with renal calcium stones and/or bone demineralization. Two different heterozygous mutations in the sodium-linked phosphate transport protein encoded by the NPT2a gene have been associated with this disorder.[33] Subsequent studies have shown that although genetic variants of NPT2a are not rare, they do not seem to be associated with clinically significant renal phosphate or calcium handling anomalies in a large cohort of hypercalciuric stone-forming pedigrees.[34]

Renal Hypercalciuria

Diagnostic criteria include: calcium nephrolithiasis, normocalcemia, normophosphatemia, hypercalciuria on the restricted diet, elevated fasting urinary calcium (>0.11 mg per dL GF), and elevated serum parathyroid hormone (PTH).[35]

Primary Hyperparathyroidism (Resorptive Hypercalciuria)

Criteria for diagnosis include: nephrolithiasis, hypercalcemia, hypercalciuria, and high serum PTH with surgical confirmation of abnormal parathyroid tissue.[23–26,28,36,37]

20.1 Urinary Chemistries in Evaluation of Nephrolithiasis[a]

Category	Seltzer and Hruska[80] Jewish Hospital (n = 587)		Levy et al.[79] University of Texas Southwestern (n = 1270)	
	Sole occurrence (%)	Combined occurrence (%)	Sole occurrence (%)	Combined occurrence (%)
Hypercalciuria	14	51	—	—
Male (>250 mg/24 hr)	8	34	—	—
Female (>225 mg/24 hr)	6	17	—	—
Absorptive hypercalciuria	—	37	6.1	23.1
Fasting hypercalciuria	—	14	4.3	13.9
Renal hypercalciuria	—	1	0.3	1.3
Renal phosphaturia	—	2	2.1	7.6
Primary hyperparathyroidism	—	1	0.8	1.3
Hyperuricosuria	8	42	—	—
Male (>0.75 g/24 hr)	6	30	—	—
Female (>0.70 g/24 hr)	2	12	—	—
Hyperuricosuric calcium nephrolithiasis	—	—	8.3	27.6
Gouty diathesis	—	—	3.1	6.9
Hypocitraturia	9	34	—	—
Male (<250 mg/24 hr)	5	18	—	—
Female (<300 mg/24 hr)	4	16	—	—
Complete distal RTA	—	—	0.08	0.16
Incomplete distal RTA	—	—	0.0	1.1
Chronic diarrheal syndrome	—	—	0.2	1.8
Idiopathic	—	—	3.5	24.4
Hyperoxaluria (>40 mg/24 hr)	8	34	—	—
Enteric hyperoxaluria	—	—	0.2	1.4
Primary hyperoxaluria	—	—	0.0	0.4
Dietary hyperoxaluria	—	—	0.4	5.7
Hypomagnesuria (<5 mEq/24 hr)	5	26	0.3	6.5 (<50 mg/24 hr)
Low urinary volumes (<1500 mL/24 hr)	26	61	1.7	13.5 (<1,000 mL/24 hr)
No diagnosis/difficult to classify	—	2	—	4.0

[a]The category definitions in the table refer to Jewish Hospital, Washington University, St. Louis. Criteria for the University of Texas Southwestern data are provided in the text.
RTA, renal tubular acidosis.

Fasting Hypercalciuria and Elevated Fasting Urinary Calcium

Calcium nephrolithiasis and hypercalciuria on a restricted diet can be categorized into a resorptive form because of fasting hypercalciuria. Fasting hypercalciuria is further characterized by normal to suppressed parathyroid function, eliminating renal calciuria, normocalcemia, and normophosphatemia (>2.0 mg per dL).

Hyperuricosuric Calcium Nephrolithiasis

The diagnostic criteria for hyperuricosuric calcium nephrolithiasis (HUCN) include: calcium nephrolithiasis, hyperuricosuria

(>700 mg per day for females; >750 mg per day for males), and frequently a low urinary pH of ≤5.5.[38–40]

Gouty Diathesis

Criteria include uric acid or calcium nephrolithiasis and low urinary pH (<5.5) in the absence of excessive gastrointestinal alkali losses[41–43] or animal protein excess. Hyperuricemia, hypertriglyceridemia, and a history of gouty arthritis may be present.

Hyperoxaluric Calcium Nephrolithiasis

Criteria include calcium nephrolithiasis and hyperoxaluria (>44 mg per day). The three forms of hyperoxaluric calcium nephrolithiasis are:

1. Enteric hyperoxaluria, defined as the presence of ileal disease (Crohn disease, ulcerative colitis, jejunoileal bypass, or intestinal resection), or fat malabsorption with hyperoxaluria on the random and restricted diets.[44–47]
2. Primary hyperoxaluria, consisting of marked hyperoxaluria (>80 mg per day) without evidence of bowel disease, high oxalate diet, low calcium diet, treatment with calcium-binding agents, enhanced oxalate absorption, or high doses of vitamin C.[48]
3. Dietary hyperoxaluria, marked by high oxalate diet, hyperoxaluria on a random diet, and normal urinary oxalate excretion on the restricted diet.[47,49,50]

Enteric hyperoxaluria is typically associated with hypocitraturia due to intestinal loss of HCO_3, low urinary volume, and low normal urinary calcium excretion.

Hypocitraturic Calcium Nephrolithiasis

Diagnostic criteria include calcium nephrolithiasis and hypocitraturia (<320 mg per day),[39] that compose:

1. Distal RTA, which is characterized by systemic metabolic acidosis or defective urinary acidification following an ammonium chloride load and urinary pH above 6.5. The acidosis is a hypokalemic, hyperchloremic nonanion gap metabolic acidosis.[50] In the complete form, metabolic acidosis is present before an ammonium chloride load, whereas in the incomplete form, urinary acidification following an ammonium chloride load is impaired despite normal serum electrolytes before the load.
2. Chronic diarrheal syndrome, which is defined as chronic diarrhea with excessive alkali loss from various gastrointestinal disorders (e.g., gastric resection, ileal disease, Crohn disease, and ulcerative colitis).[51]
3. Idiopathic hypocitraturia of unknown etiology.

Hypomagnesiuric Calcium Nephrolithiasis

Criteria for diagnosis include: nephrolithiasis, hypomagnesuria (<50 mg per day) on the random diet, and absence of a diarrheal disorder.[52]

Infection Stones

Criteria for diagnosis include struvite or carbonate-apatite nephrolithiasis.[53]

Cystinuria

Diagnosis is based on cystine nephrolithiasis and urinary cystine level higher than 200 mg per day.[54]

Low Urine Volume

Diagnosis is based on calcium or uric acid nephrolithiasis and urine volume less than 1 L per day.[55]

No Metabolic Abnormality

This is attributed to calcium nephrolithiasis and a normal biochemical evaluation.[56]

Difficult to Classify

Those that fall into this category are generally nephrolithiasis with recognized stone risk factors.[57] A definitive diagnosis cannot be made due to borderline or inconsistent laboratory values or to the absence of critical data (e.g., stone analysis or roentgenographic visualization).

HYPERCALCIURIC NEPHROLITHIASIS

The majority of patients with calcium nephrolithiasis exhibit hypercalciuria (Table 20.1) and have idiopathic hypercalciuria, which is a term used to describe recurrent nephrolithiasis associated with hypercalciuria, and is probably a distinct entity. However, most of the data suggest that multiple metabolic abnormalities besides excess calcium excretion contribute to nephrolithiasis associated with hypercalciuria. Furthermore, there is a much higher incidence of idiopathic hypercalciuria than nephrolithiasis in the general population.[58] Several estimates place the incidence of idiopathic hypercalciuria at 2% to 4%,[59] whereas the incidence of nephrolithiasis is no more than 0.5% to 1.0%. Between 40% and 50% of calcium stone formers excrete excess calcium in their urine, defined as more than 300 mg per 24 hours (men), 250 mg per 24 hours (women) on ≥1,000 mg calcium intake, or 4 mg per kg body weight per 24 hours (either sex). The term idiopathic hypercalciuria applies if the serum calcium level is normal and sarcoidosis, RTA, hyperthyroidism, malignant tumors, rapidly progressive bone disease, immobilization, Paget disease, Cushing disease (or syndrome), and furosemide administration have been excluded. Virtually all normocalcemic hypercalciuria encountered in patients with nephrolithiasis falls under the umbrella of "idiopathic hypercalciuria."[19]

Among patients with recurrent calcium stones who have served as control subjects in randomized, controlled trials of interventions, new stones formed in 43% to 80% of subjects within 3 years.[14,60–62]

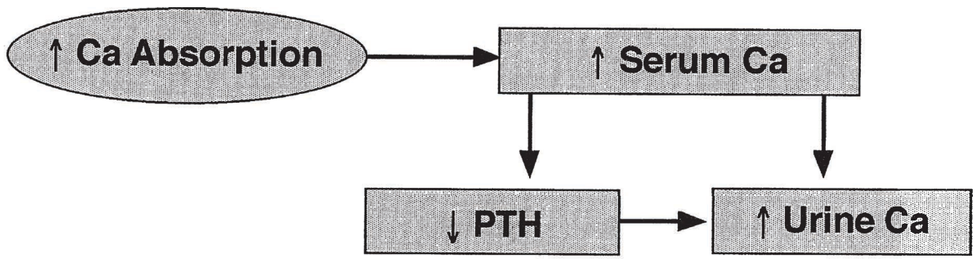

FIGURE 20.1 Pathogenesis of absorptive hypercalciuria. Intestinal hyperabsorption of calcium leads to an increase in serum calcium and a reduction of parathyroid hormone (PTH). The increase in the filtered load of calcium and the reduced calcium reabsorption produced by loss of PTH activity lead to hypercalciuria.

Idiopathic Hypercalciuria

Idiopathic hypercalciuria is an inherited syndrome. Studies of families of patients with hypercalciuric nephrolithiasis reveal a high incidence of hypercalciuria in first-degree relatives.[63] The pattern of inheritance in consecutive generations with high frequency is compatible with an inherited trait with the broad characteristics of autosomal dominant transmission. This pattern of inheritance was demonstrated in large kindred.[64] Hypercalciuria also occurs in children at the same rate as in adults.[65] Spontaneous hypercalciuria similar to that observed in hypercalciuric nephrolithiasis of humans has been demonstrated in the laboratory rat,[66,67] which has become an animal model of the human disorder.

The pathogenesis of idiopathic hypercalciuria involves excessive intestinal calcium absorption and depressed renal tubular calcium reabsorption (Fig. 20.1). The latter is largely due to suppression of PTH[68,69] and can be considered as a major factor in preventing hypercalcemia associated with increased intestinal absorption. When placed on low calcium diets, patients with hypercalciuric nephrolithiasis often demonstrate a negative calcium balance.[70] This could be due to defective renal tubular calcium reabsorption, but renal hypercalciuria should produce secondary hyperparathyroidism, which is rarely observed.[19,20] In addition, considerable evidence indicates that PTH levels are suppressed and that the negative calcium balance stems from excessive skeletal remodeling and bone resorption.[68,69] The question of whether depressed renal tubular calcium reabsorption greater than that expected with PTH suppression contributes to the hypercalciuria of nephrolithiasis remains unanswered. It is supported only by data from a few patients in whom secondary hyperparathyroidism has been documented (see "Renal hypercalciuria" in Table 20.1).

In hypercalciuric calcium oxalate stone formers, the initial site of calcium/phosphate crystal deposition is the basement membrane of the thin limbs of Henle's loop. There is subsequent extension to the vasa section, then the interstitium and, in the most severe cases, to the papillae. Alternatively, in patients with hyperoxaluria secondary to intestinal bypass and idiopathic calcium phosphate stones, the initial crystals were again calcium/phosphate complex, but these arose within the tubule lumens of terminal collecting ducts. Non–stone formers, when subjected to nephrectomy, had neither plaque nor crystals. Thus, there are different sites of crystallization depending on the metabolic abnormalities leading to stone formation.[71,72]

Additionally, in patients with idiopathic hypercalciuria, there was evidence for crystal-induced cell injury in areas of dense crystal deposition, whereas in the bypass patients there was not only cell injury, but also cell death.[11]

The genetically hypercalciuric stone forming rats spontaneously form calcium/phosphate stones unless their diet is augmented with an oxalate precursor.[73]

In the genetic hypercalciuric stone forming rats, calcium oxalate (but not calcium/phosphate) stones induce marked proliferation of the urothelium resulting in sequestration of stones.[67]

Thus, rats and humans appear protected against calcium oxalate stone formation unless a nucleation site, such as the calcium/phosphate crystal, is present.

Absorptive Hypercalciuria

Increased intestinal absorption of calcium is a uniform finding in patients with hypercalciuric nephrolithiasis[20] (Fig. 20.1). At issue is whether increased absorption is the primary defect or caused secondarily in the idiopathic hypercalciuric syndrome (Figs. 20.1 to 20.4). All forms of hypercalciuric nephrolithiasis are associated with increased intestinal calcium absorption. Those associated with intestinal calcium hyperabsorption on a secondary basis—renal hypercalciuria, primary hyperparathyroidism, and renal phosphaturia—are relatively uncommon forms of hypercalciuric nephrolithiasis. Furthermore, fasting hypercalciuria appears to be the expression of increased skeletal remodeling and intestinal calcium hyperabsorption together. All of this indirectly suggests that a specific problem producing intestinal calcium hyperabsorption is a major, if not the basic, underlying defect in idiopathic nephrolithiasis.

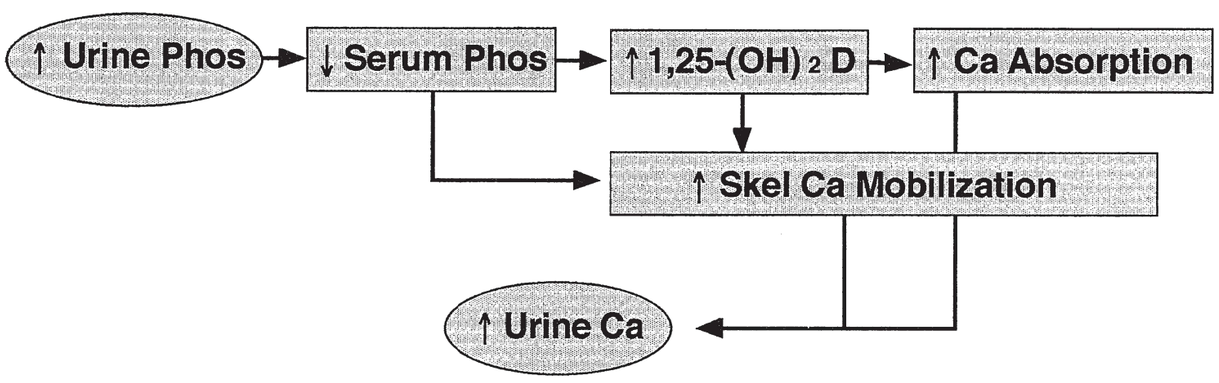

FIGURE 20.2 Renal phosphaturia. Hypophosphatemia leads to increased production of $1,25(OH)_2D_3$, intestinal hyperabsorption of calcium, and increased skeletal calcium mobilization. As a result, hypercalciuria develops.

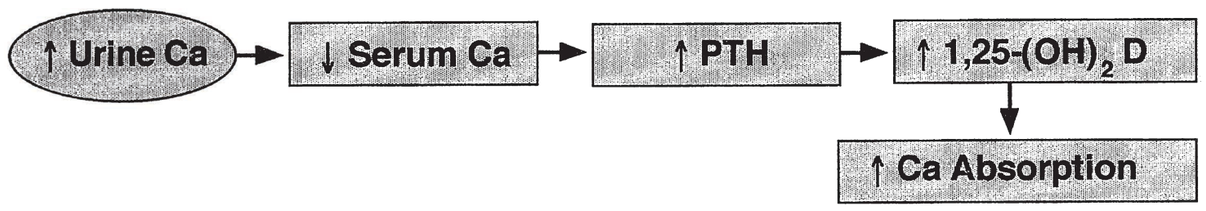

FIGURE 20.3 The pathogenesis of renal hypercalciuria. Defective renal calcium reabsorption leads to hypocalcemia and a stimulation of parathyroid hormone (PTH) secretion. The latter increases the production of $1,25(OH)_2D_3$, stimulating calcium absorption and leading to hypercalciuria. The hypercalciuria in turn compensates for the reduction in serum calcium but compensation never completely restores normal calcium, and elevated PTH values are required in this syndrome.

Intestinal Calcium Absorption

Net calcium absorption is the difference between the mucosal absorptive rate and the secretion of calcium into biliary, duodenal, and pancreatic fluids. Although calcium absorption rates may be measured using oral radiolabeled calcium, only overall balance studies in which fecal losses are measured can quantitate net calcium absorption. The mucosal to serosal absorptive rate is higher in patients with hypercalciuric nephrolithiasis than in healthy individuals[26,36,74–81] (Table 20.2), but overlap is extensive. In six studies, individuals with no signs of hypercalciuric nephrolithiasis absorbed an average of 27% to 52% of an oral dose of radioactive calcium, whereas those with hypercalciuric nephrolithiasis absorbed 22% to 80%. If one chooses only the six studies incorporating normal control subjects, the more efficient calcium absorption by hypercalciuric nephrolithiasis subjects is particularly evident. Increased mucosal-to-blood transport of calcium, but not magnesium, has also been demonstrated directly by in vivo jejunal perfusion in hypercalciuric nephrolithiasis.[29] At normal calcium intakes, <1,500 mg per day, calcium absorption in the duodenum and proximal jejunum is an active process mediated by a mucosal membrane calcium pump (ECaC)[82,83] and efficient cytosolic calcium binding proteins (calbindin),[84] both transcriptionally regulated by calcitriol. At high calcium intakes, passive transport mechanisms in the more distal small bowel and colon may account for most of calcium absorption as the proximal active calcitriol-regulated mechanisms are suppressed.

In normal individuals, urine calcium excretion rises slowly with net absorption[85] (Fig. 20.5), and calcium balance is usually positive when the absorption rate exceeds 200 mg per 24 hours. At all levels of net absorption, urinary calcium excretion was higher in hypercalciuric than in normal subjects, so much so that none of the patient data fell within the 95% confidence band derived from studies of normal individuals (Fig. 20.6). For example, in the range of 200 to 300 mg of net calcium absorption, not one of 38 normal subjects excreted as much as 300 mg of calcium in the urine, whereas 16 hypercalciuric patients did (compare Figs. 20.5 and 20.6). In other words, hypercalciuric nephrolithiasis subjects excreted in the urine an abnormally high percentage of the calcium they absorbed from the intestine. This is compatible with suppression of renal tubular calcium transport rates by low levels of PTH. Net absorption rates exceeded 200 mg per 24 hours in 55 normal subjects (Fig. 20.5). Urine calcium excretion was less than net absorption; that is, calcium balance was positive in 48 subjects. If a generous margin for error (50 mg per 24 hours) is allowed in the balance data, none of the 55 normal individuals were in negative calcium balance. Among 37 hypercalciuric patients with a calcium absorption rate above 200 mg per 24 hours, however, calcium excretion exceeded net absorption in 23 patients by more than 50 mg per 24 hours (Fig. 20.6). In other words, negative calcium balance was frequent in idiopathic hypercalciuria subjects but not in normal individuals. This is compatible with either reduced tubular reabsorption, which should produce elevated levels of PTH, or with excessive bone resorption. The latter appears most likely.

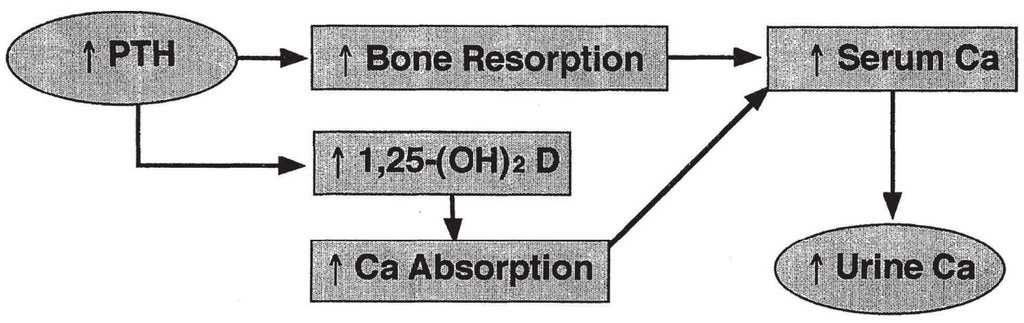

FIGURE 20.4 Pathogenesis of hypercalciuria and primary hyperparathyroidism. Excess secretion of parathyroid hormone (PTH) stimulates bone remodeling and elevations of $1,25(OH)_2D_3$ and calcium absorption. The resultant increase in serum calcium leads to an increase in the filtered load of calcium. The latter overwhelms the stimulation of renal tubular calcium transport by PTH, and hypercalciuria results.

Renal Tubular Calcium Reabsorption

Two systematic studies[86,87] have evaluated overall renal fractional calcium reabsorption (Table 20.3). In both, the filtered load of calcium was calculated from inulin clearance or creatinine clearance and ultrafilterable serum calcium concentration. The fraction of the filtered calcium load excreted was calculated for several clearance periods in normal and hypercalciuric nephrolithiasis subjects. Fractional calcium excretion was clearly high in the hypercalciuric nephrolithiasis subjects. The effects of hydrochlorothiazide and acetazolamide on the renal tubular handling of sodium, magnesium, and calcium suggested to the authors a generalized defect in proximal tubular reabsorption.[86–88] These studies did not examine the role of suppressed or elevated PTH levels in the subjects with hypercalciuric nephrolithiasis. The general finding of a tendency for PTH levels to be

20.2 Intestinal Calcium Absorption in Normal Subjects and Patients with Idiopathic Hypercalciuria

Reference (no.)	Method	Calcium intake (mg/24 hr)	Dietary calcium absorbed (%)[a]	
			Normal subjects	Idiopathic hypercalciuria
Caniggia et al.[124]	Fecal ^{45}Ca	Free diet[b]	None studied	22.0 (1)
Birge et al.[123]	^{47}Ca, PO/IV	800	52.2 ± 13.2 (6)	58.5 ± 8.6 (4)
Wills[125]	^{47}Ca, PO/IV	400	49.0 ± 10.0 (4)	76.0 ± 17.0 (5)
Pak[126]	Fecal ^{47}Ca	400	45.6 ± 9.0 (29)	69.7 ± 7.0 (9) 58.1 ± 13.0 (11)[c]
Pak et al.[86]	Fecal ^{47}Ca	400	50.0 ± 7.0 (20)	71.0 ± 7.0 (22)[d] 50.0 ± 17.0 (2)[e]
Ehrig et al.[126]	$^{47}Ca/^{45}Ca$, PO/IV	462–952	None studied	47.8 ± 11.0 (22)[f] 37.6 ± 11.0 (22)[g]
Kaplan[94]	Fecal ^{47}Ca	400	48.0 ± 8.0 (11)	80.0 ± 9.0 (211)[d] 73.0 ± 7.0 (3)[e]
Shen[128]	$^{47}Ca/^{45}Ca$, PO/IV	Free diet[b]	27.0 ± 9.0 (14)	40.0 ± 9.0 (15)
Barilla[129]	Fecal ^{47}Ca	400	None studied	69.5 ± 6.4 (10)[d] 70.1 ± 10.4 (8)[e]
Zerwekh and Pak[130]	Fecal ^{47}Ca	400	None studied	69.0 ± 7.0 (11)[d] 68.0 ± 9.0 (10)[d]

[a]Values are means ± standard deviations; numbers in parentheses represent numbers of patients studied.
[b]Usual diet but not measured.
[c]Eleven patients listed as having normocalcemic primary hyperparathyroidism may be considered hypercalciuric.
[d]Absorptive idiopathic hypercalciuria.
[e]Renal idiopathic hypercalciuria.
[f]Prior to therapy.
[g]Three to 16 months after administration of hydrochlorothiazide.
Ca, calcium; PO, orally; IV, intravenously.

suppressed and the rarity of secondary hyperparathyroidism call for a reexamination of the issue regarding renal tubular calcium fluxes.

A rare syndrome, X-linked hypercalciuric nephrolithiasis (XLHN), or Dent disease, is characterized by recurrent calcium nephrolithiasis and has been found to be due to mutations in a proximal tubular intracellular vesicle chloride transport protein, CLCN5.[89–93] Two other types of hypercalciuric nephrolithiasis, which map to the same defective gene on the X chromosome (Xp11.22) as Dent disease, X-linked recessive nephrolithiasis and recessive hypophosphatemic rickets, are associated with inactivating mutations in CLCN-5.[89]

The CLCN-5 gene is a member of a family of genes that encode voltage-gated chloride channels.[90] CLCN-5 is found in the kidney tubules and in bone cells. All mutations in the CLCN-5 gene found to date have been functional, with loss of function manifested as a lowered conductance of the mutated channel. CLCN-5 is distributed in the human kidney in the proximal tubule, in the thick ascending limb of the loop of Henle, and in the α-type intercalated cells of the collecting duct.[90] These sites are where calcium is resorbed from the filtrate. CLCN-5 knockout animals are hypercalciuric and proteinuric.[91]

CLCN-5 colocalizes with the vacuolar H+-ATPase in proximal tubular cells and a-type intercalated cells. CLCN-5 mutations are associated with modifications in the polarity and expression of H1-ATPase, but not ultrastructural alterations in proximal tubular cells.[93] The variability in diseases

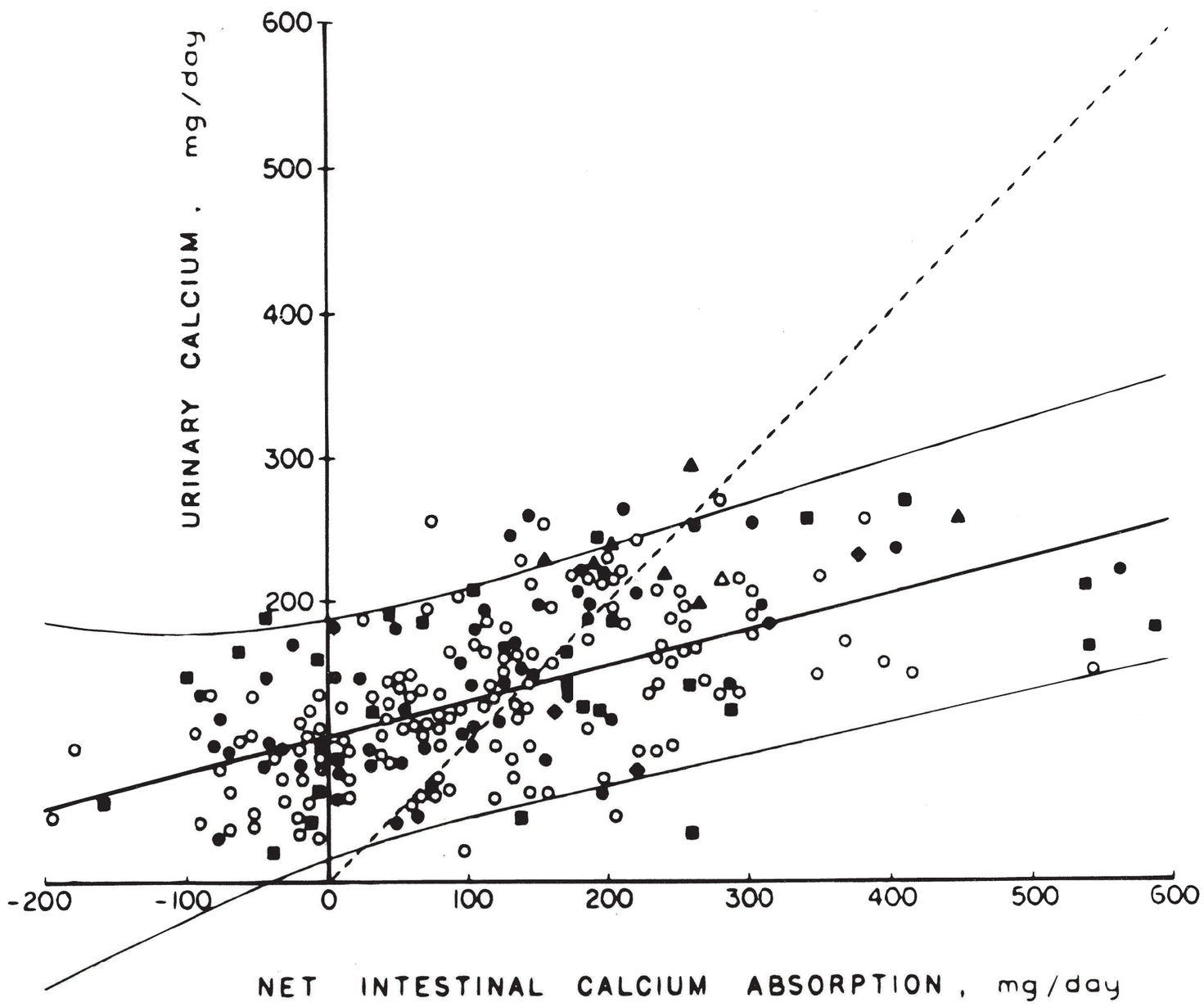

FIGURE 20.5 Urinary calcium excretion as a function of net intestinal calcium absorption. Data are derived from 6-day balance studies on 195 normal adults. Each symbol represents individual subjects from different sources: *circles,* Knapp[238]; *squares,* Lafferty and Pearson[239]; *diamonds,* Liberman et al.[240]; and *triangles,* Edwards and Hodgkinson.[241] *Open figures* represent women, and *solid figures* represent men. *Solid lines* represent mean and 2 standard deviations. The *dotted line* is the line of identity; points above the line reflect negative calcium balance. (From Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC, eds. *The Kidney,* 2nd ed. Philadelphia: WB Saunders; 1991, with permission.)

with the CLCN-5 mutations involves impaired solute reabsorption by the proximal tubule and range from kaliuresis, glycosuria, phosphaturia, and/or hypouricemia. Constant findings of Dent disease include hematuria and low molecular weight proteinuria. Dent disease progresses to renal failure due to nephrocalcinosis and tubulointerstitial nephritis.

Thiazide diuretics reduce hypercalciuria in patients with CLCN-5 mutations, but thiazides can make these patients become hypokalemic. The beneficial effect must be weighed against the potential side effect profile.[92] Langman finds little reduction in stone formation even when urinary calcium is lowered to normal in such individuals.[94]

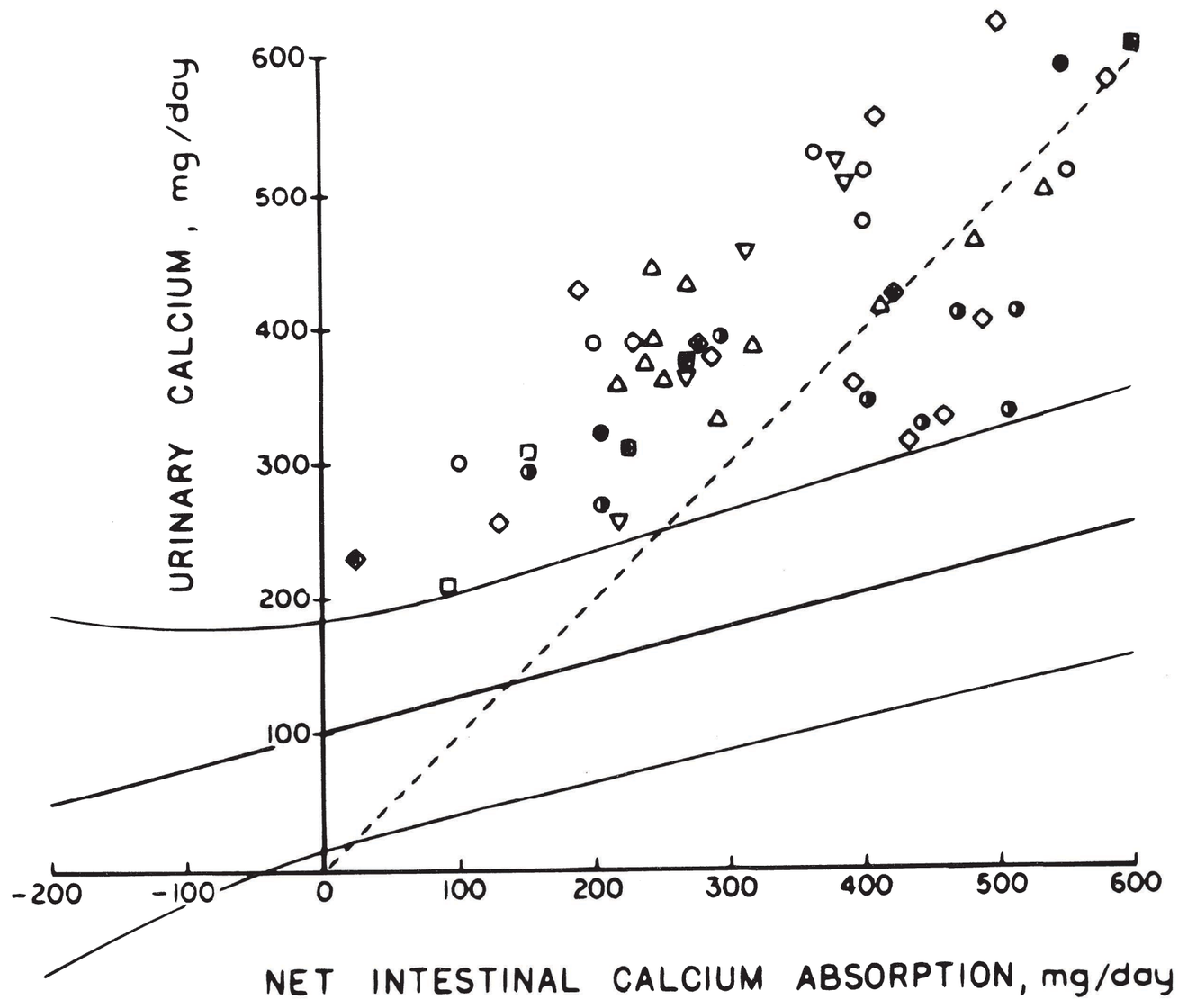

FIGURE 20.6 Urinary calcium excretion as a function of net intestinal calcium absorption from 6-day balance studies performed on 51 patients with idiopathic hypercalciuria reported as follows: *open square,* Henneman et al.[243]; *open squares with dot in center,* Jackson and Lancaster[244]; *open triangles,* Harrison[245]; *open circles with dot in center,* Dent et al.[246]; *open inverted triangles,* Parfitt et al.[247]; *closed diamonds,* Edwards and Hodgkinson[241]; *open diamonds,* Liberman et al.[240]; and *half-darkened circles,* Lemann.[248] *Solid lines* represent mean and 2 standard deviations derived from balance studies from 195 normal adults, shown in Figure 26.10. The *dotted line* is the line of identity, with positive calcium balance below the line. (From Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC, eds. *The Kidney,* 2nd ed. Philadelphia: WB Saunders; 1991, with permission.)

20.3 Fraction of Filtered Calcium Excreted in the Urine by Normal and Hypercalciuric Subjects[a]

	Normal subjects	Hypercalciuric subjects[b]
Edwards and Hodgkinson[137]	0.94% (7)	2.94% (14), $P < 0.001$
Peacock and Nordin[135]	1.27% (5)	4.25% (9), $P < 0.01$

[a]Number of subjects studied are shown in parentheses next to fractional excretion values.
[b]Urine calcium >300 mg/24 hr (men) or 250 mg/24 hr (women).

Another Voltage-Gated Chloride Channel

Bartter syndrome is a disease arising from one of three possible genes in the thick ascending limb that bear mutations in the Na^+-K^+-2Cl- gene NKCC2, in the K^+ channel ROMK, or in the chloride channel CLCNKB. Each of these mutations produces a phenotype that includes hypercalciuria and kidney stone formation with or without nephrocalcinosis. A missense mutation in the CLCNKB gene leads to disease of intrafamilial heterogeneity of urinary calcium levels. Some family members have Bartter syndrome with frank hypercalciuria, but others have hypocalciuria and a clinical phenotype of Gitelman syndrome.[95]

Hypomagnesemia/Hypercalciuria

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive tubular disorder that is frequently associated with progressive renal failure. The primary defect is related to impaired tubular reabsorption of magnesium and calcium in the thick ascending loop of Henle. Mutations in PCLN-1, which encodes the renal tight junction protein paracillin-1, were identified as the underlying genetic defects. Affected patients usually present in childhood or adolescence with symptomatic hypocalcemia.[96–98] Recurrent nephrolithiasis and nephrocalcinosis are also seen and progression to renal insufficiency and an acidification defect are common. The problem with acidification has been attributed to defective ammonia transfer to the deep nephrons and impaired medullary hydrogen ion secretion due to nephrocalcinosis.[99] Treatment with magnesium salts and thiazides seems to have no effect on the progression of the disease.

A second form of primary hypomagnesemia, Gitelman syndrome, is associated with hypocalciuria. It is due to mutations in the gene encoding the thiazide-sensitive sodium-chloride-cotransporter. Because thiazides are used to treat hypercalciuric nephrolithiasis, it is important to know that they can mimic the syndrome of hypomagnesemia including hypokalemia induced by magnesuria.[100]

Skeletal Remodeling

The high frequency of negative calcium balance in patients with idiopathic hypercalciuria on low calcium diets was the first indication that exaggerated bone resorption characterized the syndrome. Additional evidence for elevated skeletal remodeling in hypercalciuric nephrolithiasis has accrued. Several investigators[69,101] have documented reduced vertebral bone density in hypercalciuric nephrolithiasis by both CT and dual energy X-ray absorptiometry. Patients who exhibit fasting hypercalciuria tend to have a greater reduction in trabecular bone density than do other hypercalciuric patients, but there is significant overlap, and patients with absorptive hypercalciuria and normal fasting calcium excretion exhibit a high prevalence of reduced bone mineral density. Increased rates of skeletal remodeling with resorption favored over formation are supported by the findings of increased osteocalcin secretion and increased urinary hydroxyproline levels in patients with fasting hypercalciuria.[69] The pathogenesis of exaggerated bone remodeling rates may be due to elevations in 1α,25-dihydroxycholecalciferol ($1\alpha,25[OH]_2D_3$) levels, or due to elevations in bone cytokine activity such as prostaglandin activity[102] and interleukin-1 activity.[69] The result of this exaggerated skeletal remodeling is an increase in calcium release to the systemic circulation and suppression of PTH secretion.[103] One possibility is that exaggerated skeletal remodeling is a component of the syndrome of idiopathic hypercalciuria. Activation of skeletal remodeling in the hypercalciuric patient results in increased skeletal remodeling, leading to the loss of a quantum of the skeleton before counterregulatory influences decrease remodeling rates, removing the component of fasting hypercalciuria from the hypercalciuric syndrome. Such a scenario is sufficient to explain the clinical picture, as we currently understand it. Greater clarification of the roles of fasting hypercalciuria, and of bone remodeling, and their pathogenesis is required in patients with hypercalciuric nephrolithiasis. The role of skeletal remodeling in nephrolithiasis was further clarified by the recent discovery of a mutation in the type 2a, sodium-dependent phosphate cotransporter gene found in the proximal tubule and osteoclasts.[33]

Fasting Hypercalciuria

Except for negative calcium balance, either primary intestinal calcium absorption or a primary renal calcium leak could produce the findings summarized in Tables 20.2 and 20.3 and in Figures 20.5 and 20.6. Primary intestinal overabsorption increases postprandial serum calcium levels

above normal and increases the filtered load of calcium (Fig. 20.1). PTH secretion is reduced by the hypercalcemia, and suppression of PTH secretion would reduce calcium reabsorption because PTH stimulates renal tubular calcium reabsorption. In contrast, a renal tubular transport defect (Fig. 20.2) leading to hypercalciuria would produce secondary hyperparathyroidism. PTH, in turn, would stimulate the production of $1\alpha,25(OH)_2D_3$ and produce intestinal calcium hyperabsorption. Hyperabsorption would elevate postprandial serum calcium levels, raising the filtered calcium load and decreasing the magnitude of secondary hyperparathyroidism. The only way of distinguishing one mechanism from the other is by testing specific predictions that differ in the two forms of hypercalciuria. Clinically, PTH levels are the most clear-cut basis of distinction. Fasting hypercalciuria is not a means of detecting a renal calcium leak because it can be and is caused by exaggerated bone remodeling.

Absorptive hypercalciuria is associated with low or normal fasting immunoreactive PTH (iPTH) levels. The absorptive hypercalciuria hypothesis predicts a spectrum of fasting PTH values, but it forbids the combination of elevated fasting urinary calcium-creatinine ratio, and normal-to-suppressed iPTH levels. Normal PTH levels are typically observed in patients with fasting hypercalciuria and hypercalciuric nephrolithiasis. The renal model requires elevated fasting urinary calcium-creatinine ratios and a high serum iPTH level. This is seen uncommonly (Table 20.1).

On the other hand, evidence exists for suppressed PTH levels in patients with hypercalciuric nephrolithiasis who exhibit fasting hypercalciuria[103] (Fig. 20.7). When fasting hypercalciuric subjects are treated with sulindac, an NSAID agent, their urinary calcium excretion is decreased but, more importantly, their PTH levels are increased. This suggests that a bone resorptive process is releasing calcium to the circulation and suppressing PTH. Inhibition of bone resorption by sulindac results in an increase in PTH levels, suggesting that the levels are suppressed in patients with fasting hypercalciuria.[103]

Past studies attempting to detect low or normal PTH levels[26,35,79,104,105] suffer from difficulties with the radioimmunoassay for PTH. More recent double-antibody techniques that enable the measurement of intact hormone and the detection of low circulating PTH levels circumvent these problems and support the finding of low or normal PTH levels in patients with hypercalciuric nephrolithiasis.

Pathogenesis of Absorptive Hypercalciuria

A potential explanation for the pathogenetic process identified in absorptive hypercalciuria is abnormally elevated $1,25(OH)_2D_3$ levels. Patients with idiopathic hypercalciuria tend to exhibit elevations in $1,25(OH)_2D_3$ levels.[32,36,70,79,106] The frequency of high $1,25(OH)_2D_3$ levels in idiopathic hypercalciuria is controversial, but it appears to range from 30% to 40%. Kaplan[36] demonstrates that fractional calcium absorption correlates with the serum concentration of $1,25(OH)_2D_3$. Two thirds of the patients in this study did not have elevated $1,25(OH)_2D_3$ levels.

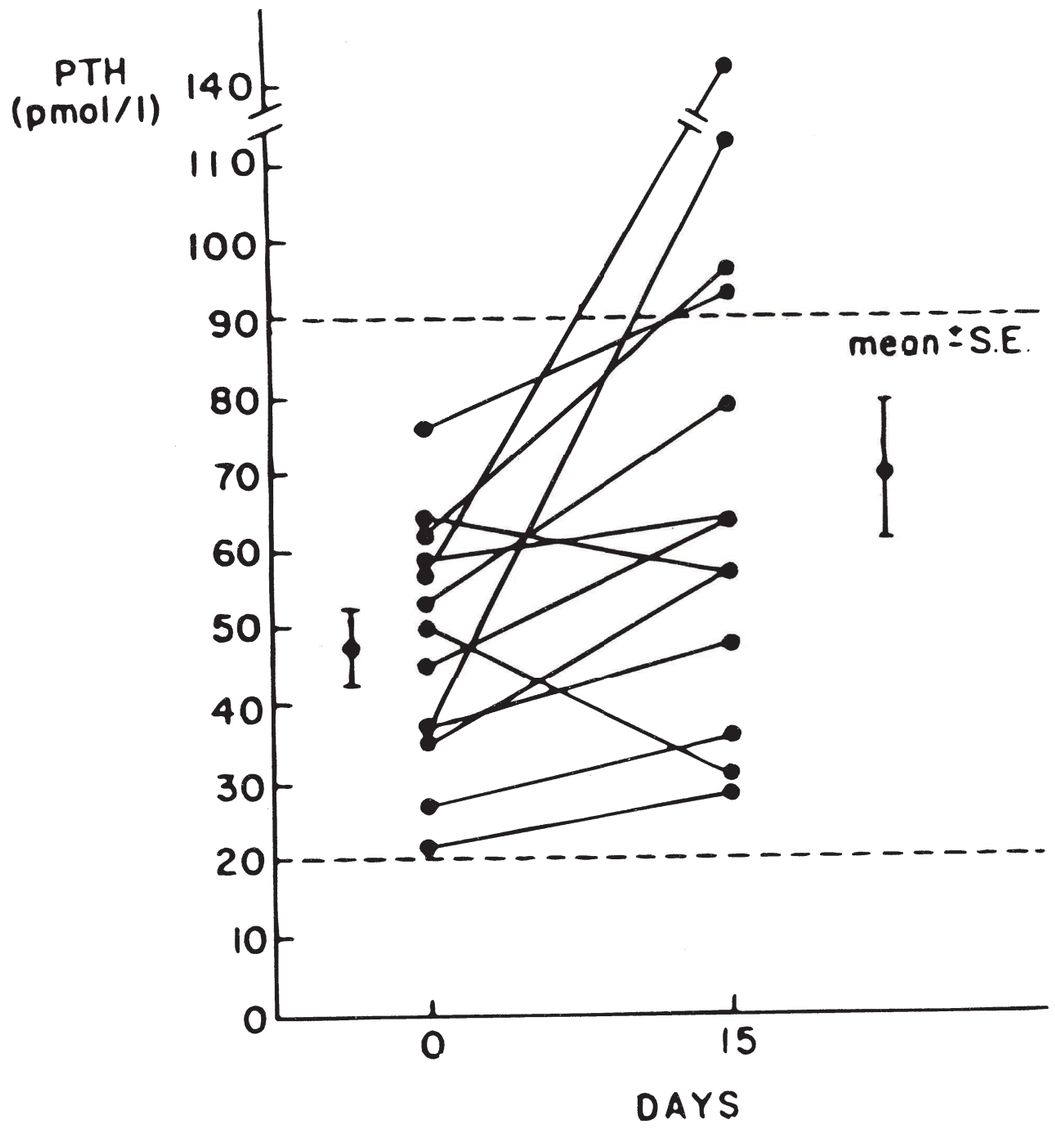

FIGURE 20.7 Changes in serum immunoreactive parathyroid hormone (PTH) in patients with fasting hypercalciuria after 15 days of diclofenac treatment. *Dotted lines* indicate the normal range of PTH. (From Filipponi P, Mannarelli C, Pacifici R, et al. Evidence for a prostaglandin-mediated bone resorption mechanism in subjects with fasting hypercalciuria. *Calcif Tissue Int.* 1988;43:61, with permission.)

On the other hand, evidence exists that shows that increased intestinal absorption of calcium may be primary and independent of vitamin D. Several studies indicated that the hypophosphatemia observed in idiopathic hypercalciuria is not sufficient to stimulate $1,25(OH)_2D_3$ levels.[107,108] Breslau[109] used ketoconazole, an imidazole antimycotic agent[110] capable of reducing serum $1,25(OH)_2D_3$ levels by 40%, in normal subjects and in patients with primary hyperparathyroidism after 1 week of therapy.[111] Ketoconazole was used as a probe to investigate the pathogenetic importance of $1,25(OH)_2D_3$ in patients with absorptive hypercalciuria. Twelve of 19 patients responded to ketoconazole with a reduction in serum $1,25(OH)_2D_3$ levels, intestinal calcium absorption, and 24-hour urinary calcium excretion. In the responding patients, intestinal calcium absorption was directly correlated with serum $1,25(OH)_2D_3$ levels and 24-hour urinary calcium excretion. In seven nonresponders, a reduction in $1,25(OH)_2D_3$ produced no change in intestinal calcium absorption or 24-hour urinary calcium excretion. The authors conclude that absorptive hypercalciuria is a heterogeneous disorder composed of both vitamin D–dependent and vitamin D–independent subsets.[109] The vitamin D–dependent subsets incorporate patients with elevated $1,25(OH)_2D_3$ levels, patients with abnormally responsive vitamin D receptors, and patients with allelic variations

20.4 Types of Renal Stones Formed and Frequency of Occurrence[a]

	CaOx and Cap	CaOx	Cap	Uric acid	Cystine	Struvite	Number of stones
Nordin and Hodgkinson[1]	46.0	14.7	8.0	2.9	3.3	25.1	243
Lagergen[2]	44.2	15.1	7.6	3.6	1.1	28.1	460
Melick and Henneman[3]	30.3	27.1	20.6	12.9	2.6	14.8	155
Prien[4]	34.3	32.7	5.3	5.8	2.9	19.0	1,000
Sutor et al.[5]	35.9	28.5	7.4	2.47	1.61	24.1	810
All series	37.2	26.3	7.4	4.5	2.2	22.3	2,668

[a]Numbers represent the percentage of each stone type in the series. Total numbers of stones surveyed are shown in the last column.
CaOX, calcium oxalate; Cap, calcium phosphate.
From Coe FL, Favus MJ. Nephrolithiasis. In Brenner BM, Rector FC Jr., eds. The Kidney, 2nd ed. Philadelphia: WB Saunders; 1991, with permission.

in the vitamin D receptor that have been incriminated in causing osteoporosis.[112] Animal studies in the genetically hypercalciuric rat support the possibility that an abnormal vitamin D receptor could contribute to the pathogenesis of absorptive hypercalciuria.[113]

Pathogenesis

The crystals that form into renal stones consist of calcium salts, uric acid, cystine, or struvite (magnesium ammonium phosphate). Calcium stones are the predominant variety (Table 20.4) and they are composed of calcium oxalate (CaOx), CaOx and calcium phosphate as apatite, or apatite alone.[12,114–119] Two forms of CaOx crystals—monohydrate and dihydrate—differ in their lattice structure and microscopic appearance[120] and this may be relevant to pathophysiology. The calcium phosphate crystals are most commonly apatite or hydroxyapatite. Calcium carbonate is a crystal form usually found mixed in struvite stones or a high pH environment. Occasionally, brushite (calcium hydrogen phosphate), whitlockite (calcium orthophosphate), and octacalcium phosphate are found.[118] Calcium phosphate crystals are as common in stones as are CaOx crystals (Table 20.4), but the amount of CaOx in mixed stones generally exceeds that of calcium phosphate. Additionally, pure CaOx stones are much more frequent than are pure calcium phosphate stones.

Formation of Renal Stones

The formation of renal stones composed of calcium salts is a complex process that remains poorly understood despite considerable efforts over many centuries. The process consists of a calcium salt precipitating from solution (nucleation) forming a crystal. Subsequent crystal growth and aggregation lead to a stone nidus. When the aggregate adheres to the tubulopelvic uroepithelium, continued epitaxial growth of the crystal aggregate eventually leads to a detectable size, making it a renal stone.

Nucleation

Nucleation describes the process that occurs when the activity of calcium salts reaches the level at which the solid phase begins. If one compares urine to an aqueous solution, it quickly becomes apparent that urine is able to hold much higher levels of calcium salt in solution than is water. If one considers an aqueous solution containing crystals of a calcium salt when the crystal neither grows nor shrinks, the solution is in equilibrium. The product of the free ion concentrations (activity product) at this equilibrium determines the equilibrium solubility product (SP) of the salt (Fig. 20.8). Solutions with concentrations of salt less than the equilibrium SP are undersaturated. A higher free ion activity product will cause the solid phase, the crystals, to grow (epitaxy). However, if the crystals are removed from a

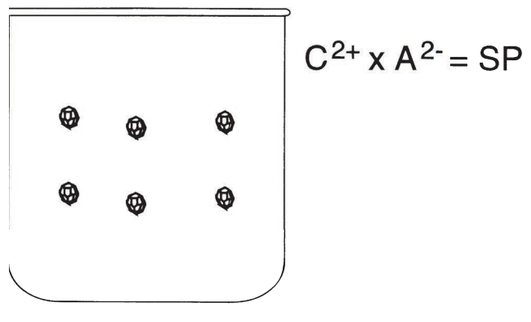

FIGURE 20.8 A solution containing calcium salt (a calcium salt consists of cations, C^{2+}, and anions, A^{2-}) crystals is in equilibrium when the crystals neither grow nor shrink. At this point, the product of the free ion concentration (activity product) is the equilibrium solubility product (SP) of the salt.

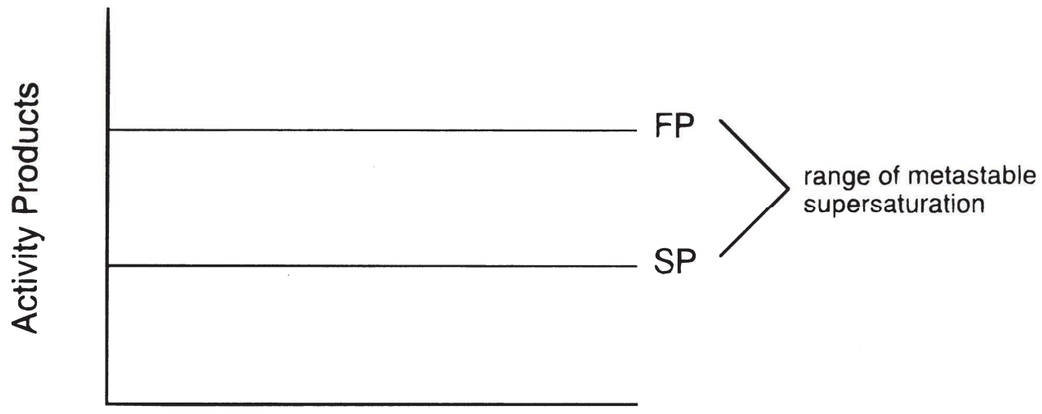

FIGURE 20.9 When calcium salts are added to a solution, precipitation (nucleation) does not occur until free ion activity products well above the solubility product are reached. The activity product at which solid phase begins to form is the formation product.

solution at the level of the equilibrium SP and then the ion activity product is elevated, the activity product that would have caused growth of preformed crystals now results in no appearance of a new solid phase. This solution is called metastably supersaturated (Fig. 20.9). The activity products of calcium salts in urine are almost constantly in the range of metastable supersaturation. In the range of metastable supersaturation, if the activity products are raised sufficiently, new crystals will appear. The activity product at which new crystals form is called the formation product (FP), or the upper limit of metastability (Fig. 20.9). Above the level of the FP, a solution is unstable, creating new crystal nuclei. Urine may be undersaturated, metastably supersaturated, or unstable with respect to CaOx or the stone-forming calcium phosphate crystals (brushite, octacalcium phosphate, hydroxyapatite, and apatite), but most of the time it is metastably supersaturated and, particularly for brushite, close to the FP.

Factors Influencing Urinary Supersaturation

The multiple factors influencing urinary supersaturation in a clinical setting are shown in Table 20.5. The renal excretion of calcium salts that precipitate and take part in stone formation is a primary determinant of urinary supersaturation. Thus, urinary volume, calcium, oxalate, and phosphate ions all participate in the risk of calcium stone formation. In addition, binding of calcium and oxalate to cells, or the solid phase, and urine pH (which influences relative amounts of monohydrogen phosphate and dihydrogen phosphate) drastically alter free ion concentrations and have great importance in regulating saturation—at least equal to the role of the total concentrations of the respective substances. This is the reason why hypercalciuria, oxaluria, unduly alkaline urine, and low urinary volumes are not sufficient to ensure that stones will form in and of themselves. Binding of the components of calcium salts also complicates the measurement of urine saturation, and simple concentration measurements give only small clues to actual free ion activity products.

Alternative substances to calcium salts may be considered as inhibitors of urinary saturation and contribute to the ability of urine to hold salts in solution to a much greater extent than does a simple aqueous solution. The known inhibitors of urinary saturation include the divalent cation magnesium, which forms oxalate, and phosphate salts, which are more soluble compared to those of calcium. In addition, citrate and sulfate are anions with which calcium forms soluble complexes as alternatives to phosphate or oxalate. Urine also contains substances to which calcium binds, thereby reducing the free ion activity. Pyrophosphate, nephrocalcin, and osteopontin are other inorganic and organic crystal inhibitory calcium-binding sites, and are discussed in greater detail later in this chapter. In addition, certain substances to which calcium salts may complex actually promote precipitation. In this category, uric acid and sodium urate are found. These substances are also discussed later in the chapter.

20.5 Factors Affecting Urinary Supersaturation in the Clinic

Renal excretion rates
- Calcium
- Oxalate
- Phosphate
- Protons
- Water

Inhibitors
- Magnesium
- Citrate
- Nephrocalcin
- Osteopontin
- Tamm-Horsfall protein
- Others (pyrophosphate, glycosaminoglycans)

Promoters
- Uric acid, urate
- Altered epithelial calcium oxalate binding

Measurements of Urinary Supersaturation

Because simple concentration measurements give little clue to the activity of specific ions in urine, several strategies have been designed to estimate urinary supersaturation.[61,121,122] These approaches are computer-based calculations of urinary free ion activity for calcium, oxalate, and phosphate derived from their concentrations and their known tendencies to form soluble complexes with each other and with other ligands such as citrate and sulfate. A calculated free ion activity product such as the CaOx ion product, when divided by the corresponding equilibrium SP, yields an activity product ratio (APR), which estimates the degree of saturation (Fig. 20.10). A ratio above one indicates urinary supersaturation. Ratios below one represent undersaturation. The upper limit of metastable supersaturation can be

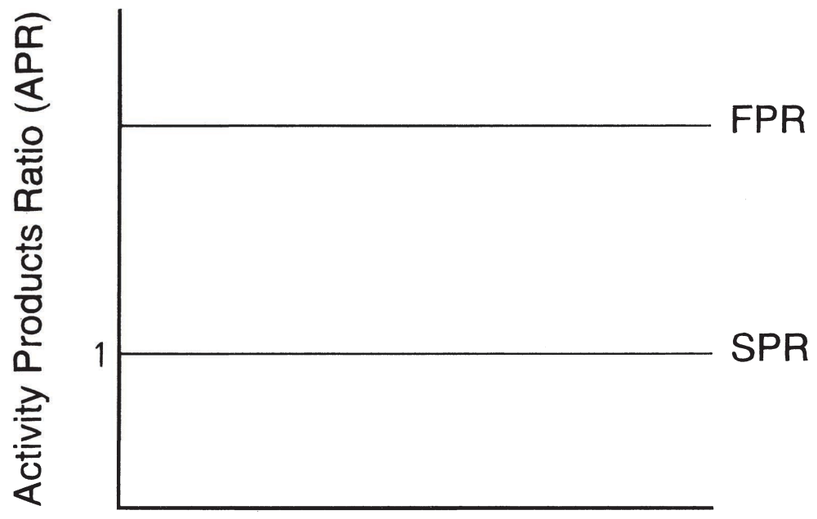

FIGURE 20.10 The calculated activity product of a calcium salt factored by its solubility product (SP) yields a ratio (R) describing undersaturation (<1), saturation (1), metastable supersaturation (>1), and the formation product ratio (FPR) at the point when the solid phase begins to form. The FPR determined for calcium oxalate (CaOx) by Pak and Holt[23] was 11 ± 3.

determined by raising the APRs to the point at which precipitation or solid phase formation begins to appear. The APR at this point is called the formation product ratio (FPR).[61] Pak and Holt[123] modified the approach to measuring urinary supersaturation by adding seed crystals to urine and incubating at 37°C with stirring at constant pH, for 2 days. The ratio of concentration products at the start and end of the incubation must equal the APR, even though the concentration products themselves do not equal the activity products. Pak and Holt[121,123] show that the assumption of stable activity coefficients is valid, so that that the empirical concentration product ratio is a valid estimate of the APR, within limits.

Observations of Urinary Supersaturation

Several investigators[124–128] with varying approaches have accumulated evidence indicating that urine from stone formers is more supersaturated than normal (Table 20.6). Because of differences in methodology, the absolute values of activity products differ among investigators. However, stone formers, whether hypercalciuric or normocalciuric, had higher average values of urine saturation than did those who did not form stones. This held whether saturation was measured with respect to CaOx, brushite, octacalcium phosphate, or hydroxyapatite.

An important observation common to approaches both with and without the use of seed crystals is that activity products of normal urine, on average, are above the equilibrium SP (Fig. 20.9) or oversaturated, except with respect to brushite. In the data from Pak and Holt[123,127] and Weber,[128]

20.6 Urine Calcium Oxalate and Calcium Phosphate Activity Product Ratios in Normal Subjects and in Stone Formers[a]

	Normal subjects	Hypercalciuric stone formers	TCH Normocalciuric TCH stone formers
Calcium oxalate monohydrate			
Robertson[b]	3 ± 1.2 to 10.7 ± 1.3	5.5 ± 1.3 to 18.2 ± 1.3	—
Pak[c]	1.45 ± 0.70	2.8 ± 1.4	2.2 ± 6.1
Weber[d]	1.97 ± 0.90	3.3 ± 2.2	2.2 ± 1.0
Brushite			
Pak[e]	0.35 to 0.26	1.74 ± 0.79	0.9 ± 0.5
Marshall[f]	1.15 ± 0.60	1.35 ± 0.70	4 ± 1.4
Octacalcium phosphate	63	79	200
Hydroxyapatite	4.6×10^5	9.1×10^5	2.9×10^8

[a]All values are means ± standard deviations.
[b]From Robertson et al.[20,24,25] and Marshall et al.[26]; values of APR were calculated from activity products; the equilibrium solubility product (K_{sp}) was taken as 1.7×10^{-9} m^2.
[c]From Pak[27]; values of APR were measured by experiments.
[d]From Weber[28]; values of concentration product ratio (see text) were measured by seeding experiments.
[e]From Pak et al.[23]; K_{sp} of brushite was taken as 9.32×10^{-7} m^2; values of APR were calculated.
[f]From Marshall[26]; K_{sp} of octacalcium was taken as 2.3×10^{-18} m^2 and of hydroxyapatite as 1.1×10^{-56} m^2; values of APR were calculated.
APR, activity product ratio.
Modified from Coe FL, Favus MJ. Nephrolithiasis. In: Brenner BM, Rector FC Jr., eds. The Kidney, 2nd ed. Philadelphia: WB Saunders; 1991, with permission.

this is a visible fact: Added crystals grew in urine from most normal persons. The use of urine measurements to assess supersaturation may be insufficient to reveal the full crystallization potential that exists in the renal tubule. Hautmann and colleagues[129] studied the calcium and oxalate concentrations in tissue from cortex, medulla, and papillae of human kidneys. The CaOx concentration product in the papillae exceeded that of urine and the concentrations in the medulla and cortex. If the high chemical concentration product in the papillae reflects a high free ion product in tubular fluid or interstitium, CaOx crystallization in this region may occur more rapidly than would be predicted from the ion product of the final urine.

Formation Products

The urinary APR at which urine produces new crystals has been measured for CaOx and brushite for those with no signs of stone formation and hypercalciuric, normocalciuric, and hyperparathyroid stone formers. Surprising variability was reported by Pak[127] (Fig. 20.11). However, the APR at the limit of metastability, the FPR, is higher in normal urine than in urine from stone formers. Furthermore, the FPR in urine from patients with hyperparathyroidism may be below the value observed for simple aqueous salt solution. This low of a value of FPR suggests facilitation of crystal formation. This type of data from several investigators yielded several conclusions. The first is that urine is abnormally supersaturated in stone formers. The values of APRs lie close enough to the FPR, at least for CaOx, so that new crystal formation would be expected. Most urine, even from those without stone formations, is metastably supersaturated with respect to CaOx so that growth of crystal nuclei into a significant mass is predictable.

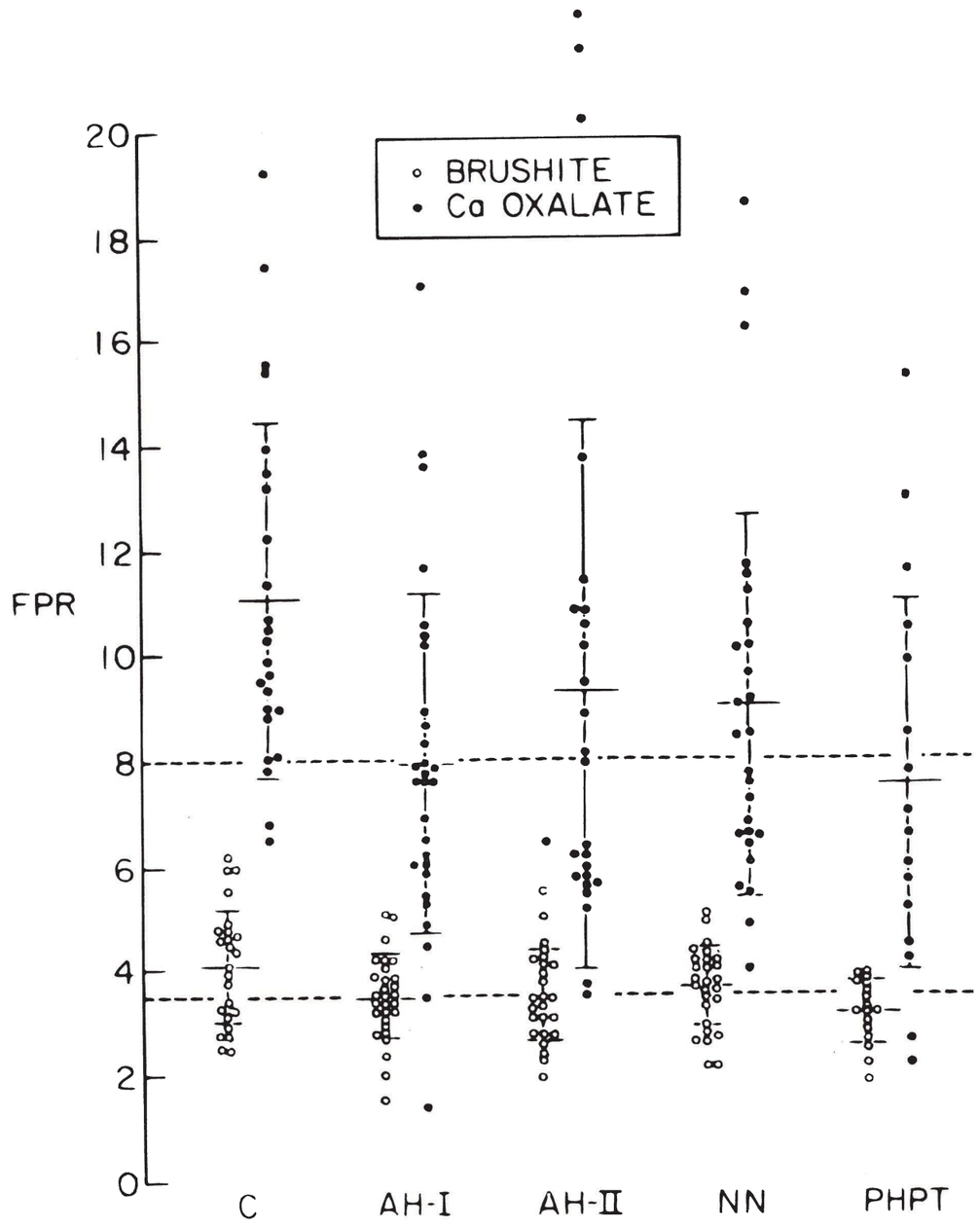

FIGURE 20.11 Formation product ratios (FPRs) for calcium oxalate (CaOx) and brushite of urine from normal subjects and stone formers. Each point shows the value for a single urine sample. For an aqueous solution, the activity product ratios at which spontaneous crystallization of CaOx and brushite occurs, the so-called FPRs, are shown by *dotted lines* at 8 and 3.6, respectively. *C*, control subject; *AH-1* and *AH-2*, severe and mild absorptive hypercalciuria; *NN*, normal calciuric stone formers; *PHPT*, primary hyperparathyroidism. Mean values in standard deviations (SD) are shown by *horizontal lines*. (From Pak CYC, Holt K. Nucleation and growth of brushite and CaOx in urinary stone-formers. *Metabolism*. 1976;25:665, with permission.)

Homogeneous Versus Heterogeneous Nucleation

Nucleation, the initial precipitory event in stone formation, may be homogeneous or heterogeneous. In an unstable solution, crystals form spontaneously by homogeneous nucleation. Much higher levels of supersaturation are required to produce homogeneous nucleation than heterogeneous nucleation.[130] The latter occurs in metastably supersaturated urine as certain macromolecules, or other crystals that can act as nuclei, stimulate precipitation. Because urine contains a number of macromolecular and cellular degradation products, crystallization is most often heterogeneous.[130,131]

The efficiency of heterogeneous nucleation depends on the similarity between the spacing of charged sites on the preformed surface and the spacing in the lattice of the crystal that is to grow on that surface. This matching is referred to as epitaxis, and its extent is usually referred to as a good or poor epitaxial relationship.[132] A number of urine crystals have good epitaxial matching and behave toward one another as heterogeneous nuclei. Monosodium urate and uric acid are excellent heterogeneous nuclei for CaOx,[133,134] so uric acid or urate could, by crystallization, lower the FPR for CaOx. Heterogeneous nucleation is thought to play a role in linking hyperuricosuria to CaOx stones,[38,135–138] a matter discussed later in this chapter. Epitaxial overgrowth of CaOx on a surface of uric acid has been experimentally documented.[139] At a pH above 6.9, brushite may transform to hydroxyapatite, which can serve as a nucleus for CaOx.[140] Based on observations that calcium phosphate is the most common crystal in human urine,[141] is ubiquitous in human urinary stones, and is often seen at the center of mixed CaOx/calcium phosphate urinary stones,[142] nucleation of CaOx crystals is proposed to be induced by calcium phosphate.[11,12] In addition, both apatite and brushite crystals induce crystallization of CaOx in vitro from metastable solutions of CaOx.[143,144] The other possible epitaxial relationships have not yet been linked explicitly to particular varieties of stone disease.[145] However, the low FPRs in primary hyperparathyroidism suggest that heterogeneous nucleation may be occurring.

Crystal Growth

Once present, crystal nuclei grow if suspended in urine with an APR above one (Fig. 20.12). Crystal growth is critical to stone disease, because microscopic nuclei are too small to cause obstruction. Crystals are regular lattices, composed of repeating subunits, and they grow by incorporation of calcium and oxalate, or phosphate, into new subunits on their surfaces. In metastable solutions at 37°C, growth rates of CaOx and the stone-forming calcium phosphate crystals are rapid. Appreciable changes in macroscopic dimensions occur over hours to days. Growth rate increases with the extent of oversaturation and tends to be most rapid in urine having the highest APR.

Factors Influencing Crystal Growth

In urine, the upper limit of metastability is higher and crystal growth rates are lower than in a salt solution with the same APR. The nature of the materials that confer crystal growth rate inhibition on urine is incompletely known. Crystal growth inhibitors for calcium phosphate crystals may not be the same as the substances that affect CaOx crystal growth.

Inorganic pyrophosphate increases the FPs of calcium phosphate and CaOx in salt solutions and, by absorbing their surfaces, retards the growth of hydroxyapatite[138] and CaOx crystals.[140] Urinary pyrophosphate concentrations range from 20 to 40 μM in adults. This concentration is sufficient to inhibit crystal growth.[146] Fleisch and Bisaz[137] suggest that urine raises the FP for calcium phosphate above the level expected from the pyrophosphate it contains. They suggest that other inhibitors accounted for approximately 50% of the total inhibition of calcium phosphate crystal growth. Smith and colleagues[147] have produced similar estimates. Bisaz[148] suggests that pyrophosphate, citrate, and magnesium ions contribute about 77% of the total calcium phosphate crystal inhibition capacity of urine. However, several investigators[140,149] concluded that urine pyrophosphate contributes insignificantly to CaOx crystal growth inhibition.

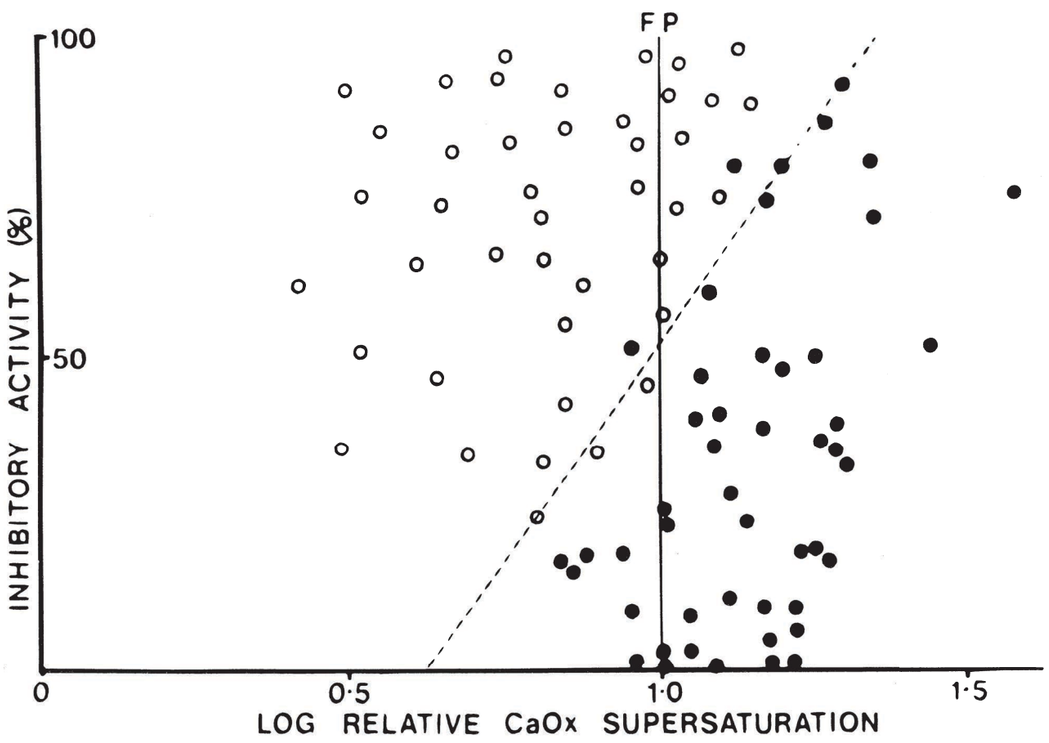

FIGURE 20.12 Saturation-inhibition index of urine from stone formers in normal subjects. The ability of dilute urine to prevent crystal aggregation in an in vitro system (inhibitory activity) is shown as a function of relative supersaturation with respect to calcium oxalate (CaOx) (*x* axis). A value of 1.0 represents the saturation at the level of the formation product (FP); a value of 0.1 would be near the solubility product. Stone formers (*solid circles*) and normal subjects (*open circles*) fall into separate zones; the *dotted line*, obtained by statistical analysis, is the best plane of separation. (From Robertson WG, Peacock M, Marshall RW, et al. Saturation inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. *N Engl J Med*. 1976;294:249, with permission.)

Inhibitors of Calcium Oxalate Crystal Growth

Some progress has been made describing the urinary inhibitors of CaOx crystal growth. The studies of Robertson and associates[150,151] suggesting that a urinary proteoglycan may significantly contribute to CaOx crystal growth inhibition have not been further supported. Strongly acidic peptides such as nephrocalcin and osteopontin have been described as important inhibitors of CaOx crystal growth.[59,146,152–155] Strongly acidic peptides such as poly-L-aspartate and poly-L-glutamic acids inhibit CaOx crystal growth, and urine appears to contain several glycopeptides unusually rich in these two amino acids. Two of the best known of these are nephrocalcin and osteopontin.[155] Treatment of urine with nonselective proteases diminishes the inhibition of CaOx crystal growth,[146] which may be related to urinary glycoproteins. Nephrocalcin contains γ-carboxyglutamic acid (Gla), and is an amphiphilic molecule with a molecular mass of about 15 kd. It tends to self-aggregate into a series of higher molecular mass polymers.[59,152]

Nephrocalcin from stone formers lacks the Gla residues, which is associated with a loss of ability to form stable film at an air-water interface, perhaps reflecting decreased amphiphilicity. The nature of the molecular abnormality leading to decreased inhibition of CaOx crystal growth is unknown. A separate abnormality in nephrocalcin has been identified from the urine of patients with X-linked recessive nephrolithiasis.[156] Nephrocalcin from affected males and carrier females with X-linked recessive nephrolithiasis is poorly phosphorylated, with decreased ability to inhibit crystal growth.

Osteopontin (OPN) is a more recently isolated CaOx crystal growth inhibitor.[153] Expression of OPN under basal conditions is limited to bone matrix, kidney, inner ear, decidual glands, and smooth muscle.[157] OPN is found in breast milk and serum.[158,159] It is expressed in response to various mitogens and growth factors, including phorbol esters and transforming growth factor-beta (TGF-β)[160,161] and is also expressed by cells in response to injury. Kleinman[162] finds that renal tubular OPN expression increases markedly after ischemic injury. However, OPN excretion is not decreased in renal stone formers. It appears that the level of phosphorylation is the critical issue in regard to the function of OPN as a urinary crystal inhibitor.[155] The full OPN protein is not required for functional activity. Small domains of the OPN primary sequence were able to independently inhibit

calcium oxalate monohydrate (COM) crystal growth. The phosphorylated OPN peptides are potent inhibitors of COM crystal growth, but not COM crystal aggregation.[155] The extent of phosphorylation of OPN is responsive to hormonal influences such as those of vitamin D.[163] The role of OPN in the kidney is not fully understood, although it is protective against CaOx monohydrate crystal aggregation and retention in the kidney as demonstrated by studies in OPN null mice, genetic hypercalciuric stone-forming rats, and humans.[67,164]

The role of OPN in biology is complex.[165] It is an osteoclast autocrine regulating motility and bone resorption.[166] OPN is a ligand for the cell surface receptors of $\alpha_v\beta_3$ and CD44, and it stimulates osteoclast signal transduction upon binding.[166,167] In the kidney $\alpha_v\beta_3$ is found largely on the basolateral surface of the distal nephron[168] and CD44 localization is unknown, whereas OPN is secreted mainly by the thick ascending limb into the tubular fluid at the luminal surface of the epithelial cell. OPN is structurally related to proteins found in other mineralized tissues, notably mollusk shells, which have contrasting effects on crystallization depending on whether they are in solution or immobilized on a surface. When in solution, these proteins inhibit calcite, CaOx, and apatite crystal growth.[169] However, if these proteins are immobilized on a support before incubation with a supersaturated solution of the mineral phase, they are able to initiate crystal nucleation in specific orientations.[170] It is believed that OPN serves as a modulator of crystallization and is important for ordered crystal structure of the bone.[171] In its phosphorylated state, it inhibits and thereby regulates apatite formation.[172] OPN is also found in association with the pathologic calcification of stone matrix[173] and atherosclerotic plaques.[160,174] Phosphorylation of OPN renders it inhibitory to vascular calcification[175] and skeletal mineralization.[171]

Tamm-Horsfall protein (THP) is the major urinary glycoprotein of normal urine. THP from stone formers is abnormal, with a higher tendency to aggregate under conditions of increased ionic strength and low pH.[176] Normal THP is an inhibitor of crystal aggregation, but THP from stone formers is less active in preventing aggregation and, under some conditions, THP from stone formers may promote the formation of crystal aggregates, especially in the presence of high concentrations of calcium. The structural abnormalities responsible for impaired inhibitory activity are not completely understood.[177] One study demonstrated that Tamm-Horsfall glycoprotein and citrate concentrations are linearly related to CaOx monohydrate agglomeration inhibition.[178] The effects of the two substances are synergistic. Tamm-Horsfall glycoprotein removal from the urine dramatically reduced CaOx agglomeration.[178]

Hallson and Rose[179] suggested that certain materials in urine, which they refer to as uromucoids, might promote calcium phosphate crystallization and aggregation. The significance of these findings is unclear. Of greater interest, urate anions in urine appear to bind and adsorb inhibitor substances, suggesting that hyperuricosuria could promote CaOx stones by reducing levels of urine crystal growth inhibitors.[150] When incubated in vitro, monosodium urate is able to bind heparin, a potent proteoglycan inhibitor of CaOx crystallization.[180] This demonstrates that at least one specific polyanion inhibitor could be adsorbed by a solid phase of uric acid.

Relationship of Oversaturation and Crystal Growth Inhibition to Clinical Nephrolithiasis

The force that drives calcium salts out of solution, into the solid phase, is oversaturation. Compared to homogeneous nucleation, heterogeneous nucleation facilitates stone formation by decreasing the degree of oversaturation required for nucleation. Inhibitors such as magnesium, pyrophosphate, osteopontin, and nephrocalcin suppress nucleation; increase the supersaturation needed to produce the solid phase; and retard the growth of nuclei already formed. In the most important stone-forming conditions, oversaturation, heterogeneous nucleation, and reduced inhibitors have documented, or at least postulated, roles that vary from one disease to another (Table 20.7). Treatment is often successful in reversing stone formation by eliminating the disturbances that enhance the risk of stones or, in some cases, by introducing secondary biochemical changes that compensate for the underlying defect.

Oversaturation occurs in idiopathic hypercalciuria, primary hyperparathyroidism, and hyperoxaluria because of overexcretion (Table 20.7). Both hypercalciuria and phosphaturia occur in RTA. Oversaturation with respect to calcium phosphate salts, which make up most of the stones in RTA, is also increased by alkaline urine and by low levels of urinary citrate, an important calcium-binding agent.

The finding of urine formation products below those of simple salt solutions provides evidence for heterogeneous nucleation in hyperparathyroidism. The basis for this finding is unknown. Hyperuricosuria is thought to engender urine crystals of uric acid or sodium hydrogen urate, which are efficient heterogeneous nuclei for CaOx.[38,133,134] It is uncertain whether these crystals are in a gel state.[150]

Low levels of urine inhibitors have been demonstrated in some hypercalciuric and normocalciuric stone formers.[125] Robertson[125] reports lower inhibitor levels in hyperuricosuric stone formers. In general, the levels of crystallization inhibitors in the urine of stone formers differ from those in non–stone formers and, consequently, their urine samples can be distinguished from samples of normal people more reliably when inhibitor content is measured than by the use of supersaturation measurements alone (Fig. 20.13). This fact highlights the presence of low inhibitor levels in stone-forming patients and suggests that inhibitors are very important in preventing stones.

More recent measurements of urinary inhibitors of CaOx monohydrate crystal growth show that the lowest inhibitor levels occur in patients with hypercalciuria, but not hyperuricosuria,[180] and that samples from normal subjects can be distinguished from those of stone formers no more

20.7 Pathogenetic Mechanisms in Some Established Forms of Calcium Nephrolithiasis

	Mechanisms		
	Overexcretion	**Heterogeneous nucleation**	**Reduced inhibition of stone formation**
Idiopathic hypercalciuria	+	−	Unknown
Primary hyperparathyroidism	+	+	−
Hyperuricosuria	−	+	−
Renal tubular acidosis	+	Unknown	−
Hyperoxaluria	+	−	−
No detectable metabolic abnormality	−	−	−

reliably by a combination of inhibitor and supersaturation measurements than by measurements of inhibition alone. Nephrocalcin from urine of stone-forming patients[181] or CaOx stones[182] seems abnormal; it lacks Gla and forms weak air-water films. The difference between these results and those of Robertson[125] is probably related to differences in methodology. Hallson and Rose[179] present additional evidence that inhibitors of crystallization are functionally important and differ in stone formers from those in normal subjects.

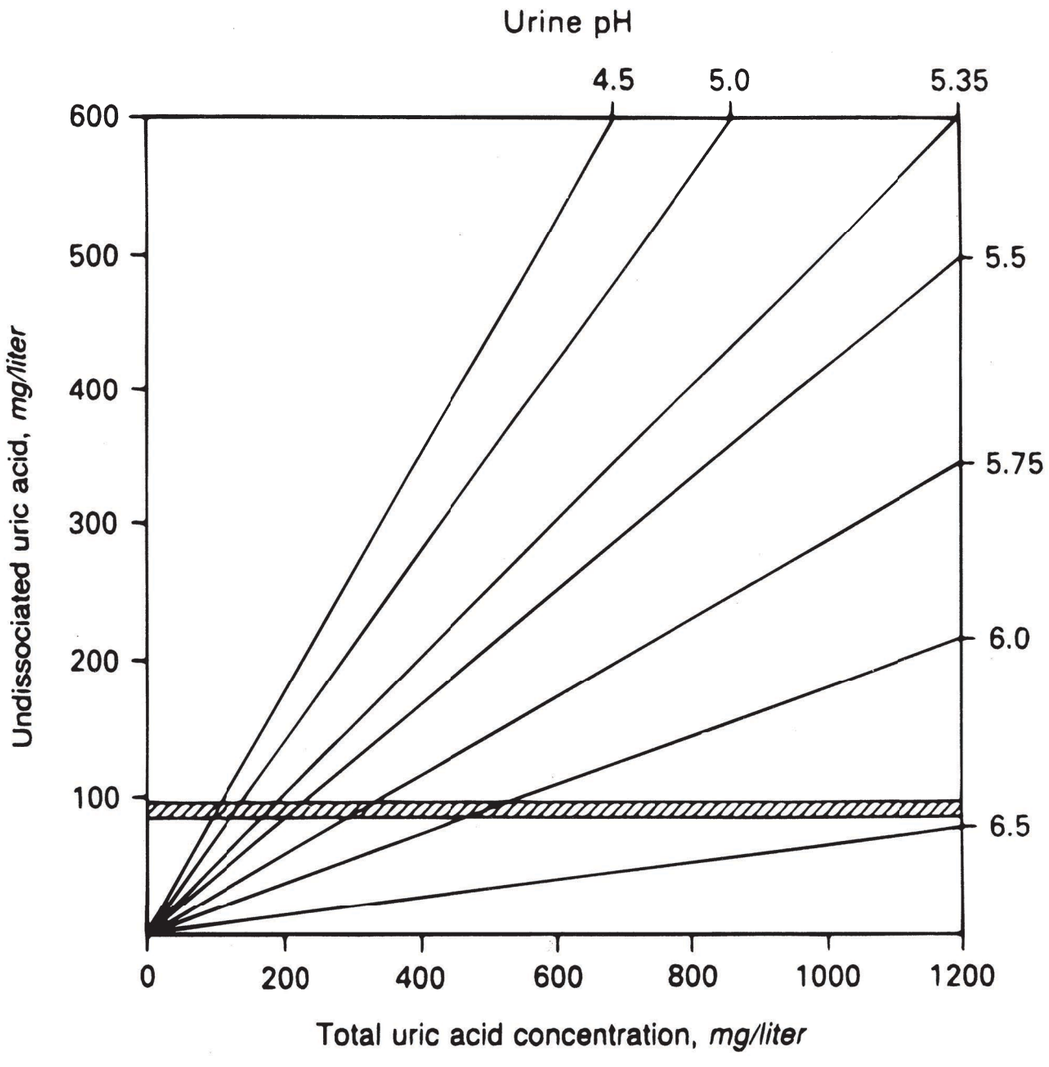

FIGURE 20.13 Nomograms showing undissociated uric acid concentration at values of urine pH and total uric acid concentration. The solubility limit for uric acid is shown by crossed hatched bars (96 ± 2 mg per L). (From Coe FL. Uric acid and calcium oxalate nephrolithiasis. *Kidney Int.* 1983;24:392, with permission.)

Medical Management of Hypercalciuric Nephrolithiasis

The conservative recommendations made for all patients, regardless of the underlying etiology of stone disease, are affected somewhat by the diagnosis of hypercalciuria. Measures undertaken to increase urinary output to more than 2 L per day, to avoid high oxalate and sodium intake, and to restrict animal proteins with extremely high purine levels are generally recommended in any patient with recurrent nephrolithiasis. With these conservative measures alone, a significant number of patients are able to normalize urinary risk factors for stone formation and reduce the incidence of recurrence. After 3 to 4 months of conservative therapy, the patient needs reevaluation. If the patient's metabolic or environmental abnormalities have been corrected, the conservative approach to therapy should be considered and the patient followed every 6 months with repeat 24-hour urine testing. Follow-up is essential, not only to monitor the efficiency of treatment but also to encourage patient compliance. However, if a metabolic defect persists despite conservative treatment, medical therapy may be instituted. For example, if significant sodium-dependent hypercalciuria persists despite dietary recommendations of restricting salt intake, medical therapy with thiazides or citrate may be instituted.

The issue of dietary calcium in patients with hypercalciuric nephrolithiasis has become an important one. It is clear that adequate calcium intake is needed to maintain skeletal homeostasis during adulthood and old age. However,

high calcium intakes are not tolerable in patients with hypercalciuric nephrolithiasis and absorptive hypercalciuria. Nevertheless, a low-calcium diet promotes oxalate absorption by decreasing CaOx salt formation in the intestine. In the absence of calcium, increased free oxalate anion is available for absorption. The impact of low-calcium diets favoring hyperoxaluria has been tested in a large population study from Framingham, Massachusetts.[183] Curhan and coworkers[183] show that dietary calcium intake is inversely associated with the risk of kidney stones in patients who did not have a prior history of kidney stones. Thus, low-calcium diets increased the risk of the development of a kidney stone. It is important to realize that this study specifically excluded most patients with idiopathic hypercalciuria who had already had a renal stone. It is a mistake to recommend high-calcium diets in patients with hypercalciuric nephrolithiasis, especially those who have absorptive hypercalciuria.

Despite improved elucidation of pathophysiology and formulation of diagnostic criteria for nephrolithiasis of different causes, selective medical treatment programs for nephrolithiasis have not been widely implemented. Confusion and controversy regarding the pathogenesis of hypercalciuria, and the absence of an ideal therapeutic approach to absorptive hypercalciuria, have contributed to this situation. Thiazide diuretic therapy remains the mainstay of the medical approach to hypercalciuria. Treatment with a thiazide is ideal, pathophysiologically, for renal hypercalciuria. Thiazides directly stimulate renal tubular calcium transport and suppress the secondary hyperparathyroidism associated with the condition. However, renal hypercalciuria, as previously discussed, is an uncommon form of hypercalciuria associated with nephrolithiasis. Because idiopathic hypercalciuria is largely an intestinal overabsorption problem, an ideal treatment would be one that directly and specifically inhibits calcium absorption. Such a pharmacologic agent is not available.

Sodium Cellulose Phosphate

Sodium cellulose phosphate reduces urinary calcium excretion, especially in patients with intestinal hyperabsorption. However, sodium cellulose phosphate induces a reciprocal increase in oxalate excretion due to the binding of intestinal calcium. The resultant hyperoxaluria offsets the beneficial effects of the hypocalciuria induced. In one study, the oxalate excretion rate rose from 30 to 60 mg per 24 hours[184] and the mean APR for CaOx fell only 20%, from 2.75 to 2.19. Other investigators[185] described the clinical response of recurrent CaOx stone disease to cellulose phosphate. The therapy reduced stone recurrence in patients who received cellulose phosphate to the same degree accomplished by just reducing dietary calcium intake. The latter is known to be ineffective as a single therapeutic approach. Thus, cellulose phosphate is not a treatment for the typical calcium stone former. Although the rationale for its use might seem reasonable, it is flawed by the reciprocal hyperoxaluria. In addition, the high frequency of negative calcium balance in patients with idiopathic hypercalciuria must also be of concern with use of this type of agent.

Thiazides

Thiazides have been extensively used for the treatment of hypercalciuric nephrolithiasis.[186–188] Hydrochlorothiazide is most effective in patients with renal hypercalciuria because, by reducing the high PTH levels, hydrochlorothiazide inhibits its $1\alpha,25(OH)_2D_3$ production and reduces intestinal calcium absorption. Thiazides exert significant hypocalciuric action in patients with intestinal hyperabsorption without reducing intestinal calcium absorption.[30] Nonetheless, thiazides may show long-term effectiveness in absorptive hypercalciuria. Despite the hypocalciuric action of thiazides and the resultant increase in serum calcium, intestinal calcium absorption remains persistently elevated. These studies suggest that retained calcium is added to the skeleton at least during the first few years of therapy. Bone density, determined in the distal third of the radius by photon absorptiometry, increases significantly during thiazide treatment and absorptive hypercalciuria, with an annual increment of 1.34%.[189] With continued therapy, however, the increase in bone density stabilizes and the hypocalciuric effect of thiazide becomes attenuated. These results suggest that thiazide treatment causes low turnover of bone, which interferes with the continued calcium accretion in the skeleton. The resulting hypocalciuric effect of thiazide is blunted by an increase in the serum calcium level and the resultant increase in filtered calcium load.[189]

In addition to the aforementioned studies, several controlled and uncontrolled studies documented the effectiveness of thiazide therapy in hypercalciuric nephrolithiasis.[119] Thiazides directly inhibit the sodium chloride cotransport protein for apical sodium entry in the diluting segment of the cortical thick ascending limb and the early distal nephron. This transport protein is also expressed in the osteoblast, which may further contribute to the positive actions of thiazides in patients with absorptive hypercalciuria leading to increased skeletal calcium accretion. Additional studies will be required to delineate the role of this potential mechanism in the therapeutic benefits resulting from thiazides.

Orthophosphate

Orthophosphate (neutral or alkaline salt of sodium/potassium phosphate given in doses of 0.5 g of phosphorus three to four times per day) reduces $1,25(OH)_2D_3$ synthesis. However, no convincing evidence exists that this treatment restores normal intestinal calcium absorption. Orthophosphate reduces urinary calcium by directly impairing the renal tubular reabsorption of calcium and by binding calcium in the intestinal tract. Urinary phosphorus is markedly increased during therapy—a finding reflecting the absorbability of soluble phosphate. Physicochemically, orthophosphate reduces the urinary saturation of CaOx, but increases that of brushite. Moreover, the urinary inhibitor activity is increased, probably owing to the stimulated renal excretion of pyrophosphate and citrate. The results of studies on the efficacy of orthophosphate are mixed.[189–191] Although reports to the

contrary have appeared, orthophosphate therapy can cause soft tissue calcification and PTH stimulation.[192] Furthermore, orthophosphate therapy is contraindicated in patients with nephrolithiasis complicated by urinary tract infection and in patients with renal insufficiency.

PRIMARY HYPERPARATHYROIDISM

In the past, primary hyperparathyroidism was a major cause of hypercalciuric nephrolithiasis. The advent of routine screening of serum chemistries in hypercalcemic patients led to early detection of primary hyperparathyroidism. Consequently, most patients with primary hyperparathyroidism are detected in an asymptomatic phase before nephrolithiasis becomes a problem. Currently, primary hyperparathyroidism accounts for less than 1% of hypercalciuric nephrolithiasis[19,20,193] (Table 20.1).

The pathogenesis of nephrolithiasis in primary hyperparathyroidism is a direct response to the presence of excessive PTH levels in the circulation. PTH is the primary regulator of renal tubular calcium transport and one of the primary mechanisms regulating bone remodeling. PTH regulates intestinal calcium absorption secondarily through $1,25(OH)_2D_3$. PTH acts on the proximal nephron by decreasing phosphate reabsorption increasing the production of $1,25(OH)_2D_3$ (see Chapter 72). In the thick ascending limb and the distal nephron, PTH directly stimulates tubular calcium reabsorption by regulating both transcriptional and posttranslational modifications of transport proteins both at the entry step in the luminal membrane and the exit step, the calcium ATPase, on the basolateral membrane. Measurement of tubular calcium reabsorption in hypercalcemic patients is technically difficult, but perhaps of a diagnostic value.[194] Enhanced tubule reabsorption of calcium is responsible for normal urinary calcium excretion rates in some hyperparathyroid patients,[195,196] and for the observation that for any given level of serum calcium, urinary calcium excretion is lower in hyperparathyroidism than in other nonparathyroid types of hypercalcemia (i.e., sarcoidosis and multiple myeloma).

Although PTH stimulates tubule calcium reabsorption, urinary calcium excretion is greatly elevated in primary hyperparathyroidism due to the increase in filtered calcium load.[197] The elevation of circulating $1,25(OH)_2D_3$ contributes to the hypercalcemia and. as a result, adds to the hypercalciuria seen in patients with primary hyperparathyroidism.

A major component of hypercalcemia stems from PTH stimulation of bone remodeling. PTH affects both bone formation and bone resorption through its direct actions on marrow cells at varying stages of osteoblast differentiation. Stimulation of osteoprotegerin ligand in the osteoblast and marrow stromal cells stimulates osteoclast differentiation.

Renal stones observed in patients with primary hyperparathyroidism are usually composed of hydroxyapatite and CaOx. Stones often recur and become bilateral if the diagnosis is not made early in the course of the disease. Nephrocalcinosis may be the only renal manifestation of hyperparathyroidism. PTH increases the APRs for CaOx and brushite mainly due to hypercalciuria. Phosphate overexcretion may be observed, but it also may be absent because chronic phosphaturia tends to cause a negative phosphorus balance. The urine pH is not abnormally alkaline in primary hyperparathyroidism and the magnitude of the acidosis is negligible when glomerular function is normal. This suggests that altered acid-base metabolism in hyperparathyroidism does not contribute significantly to stone formation.[120] Pak and Holt[123] demonstrate an unexplained reduction of the FPR for CaOx and brushite in primary hyperparathyroidism (Fig. 20.11). The diagnosis of primary hyperparathyroidism rests with repeated determination of the serum calcium and iPTH levels. The assays for circulating PTH levels using double-antibody techniques enable the detection and measurement of the intact hormone.[198,199] More recently, the "intact" hormone assays have been recognized as also detecting PTH inhibitory peptides lacking the first few amino acids from the NH_2-terminus.[200] Assays that specifically recognize PTH and its first amino acid have been developed and are referred to as "bioactive" PTH assays.[200,201] In the diagnosis of primary hyperparathyroidism, detection of familial hypocalciuric hypercalcemia is critical. This inherited disease is produced by a mutation in the PTH gland calcium sensor such that PTH secretion is not adequately sensitive to the serum calcium levels. Affected patients may exhibit mild hypercalcemia and mild hyperparathyroidism but do not have complications related to the hyperparathyroid state. Thus, detection of patients with the familial disease is crucial so that they are not exposed to surgical therapy for primary hyperparathyroidism. The very low urinary clearance of calcium factored by creatinine excretion is currently the most sensitive means of separating primary hyperparathyroidism from familial hypocalciuric hypercalcemia.[202]

Surgical removal of adenomas or excess parathyroid tissue is indicated in patients with primary hyperparathyroidism who have sustained complications of the disease. Nephrolithiasis is one of the main complications of primary hyperparathyroidism, and any patient who has had a renal stone should undergo surgical correction of the disease state.

HYPERURICOSURIA

Hyperuricosuria can be a significant factor in the formation of calcium stones. Reduction in calcium stone formation in hyperuricosuric patients treated with allopurinol establishes hyperuricosuria as a contributory agent.[135,186,203] Hyperuricosuria is defined as a urinary excretion rate of uric acid that exceeds 700 mg per 24 hours in women or 750 mg per 24 hours in men.

Hyperuricosuria is not the cardinal risk for the development of uric acid stones. Instead, the overriding pathophysiologic process is the unduly acidic urine.[204] Epidemiologic data from converging sources have featured obesity, type II diabetes mellitus, and the metabolic syndrome as strong

associations with low pH.[205–208] With the obesity epidemic in the United States, the incidence of nephrolithiasis has grown in parallel.[209,210] Increased calcium excretion in the obese covaries with intake of animal protein and sodium. No relationship was found between body mass index (BMI) and urinary supersaturation of calcium oxalate. These results suggest that the augmented incidence of nephrolithiasis in the obese is secondary to uric acid stones.[205]

Either of two theories may explain the mechanism by which uric acid promotes calcium stone formation. The prevailing hypothesis states that through epitaxy,[211] the growth of one crystal on the substance of another, uric acid can serve as a seed for precipitation of a calcium salt. Studies have demonstrated that sodium urate accelerates precipitation of CaOx or calcium phosphate in vitro.[133,134] This has been attributed to physiologically relevant urinary concentrations of monosodium urate (0.1 mg per mL).[133] An alternative hypothesis states that urate can complex with and thus neutralizes endogenous urinary acid mucopolysaccharides, which normally retard the crystallization of CaOx.[212]

Urinary uric acid depends on both the renal filtered load of uric acid and its subsequent tubular transport. Hyperuricosuria is usually due to a high filtered load. The source of this uric acid is purines, which come from the dietary intake of purine-rich foods, endogenous synthesis of purines from nonpurine precursors, and salvage of purine bases from tissue catabolism.[213] Coe and colleagues[214] have studied the contribution of dietary purine overconsumption to hyperuricosuria in CaOx stone formers. They find that hyperuricosuric patients consume more purine than control subjects eating isocaloric diets. Overall, a very close correlation exists between urine uric acid and dietary purine intake. Coe and colleagues[214] also find that excessive purine intake does not fully account for the hyperuricosuria, as some patients excrete more urate than normal subjects consuming equivalent amounts of purine. A purine-rich diet is also rich in protein. Gutman[215] hypothesizes that, in some instances, a high-protein diet causes overproduction of uric acid, as the increase in urinary uric acid during a high-protein diet is only partially accounted for by the purine content. It is possible that abnormal tubular handling of urate resulting in hyperuricosuria and normal or low serum urate levels also occurs. Patients with such a defect and stones have been described.[213] Similarly, the Dalmatian dog, which has been well studied, can have hyperuricosuria due to enhanced tubular secretion of urate.[216] Although purine load is the most common mechanism for hyperuricosuria, other factors are contributory.

The treatment of hyperuricosuric calcium stone formers focuses on decreasing the hyperuricosuria. All of these patients should be administered a low-purine diet, which will decrease urinary uric acid excretion,[39,214,217] although in some patients the effectiveness in stone prevention is unproven. A low-purine diet involves avoiding meat, fish, and poultry. Hyperuricosuric calcium stone formers may also be treated with allopurinol, which blocks xanthine oxidase, the last enzymatic step in the purine degradative pathway before uric acid is produced. Very good evidence shows that allopurinol decreases stone formation in this group of patients,[135,186] including the results of a prospective controlled study by Ettinger and colleagues.[203] The usual starting dose of allopurinol is 300 mg per day. A follow-up measurement of 24-hour urinary uric acid excretion after institution of allopurinol will determine the adequacy of the dose. Potassium citrate may be an alternative to allopurinol in patients with mild hyperuricosuria (600 to 1,000 mg per day) in whom hypocitraturia is present, as citrate will inhibit CaOx precipitation.[218]

Uric acid transporters are localized to both the apical and basolateral membranes of the PCT epithelial cells and are coupled to sodium and organic ion transport. The inhibition of the apical membrane URAT1 transporter by probenecid, NSAIDs, salicylates, and losartan can worsen hyperuricosuria.[219]

RENAL TUBULAR ACIDOSIS

RTA is a known risk factor for both nephrocalcinosis and calcium stones. The patients may have severe disease, as in one study by Caruana and Buckalew[220] in which the patients had a mean of 51 ± 14 stone episodes. Only the hypokalemic form of distal RTA (type I RTA) is associated with kidney stones, but the cause of the RTA may be idiopathic or secondary to systemic diseases.[220] Most patients present with nephrolithiasis, a normal serum bicarbonate level but abnormal urinary acidification in response to an ammonium chloride challenge. These patients are believed to have incomplete RTA.[221] The complete form of RTA is often accompanied by nephrocalcinosis. An early description by Albright[222] was of a 13-year-old girl severely affected by RTA with renal calculi, but also with rickets combined with dwarfism. The calculi may be CaOx, calcium phosphate, or a mixture of both, but the presence of calcium phosphate makes RTA a likely etiology.[220,223,224] When both nephrocalcinosis and RTA appear in concert, it may be difficult to define the primary event because nephrocalcinosis can both cause RTA or occur as a result of it. For example, when medullary sponge kidney is associated with kidney stones, nephrocalcinosis, and RTA, it is unclear which is the primary problem.[225]

RTA results in hypercalciuria and hypocitraturia, both of which predispose to nephrolithiasis. Induction of metabolic acidosis with ammonium chloride results in hypercalciuria,[226] which is associated with a net systemic positive acid balance with a stable serum bicarbonate level, suggesting that the acid load is buffered. Because gastrointestinal absorption of calcium does not increase with metabolic acidosis, bone is the likely source of both the urinary calcium and the buffering capacity. This is most likely the link between distal RTA and rickets.[222] Furthermore, as expected, alkali therapy corrects the acidosis and the osteomalacia.[227] Citrate excretion, a known inhibitor of calcium precipitation, is low in patients with RTA due to increased reabsorption.[220]

Simpson[228] demonstrated in vitro inhibition of citrate oxidation by renal cortical slices incubated in medium with a high pH. Furthermore, hypokalemia, a frequent accompaniment of distal RTA, also results in hypocitraturia due to increased reabsorption.

Distal RTA often presents as a familial disease. Primary distal RTA is inherited as either an autosomal dominant or autosomal recessive trait (OMIM179800, 276300, and 602722). Autosomal recessive distal RTA often presents in infancy, whereas autosomal dominant distal RTA may not present until adolescence or young adulthood.[229] Patients with autosomal dominant and recessive distal RTA have been shown to harbor mutations in the gene encoding the chloride-bicarbonate exchanger AE1 (SLC4A1).[229–235] Mutations in ATP6N1B, encoding a kidney vacuolar proton pump protein 116kD subunit, cause recessive distal RTA with preserved hearing.[229,236] On the other hand, mutations in the gene encoding B1 subunit of the proton pump (ATP6V1B1) and (ATP6V0A4) cause renal tubular acidosis with sensory neurodeafness.[229,237,238] Mutations in the genes encoding carbonic anhydrase (CA) II,[239–241] kidney anion exchanger 1 (K AE1),[231,234–236,242] and subunits of the H+-ATPase[229,238,243] have also been identified in patients with distal RTA.[229] In the familial forms of RTA, renal deposition of calcium salts (nephrocalcinosis) and renal stone formation commonly occur. Replacement of alkali corrects the systemic metabolic defects and improves the nephrocalcinosis and nephrolithiasis. Such treatment does not affect the hearing loss of the patients with recessive distal RTA.

Pseudohypoaldosteronism type II is a genetic disorder that produces a clinical phenotype that includes hypertension, hyperkalemia, and metabolic acidosis. It has been linked to mutations in the gene encoding WNK-1 or -4 and is localized to the distal nephron.[244] A recent study demonstrated that in an affected family with a mutation in the WNK4 gene, there was marked hypercalciuria and osteopenia. Thiazide diuretics completely reversed both the urine and bone abnormalities.[244] Distal RTA is possible only if fasting urine pH is above 5.3. If so, an ammonium chloride challenge is necessary to prove the diagnosis. A urine pH above 5.3 after ammonium chloride–induced (100 mg per kg) acidification indicates the presence of RTA.

The treatment of RTA-associated nephrolithiasis consists of alkali therapy. Coe and Parks[245] and Wilansky and Schneiderman[246] document dramatic decreases in stone disease when patients with RTA are treated with alkali. This treatment may also result in an associated decrease in nephrocalcinosis. The dose of alkali is 1 mEq per kg (approximately 80 mEq per day) in four divided doses orally, but the dose should be adjusted to normalize 24-hour urinary citrate excretion. The alkali is usually given in the form of citrate, which both normalizes urinary citrate[247] and decreases urinary calcium.[227,246,247] Potassium citrate is preferable over sodium citrate, because potassium citrate causes a greater decrease in calcium excretion.[247] By replacing buffer capacity with oral alkali, potassium citrate causes calcium excretion to fall as less bone buffers are mobilized. It is likely that sodium citrate does not decrease hypercalciuria as much as the potassium salt, because the extra sodium may have a calciuric effect. Potassium citrate is contraindicated in severe renal insufficiency, and care should be taken to avoid hyperkalemia in the presence of mild or moderate renal insufficiency.

HYPEROXALURIA

Excessive urinary oxalate excretion (>40 to 45 mg per day) contributes to stone formation by increasing the saturation of urine with respect to CaOx. Signifying the importance of hyperoxaluria, increased urinary concentration of oxalate has a greater impact than does urinary calcium on the saturation of CaOx.[248] Hyperoxaluria is a relatively frequent finding in patients with kidney stones and was detected in 34% of 587 consecutive patients evaluated for recurrent nephrolithiasis at the Jewish Hospital of St. Louis Kidney Stone Center from 1987 to 1993. In 8% of these patients, hyperoxaluria was the only identifiable defect (Table 20.1).[249]

Oxalate is an end product of metabolism excreted primarily by the kidneys. Under normal conditions, oxalate is poorly absorbed from the gastrointestinal tract and only about 10% of urinary oxalate can be accounted for by dietary intake.[250] Urinary oxalate excretion varies among patients and is determined by intrinsic oxalate production and metabolism and gastrointestinal oxalate absorption.

Oxalate Production and Metabolism

Oxalate production occurs through a number of metabolic pathways, some of which remain incompletely characterized (Fig. 20.14). The oxidative metabolism of glyoxylate is a major contributor to oxalate production; in addition, ascorbic acid and tryptophan are converted directly to oxalate. Pyridoxine (vitamin B_6) is required as a cofactor for the transamination of glyoxylate to glycine. Moreover, deficiency of vitamin B_6

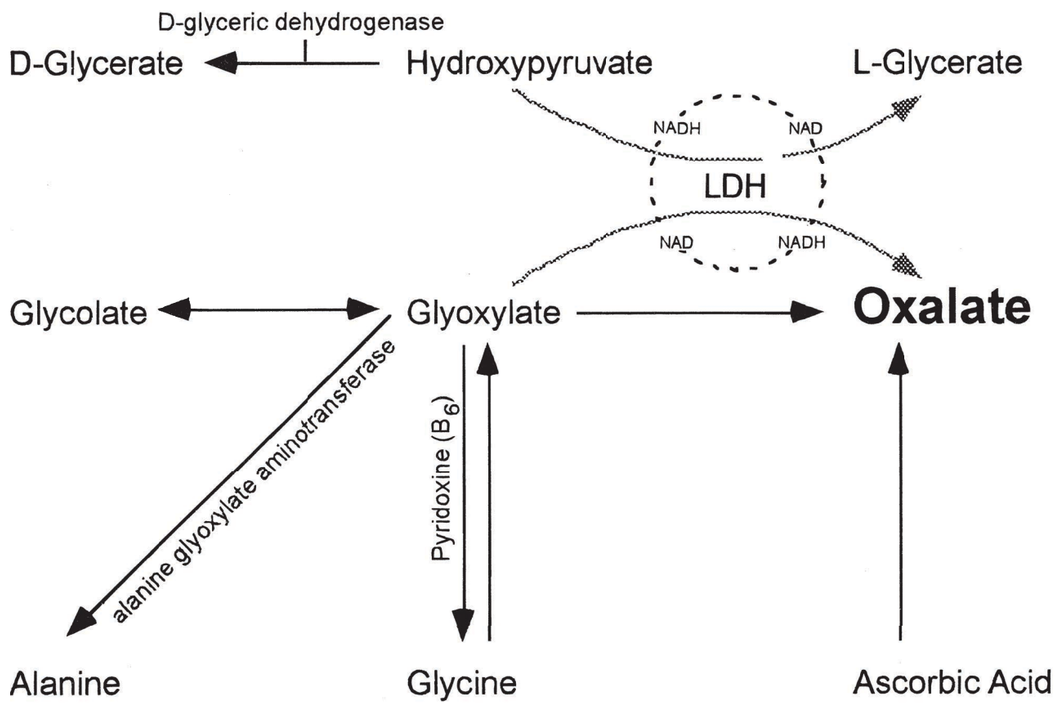

FIGURE 20.14 Metabolic pathways of oxalate production. Oxidative metabolism of glyoxylate is the major endogenous source of oxalate. Ascorbic acid (vitamin C) is also directly converted to oxalate.

may result in accumulation of glyoxylate, increased production of oxalate, and hyperoxaluria.[251] Disordered red cell oxalate exchange and defective oxalate transport may occur as an inherited trait and has been proposed to be a factor in hyperoxaluria within certain families.[252]

Increased availability of substrate for oxalate production can occur clinically in patients taking large doses of ascorbic acid (vitamin C) and in those who ingest ethylene glycol. Metabolism of ethylene glycol results in increased production of glycolate, increased glyoxylate, oxalate formation, and hyperoxaluria. Ascorbic acid, when taken in large doses (4 to 8 g per day), may lead to marked increases in urinary oxalate[253]; however, in some patients, hyperoxaluria may develop with doses as small as 500 mg per day.

Primary hyperoxaluria (PHO) is a rare metabolic disorder with autosomal recessive inheritance. PHO is induced by one of two enzymatic defects, both of which result in markedly enhanced conversion of glyoxylate to poorly soluble oxalate which is then excreted in the urine. Glyoxylate is normally metabolized in three ways. Glyoxylate is converted to glycine by the hepatic peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT). AGT is abnormal in type I PHO.[254] Pyridoxine (vitamin B_6) is a coenzyme of AGT. Glyoxylate is converted to glycolate by the cytosolic enzyme glyoxylate reductase/D-glycerate dehydrogenase (GRHPR). GRHPR is deficient in type II PHO.[255] Patients with this disorder excrete increased amounts of L-glyceric acid as well as oxalate. Glyoxylate is also converted to oxalate by lactate dehydrogenase.

A small number of patients fit the diagnostic criteria for having primary hyperoxaluria (PH), but when studied in vitro, they demonstrate full function of the two now enzymatic defects. This group constitutes about 7% of the 95 patients reported in the Mayo Clinics International Registry and such patients are classified as type III.[256–259]

Type I PHO is much more common than type II. The defect in AGT, which normally converts glyoxylate to glycine, results in an increase in the glyoxylate pool available for conversion to oxalate. The AGT gene maps to chromosome 2q36–37 and encodes for a 43 kDa protein. A number of different mutations have been identified in the coding region of the AGT gene in type I PHO. These defects lead to absent AGT protein, and/or absent AGT catalytic activity.[254] The GRHPR gene has been mapped to chromosome 9 and contains nine exons, spanning 9 kilobases.[260]

In most patients with type I PHO, glycolate excretion is increased. Practically all patients with type II disease have increased excretion of L-glyceric acid. In general, hyperoxaluria plus increased urinary excretion of glycolate or L-glyceric acid is strongly suggestive, but not absolutely diagnostic, of either type I or II PHO. In addition, hyperoxaluria alone, without increased glycolate or L-glyceric acid excretion, does not exclude the diagnosis of PHO.[48]

The presence of AGT deficiency can be confirmed by liver biopsy. Evaluation of the hepatic tissue includes quantification of enzymatic activity, an immunoblot to analyze the protein, and an immunoelectronic examination to demonstrate the virtual absence of AGT in the peroxisomes. AGT activity is less than 2% of normal in one-third of cases and ranges from 2% to 48% of normal in the remaining patients.[255] The relative ease in modern laboratory medicine with performing sequence analysis, and the delineation of the molecular basis of many of the mutations behind PHO type I and PHO type II, has led to the proposal of molecular diagnostic algorithms that may obviate the need for invasive biopsy procedures.[261,262]

Phenotypically, type I PHO is heterogeneous, ranging from severe infantile oxalosis and death to milder forms with renal stone disease in later life. Type II PHO has a less severe clinical course, but there may be a decline in renal function associated with the presence of nephrocalcinosis. Children with nephrolithiasis secondary to hyperoxaluria should have urinary glycerate measured to exclude type II PHO.[10]

Gastrointestinal Oxalate Absorption

Increased absorption of oxalate occurs with excess dietary oxalate intake, diminished binding of oxalate by dietary calcium and magnesium, or enhanced permeability of the colon to oxalate. Foods with relatively high oxalate content include spinach, beets, rhubarb, asparagus, cranberries, wheat germ, colas, teas, chocolates, nuts, beans, and various green leafy vegetables. Once ingested, oxalate forms insoluble salts with available calcium (and magnesium) in the intestinal lumen and is poorly absorbed; any free unbound oxalate is available for absorption distally in the colon.

Disorders characterized by absent or dysfunctional small bowel, as well as any causes of fat or bile acid malabsorption, can lead to hyperoxaluria.[263] Fat malabsorption allows luminal calcium and magnesium to saponify, leaving inadequate free calcium (and magnesium) to bind oxalate. Bile acid malabsorption causes increased permeability of the colon to oxalate.[264] Thus patients with inflammatory bowel disease, those who have had ileal bypass surgery or resection, and those with disorders associated with malabsorption develop hyperoxaluria due to an increase in unbound oxalate and enhanced colonic absorption.

Patients following a low-calcium diet and those taking sodium cellulose phosphate for the treatment of absorptive hypercalciuria may also manifest hyperoxaluria as a result of such therapy. Diminished oral calcium intake (with a low-calcium diet) and binding of calcium and magnesium in the gut by sodium cellulose phosphate allow increased amounts of unbound oxalate to be presented distally in the colon where it is readily absorbed.[265,266]

Therapy

Treatment of primary hyperoxaluria is difficult. Reducing sodium intake to 2 to 3 gm per day and limiting or avoiding high-oxalate foods is recommended. Supplemental citrate, magnesium, and phosphorus may help decrease urinary oxalate crystallization. Calcium intake should not be restricted

because this can increase intestinal calcium absorption. Approximately 10% to 40% of patients respond to pyridoxine supplementation, but vitamin C and D supplements should be avoided. Dialysis does not remove oxalate adequately. Hepatic transplantation remains the only therapy capable of correcting the underlying abnormalities in these patients.[267]

Treatment of patients with enteric hyperoxaluria should include a low-fat diet with restriction of oxalate-rich foods, appropriate therapy of any underlying gastrointestinal disorders, and avoidance of a low calcium intake. Some patients may benefit from the addition of oral calcium and magnesium supplements taken with meals, which act to bind dietary oxalate in the intestinal lumen, making it unavailable for absorption. Cholestyramine may also be of some benefit in those patients with significant fat and bile acid malabsorption as it acts as a nonabsorbable resin to bind fats and bile acids. Pyridoxine supplements may be effective in patients with moderate to severe hyperoxaluria.[268]

Patients with chronic diarrhea frequently have hypomagnesemia, hypokalemia, metabolic acidosis, hypocitraturia, and low urinary volumes. For these reasons, they also are prone to the development of uric acid stones. Therapy involves increased fluid intake, correction of hypokalemia and hypomagnesemia, and oral citrate supplements. Attention must also be paid to treatment of the underlying intestinal disorder and diarrhea.

Uric Acid Stones

Uric acid stones are radiolucent stones responsible for approximately 5% of kidney stones in the United States. Other populations may have a higher relative incidence of uric acid stones as a cause of urolithiasis. Due to the difficulty in visualizing these stones on an abdominal radiograph, an intravenous pyelogram is often necessary to make the diagnosis. Stones containing some calcium may be visualized on the radiograph, which may have important therapeutic implications.

Pathogenesis

Uric acid is the normal breakdown product of purine metabolism and is a natural urinary constituent. Precipitation of uric acid to form a stone can be demonstrated best by the relationships demonstrated in Figure 20.8.[42] The solubility limit of undissociated uric acid is 96 ± 2 mg per L at 37°C. In a given sample of urine, undissociated uric acid is dependent on the total uric acid concentration and urinary pH. The clinical laboratory routinely measures total uric acid excretion, but the undissociated uric acid can be inferred from both the total uric acid concentration and the urinary pH.

The total urinary uric acid concentration is a function of both uric acid excretion and urinary volume. Hyperuricosuria is defined as a urinary excretion rate of uric acid that exceeds 700 mg per 24 hours in women or 750 mg per 24 hours in men. When patients with gout were assessed for risk factors for stone disease, it was found that the incidence of stones increases with increasing degrees of hyperuricosuria.[269] A urinary excretion rate of uric acid of more than 1 g per 24 hours is associated with a 50% incidence of stones.[269] Urinary uric acid excretion depends on both the renal filtered load of uric acid and its subsequent tubular transport. Hyperuricosuria is usually due to a high filtered load. As stated earlier, the source of this uric acid is mostly purines,[213] usually from meat, fish, and poultry. A purine-rich diet is also rich in protein and, as Gutman[215] hypothesizes, in some instances a high-protein diet also causes overproduction of uric acid as the increase in urinary uric acid during a high-protein diet is only partially accounted for by the purine content. The overriding risk for uric acid nephrolithiasis is acidic urine.[270] Low urine pH leads to stone precipitation with relatively modest amounts of uric acid excretion whereas urine pH above 6.0 requires large amounts of urinary uric acid for lithogenesis. In two large cohorts of uric acid stone formers, there was a strong negative correlation between BMI and urine pH.[208] Each component of the metabolic syndrome appears to confer a risk for more acidic urine.[206] The dysfunction is impaired ammoniagenesis and, therefore, insufficient buffering of urinary protons. Low ammonium production leaves free protons to be buffered by titratable acids (i.e., phosphates and sulfates) thereby lowering urine pH. Reabsorption of filtered citrate is stimulated in the proximal tubule in states of acidosis/acid loads and hypocitraturia results.

The link between metabolic syndrome and impaired ammoniagenesis is thought to be mediated by insulin resistance.[207,271,272] Impaired ammoniagenesis is not the only pathogenic feature of patients with abnormally low urine pH. Increased net acid excretion and titratable acidity has been observed in uric acid stone formers even on fixed diets.[272–274] The biochemical origin of these findings is unclear, but may be related to post-prandial alkaline tide.[275,276]

Diseases Associated with Uric Acid Lithiasis

Among patients with uric acid lithiasis, a family history of gout or kidney stones often exists, predominantly in men. Most cases of uric acid lithiasis are idiopathic, but some disease associations should be considered when treating a patient with uric acid stones.

Primary Gout

Twenty-two percent of patients with primary gout have uric acid stones.[269] Eighty-three percent of these stones are pure uric acid, whereas 4% are mixed stones and the rest are calcium stones. Often, the uric acid stone disease antedates the diagnosis of gout. Conversely, the stones may only appear after administration of uricosuric drugs for gout. As discussed previously, uricosuric agents may treat the gout but cause uric acid stones.

Secondary Gout

Underlying diseases that cause gout confer a higher risk for kidney stones than does primary gout (42% versus 22%).

These diseases are associated with excess generation of uric acid due to nucleotide turnover (e.g., myeloproliferative disease, polycythemia due to congenital heart disease, and chronic granulocytic leukemia).

Chronic Diarrhea

Because intestinal fluid losses may result in urinary concentration of excreted urate, chronic diarrhea can result in uric acid stones. Likewise, fecal bicarbonate loss causes renal regeneration of bicarbonate with subsequent urinary acidification and more undissociated urate.

Familial Disease

Hereditary disorders associated with overproduction of uric acid include inborn errors of metabolism such as Lesch-Nyhan syndrome and type I glycogen storage disease. Lesch-Nyhan is caused by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficiency and Kelley-Seegmiller syndrome is a partial enzymatic defect.[277] Type I glycogen storage disease is secondary to glucose-6-phosphatase deficiency. A single gene that is located distally on the long-arm of the X chromosomes codes for HGPRT. Purification of mutant HGPRT genes in patients with partial or complete deficiency of HGPRT has led to the identification of single amino acid substitutions in five known variants of HGPRT.[278] The lack of feedback control by the purine salvage pathway leads to massive overproduction of uric acid and may lead to repeated episodes of urolithiasis and renal failure. Several families with inherited predispositions to uric acid stones have been described,[279] although the underlying cause is unknown. A candidate gene for uric acid nephrolithiasis has been identified on 10g21-g22 from a local Sardinian population with known susceptibility to uric acid kidney stone disease.[280]

Treatment

The fact that urinary uric acid concentration and urine pH can influence urate precipitation underlies the following treatment modalities.

Existing stones can be dissolved with alkali (to keep the urine pH at 6.5 or above) and a large amount of fluids to keep the urine output at more than 2 L per day. Allopurinol should also be given to reduce hyperuricosuria. Uric acid stones that contain calcium may be refractory to dissolution. Furthermore, the presence of obstruction or refractory pain may necessitate more rapid therapies such as lithotripsy or invasive urologic techniques.

Stone prevention necessitates dietary counseling and alkali therapy. Dietary modification entails keeping urine output at more than 2 L per day and reducing dietary purine intake (i.e., meat, fish, and poultry). Furthermore, patients should also reduce their protein intake to decrease both urine uric acid and the acid load. Likewise, alcohol intake should be limited because it may increase uric acid production. Alkali treatment should be given, aiming for a urine pH of 6.5. Approximately 1 mEq per kg of potassium citrate given in three divided doses is effective.[215] Sodium bicarbonate may be a less desirable form of alkali because the sodium load may aggravate hypercalciuria, which may aggravate nephrolithiasis.

Allopurinol can decrease urinary uric acid excretion by blocking the conversion of xanthine to uric acid by xanthine oxidase. Allopurinol serves as a second-line agent used when patients either refuse treatment or diet and when alkali therapy fails. Furthermore, it may be of benefit when uric acid loads are large, such as prior to chemotherapy for large volume rapidly growing tumors, or if the urinary uric acid excretion rate is more than 1 g per 24 hours. In the treatment of hyperuricosuria due to chemotherapy, a brisk diuresis should still be maintained because xanthine, the precursor of urate, may accumulate and cause acute renal failure, as can other products of tumor lysis.

The potential role of Oxalobacter formigenes in the treatment of primary hyperoxaluria has shed some light on the role of the intestine in maintaining oxalate balance. The use of the intestine to alter oxalate balance when kidney excretion alone is inadequate is among the therapeutic options on the horizon for the treatment of primary hyperoxaluria. Studies in rats have shown that intestinal colonization with O. formigenes can induce colonic secretion of oxalate, in part by producing a favorable concentration gradient through oxalate degradation.[281] Hoppe et al.[282] reported efficacy of oral O. formigenes administration in humans with primary hyperoxaluria. The majority of subjects with normal kidney function showed a 22% to 92% reduction in urinary oxalate. Two of three dialysis patients had a significant reduction in plasma oxalate levels as well, and with improvement in clinical symptoms.[283]

STRUVITE STONES

Struvite stones are composed of magnesium ammonium phosphate with variable amounts of carbonate apatite. This compound forms only in the presence of chronic urinary tract infection with bacteria capable of producing urease. The action of bacterial urease on urine urea yields ammonia and carbon dioxide. These are further hydrolyzed to ammonium and carbonate, resulting in a urine pH above 7.2—ideal conditions for struvite formation. Most species of Proteus and Providencia produce urease. Klebsiella, Pseudomonas, Serratia, Haemophilus, Staphylococcus, and Corynebacterium species are all capable of urease production. Escherichia coli does not possess urease activity.

Struvite now accounts for less than 10% of all stones and occurs most often in women and patients with spinal cord injury, neurogenic bladder, urinary diversion, or chronic indwelling bladder catheters due to their increased frequency of chronic urinary tract infection. Clinical findings may include evidence of urinary tract infection, hematuria, flank pain, or obstructive uropathy. Rarely, infection stones may cause xanthogranulomatous pyelonephritis. Struvite, when calcified, presents radiographically with a

characteristic multilobulated shape and laminated appearance and may extend to involve all calyces forming so-called staghorn calculi.

Because struvite formation occurs in the region surrounding bacterial colonies, all struvite stone material is infected. In addition, antimicrobial agents are unable to adequately penetrate struvite and achieve bactericidal levels. Therefore, the only curative treatment is eradication of infection with antimicrobials and removal of all stone material. Combined percutaneous nephrostolithotomy and extracorporeal shock wave lithotripsy is recommended as the first line treatment choice.[284] In those patients who are not candidates for surgery, a conservative approach may be indicated. Chronic antibiotic therapy may limit stone growth and result in partial dissolution[285] in nonsurgical patients. Another potentially useful agent is acetohydroxamic acid (AHA), which is a potent inhibitor of bacterial urease and can limit stone growth.[286] Despite the potential usefulness of AHA, it has been associated with frequent side effects, including potentially carcinogenic effects,[287] particularly in patients with renal insufficiency. For these reasons, use of AHA should be limited to patients with normal renal function who are unable to undergo surgical intervention.

CYSTINE STONES

Cystinuria is an inherited abnormality in amino acid transport affecting the gastrointestinal and proximal renal tubular epithelia. As a result of abnormal renal tubular transport of cystine and the other dibasic amino acids (ornithine, lysine, and arginine), abnormally large amounts of the amino acids are excreted in the urine. Cystinuria accounts for 1% of renal calculi in adults, but up to 10% in children.[288] Even with medical management, long-term outcome is poor due to insufficient efficacy and low patient compliance. The solubility of cystine in urine is approximately 300 mg per L. When overexcretion leads to higher concentrations than the solubility limit, cystine stones tend to form. Biochemically, it has been known for decades that different phenotypes in cystinuric populations exist. The disease has been differentiated by amino acid excretion in obligate heterozygotes as type I (normal urinary amino acids), type II (high excretion of dibasic amino acids), or type III (modest elevation in dibasic amino acids). Given the identification of only two affected genes, a new classification scheme is now utilized.[289] Mutations in SLC3A1 and SLC7A9 are responsible for all three phenotypic subtypes of cystinuria. To date, all mutations of SLC3A1 produce the type I phenotype whereas mutations in SLC7A9 can cause all phenotypic subtypes.

In the new classification, cystinuria is defined as type A if mutations are found in both SLC3A1 alleles, type B if mutations are found in both SLC7A9 alleles, and putative type AB if one mutation is found in each gene.[289] Heterozygous type AB individuals have been identified,[290] but cystinuric patients from families of such individuals have two mutated alleles in the same gene in addition to a mutated allele in the other gene. Because type AB double heterozygous individuals do not produce stones, and two mutations in the same gene were found in patients from these families, digenic inheritance of cystinuria was ruled out.[291]

The amino acid transport system b(o)+AT is the main effector of cystine reabsorption in the kidney. This system was identified by expression cloning of renal cDNA (related to bo,+ amino acid transporter [rBAT]) that induces the $b^{o,+}$ system in Xenopus oocytes. In oocytes, the rBAT ($b^{o,+}$-like) system acts as a tertiary active transport mechanism. A cloned "light" subunit ($b^{o,+}$AT) coexpresses system $b^{o,+}$ activity in heterologous expression systems. Both proteins are expressed in brush-border membranes of proximal straight tubule and small intestinal mucosa. Whereas all renal $b^{o,+}$AT is disulfide bound with rBAT, there is an excess of rBAT covalently bound to an unidentified "light" subunit (X) in renal brush-border membranes. The rBAT/$b^{o,+}$AT heterodimer shows a gradient of expression along the proximal tubule: higher in the convoluted tubule and lower in the straight tubule. In contrast, the rBAT/X heterodimer has the opposite gradient of expression along the proximal tubule.

More than 50 unique SLC3A1 /2p16.3-p21 mutations have been identified in cystinuria patients. Missense mutations show loss of transport function in oocytes, apparently due to trafficking defects during transfer from endoplasmic reticulum to plasma membrane. Null SLC3A1 mutations are fully recessive in cystinuria heterozygotes. Mutational analysis and linkage studies have demonstrated genetic heterogeneity in cystinuria. The SLC3A1 gene is only associated with type I (fully recessive) cystinuria. A second locus, accounting for types II and III cystinuria (incomplete recessive forms), has been identified by linkage analysis at chromosome 19q13.1–13.2. The gene for the non-type I cystinuria (SLC7A9) codes for the "light" subunit ($b^{o,+}$AT) of rBAT, where 37 unique cystinuria-specific mutations have been identified. This strongly supports the notion that rBAT/$b^{o,1}$AT heterodimer (system $b^{o,+}$) is the main apical reabsorption system for cystine.

In non-type I cystinuria, the urinary hyperexcretion of cystine and dibasic amino acids among heterozygous carriers of SLC7A9 mutations correlates well with the severity of defective amino acid transport in vitro. In some cases, mild SLC7A9 mutations account for heterozygous type I cystinuria. Patients with the mixed form of cystinuria (type I/III) excrete slightly lower levels of cystine and appear to have a lower risk of nephrolithiasis in the first decade. An SLC3A1 mutation is rarely identified on the fully recessive allele in these patients.[292]

While the molecular physiology of the renal cystine transport mechanism is being worked out, additional clarification is needed. In particular, we need to understand the physiologic role of the SLC3A1 gene, why mutations in the "heavy" subunit (rBAT) of system $b^{o,+}$ produce a silent phenotype in carriers whereas mutations in the "light" subunit ($b^{o,+}$AT) produce a dominant negative effect, and which genes modulate urolithiasis in patients with cystinuria.

Additionally, three similar, but distinct, syndromes are associated with cystinuria. The 21p21 deletion syndrome, hypotonia-cystinuria syndrome (HCS), and atypical HCS are associated with deletions of/and genes contiguous to SLC3A1.[293–298]

Pathobiology

Although a kidney biopsy is not usually performed, examination of renal tissue obtained during stone removal procedures reveals associated parenchymal abnormalities that may partly explain the loss of kidney function associated with this disorder.[299]

The ducts of Bellini are plugged with cystine crystals, and apatite crystals can be seen in the inner medullary collecting ducts and in the thin loops of Henle. Focal areas of dilatation with varying degrees of surrounding interstitial fibrosis are present. These findings are in contrast to the histopathology of routine calcium oxalate formers (interstitial deposits of apatite without intratubular crystals). The crystallization of cystine in terminal ducts of Bellini may cause local obstruction and tissue injury contribution to loss of kidney function.

Pathogenesis

Cystine overexcretion raises urinary cystine concentration above the limits of solubility for this relatively insoluble amino acid. Characteristic hexagonal crystals are identified in cystinuric patients particularly in the first voided morning urine, which is concentrated and usually acidic.

All patients with an episode of nephrolithiasis before the age of 30 or a strong family history of recurrent nephrolithiasis should be screened for cystinuria. A rapid qualitative screening test with sodium cyanide-nitroprusside is used for diagnosis when the pathognomonic crystals are not visualized.

Normal adults excrete less than 30 mg of cystine in 24 hours (19 mg per g of creatinine),[300] whereas homozygous cystine stone formers usually excrete more than 350 mg of cystine per day (250 mg per g of creatinine).

Heterozygotes and patients with the Fanconi syndrome generally excrete less than 250 mg per day and usually do not form stones. Given that proximal tubular transport requires maturation, it is frequently difficult to diagnosis cystinuria prior to the age of 1 year.[301]

Treatment

Therapy is designed to reduce the excretion and increase the solubility of cystine. Methionine is the precursor of cystine and dietary restriction of methionine reduces urinary excretion of cystine.[302] However, methionine is an essential amino acid and dietary restriction is, therefore, not a practical mode of treatment. Lowering urinary cystine concentration by increasing urinary volume reduces the likelihood of precipitation and thus provides the basis for clinical treatment. An intake of more than 4 L per day may be required.

The pH of urine alters the level of cystine saturation, but until urinary pH is 7.0 or greater, the effect of pH on solubility is minimal.[303] Dent et al. showed that the solubility of cystine in urine is approximately 250 mg per L up to a pH level of 7.0, but increases to 500 mg per L or more with a pH level of 7.5 or greater. This degree of urinary alkalinization may require up to 3 to 4 mEq/kg/day of potassium citrate or potassium bicarbonate taken in three to four divided doses.[21]

If urinary alkalinization and high fluid intake are ineffective, then a cystine binding drug is added to the regimen.[304] D-penicillamine (DP) and α-mercapto-proprionyl-glycine (MPG) are equally effective in clearing the disulfide bond of cystine into cysteine, which is 50 times more soluble.[305,306] Side effects occur in 20% to 50% of patients and limit treatment success. Typical side effects for both drugs include rash, arthralgia, leukopenia, thrombocytopenia, myositis, and proteinuria (due to membranous nephropathy).

Captopril, a first generation ACE inhibitor, contains free sulfhydryl groups. Captopril cysteine disulfides are 200 times more soluble than cystine alone. Although captopril can reduce cystine excretion by more than 50%, hypotension often limits tolerance.

Medical therapy is beneficial with adequate patient adherence, but there is a high rate of continued stone formation and most patients require multiple urologic interventions. Cystine stone formers have significantly higher procedure rates than other stone formers.[307] Patients may form stones of mixed composition mandating a broader investigation during follow-up.[308]

The response to therapy is monitored both biochemically and radiologically. The preferred imaging test is spine CT. Urinary cystine concentration is measured serially to estimate whether the amount excreted can be held in solution at the urine volume and pH achieved by the patient. The measurement of urinary cystine is imprecise in the presence of thiol drugs. Solid phase assays overcome this barrier and should be used to monitor patients' response to interventions.[309,310]

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CHAPTER 21

Host–Pathogen Interactions and Host Defense Mechanisms

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Urinary tract infections (UTIs) represent the most common urologic disease in the United States based on the Centers for Disease Control's (CDC) statistics of visits to office-based physicians and hospital outpatient clinics. Women account for a majority of cases. The 2005/2006 Ambulatory Medical Care Utilization Estimates attributed an annual figure of approximately 8.1 million of all diagnosed patients with UTIs as the main ailment.[1a] Although no clear cost estimate is available currently, total expenditures in the year of 2000 alone was estimated to amount to US$3.5 billion.[2a]

The human urinary tract is a normally sterile environment that presents invading bacteria with numerous challenges of a dynamic nature. These challenges include the mechanical stress of urine flow, various physical barriers such as the mucosal epithelium, and the attack of invading immune cells that form part of the host's immune response. The dynamic nature of these challenges means that invading pathogens must rapidly adapt to their changing niche in order to enable colonization. Infections of the urinary tract occur when pathogens, often originating from fecal flora, enter the urethra. Although continuous cycles of urine production, storage, and voiding relentlessly expel invading organisms, pathogens are able to migrate to the bladder, where they may cause symptomatic cystitis or asymptomatic bacteriuria (ABU).[1] Pyelonephritis manifest when pathogens ascend further up to the kidney, colonizing the tubules of the nephrons.

Asymptomatic bacteriuria is defined as the presence of bacteria in the urinary tract, which do not cause any obvious clinical symptoms in the patient. ABU has been described as similar to a commensal state[2] where patients may carry up to 10^5 CFU per milliliter of urine without symptoms. ABU strains are genetically similar to those that cause symptomatic infections, but they notably tend to lack adhesion organelles.

Cystitis, or a lower UTI, occurs when the pathogens that have entered the urinary bladder attach themselves to cells of the bladder epithelial lining, where they start multiplying. A lower UTI often presents with clinical symptoms such as pain and urgency of urination. The urine of cystitis patients often appears cloudy due to the presence of bacteria, white blood cells (WBCs), and sloughed epithelial cells.[3] A urine examination and culture are essential for a diagnosis, and the infection is usually treated with antibiotics.

Further migration of bacteria up the ureters leads to an infection of the kidneys.[4] A bacterial infection of the kidney is medically termed pyelonephritis, indicating that the infection has reached the renal pelvis—the so-called pyelum—of the kidney (nephros). Upper UTI infections are more difficult to diagnose than cystitis. They show similar symptoms to a lower UTI but are often accompanied by a sudden increase in temperature and unilateral or bilateral flank pain.[5] Pyelonephritis is commonly defined as a tubulointerstitial disorder based on the pathologic picture observed in renal biopsies. This indicates that the tubules and interstitial tissue are most commonly involved.[6] In light of the greater level of inflammation, as compared to cystitis, pyelonephritis is considered a serious infection.[7] Gross pathology includes abscess formation in the renal parenchyma and edema, often leading to irreversible scar formation. Renal scar formation with fibrosis can contribute toward the development of renal insufficiency.[7]

The normal kidney is considered relatively resistant to infection but abnormalities in the structure and function of the urinary tract can increase susceptibility.[8] Risk factors in children include voiding dysfunction and vesicoureteral reflux, whereas in adults, genetic susceptibilities and behavioral risk factors are most relevant.[9]

An essential step in bacterial colonization and the initiation of a UTI is the bacterial binding to the urinary epithelium. However, the epithelia that line the urinary tract are far from uniform. The bladder is lined by a transitional stratified epithelium consisting of multiple layers, topped with facet or umbrella cells, and covered with apical plaques of hexagonal uroplakin.[10] The bladder epithelium, together with the transitional epithelium, covers the ureters and the renal pelvis and is also known as uroepithelium. The Bowman capsule (the epithelial structure that surrounds the glomerular capillary tufts) is lined with thin squamous epithelial cells. The tubular systems of the nephron all consist of a

single layer of epithelium, which expresses a unique structure and function depending on the tubule segment.[11,12,13,14] The proximal tubule consists of cuboidal/columnar epithelia covered with microvilli (~150 per square micrometer of cell surface), which function to increase the surface area for tubule reabsorption.[12] Distal tubule cells lack microvilli and constitute a tight epithelium, displaying low endocytic capacity and low permeability to water.[13,14] This suggests that bacteria do not only meet very different epithelial linings on their way up the urinary tract, but also that they do so while being exposed to a continuously altered composition of filtrate/urine.

Uropathogenic Escherichia coli (UPEC) are implicated as the causative agent in up to 80% of community-acquired UTIs, making it the leading urinary pathogen.[7] However, other gram-negative bacterial species are also associated with UTIs, including Klebsiella, Enterobacter, Pseudomonas, and Proteus mirabilis.[7] The latter accounts for more than 40% of UTIs in infant boys. Gram-positive bacteria implicated in UTIs include Staphylococcus strains, epidermidis, and aureus, as well as Enterococcus faecalis.[7] Using culture-based methods, a Canadian study of bacterial isolates in ambulatory patients with community-acquired UTIs revealed that 74.2% contained Escherichia coli, 6.2% contained Klebsiella pneumoniae, 5.3% contained Enterococcus, 2.8% contained Streptococcus agalactiae, 2% contained Proteus mirabilis, 1.4% contained Staphylococcus saprophyticus, 0.9% contained Viridans streptococci, 0.9% contained Klebsiella oxytoca, and 0.8% contained Pseudomonas aeruginosa.[15] Although the proportion of isolates vary depending on the region, the disease state, and the patient type, UPEC unanimously remains the major causative microbe for UTIs and, as such, has been used as the primary pathogen in a number of molecular studies of the UTI process. This chapter will accordingly focus on current knowledge gained from UPEC-induced UTIs.

VIRULENCE OF UROPATHOGENIC ESCHERICHIA COLI

Bacteria entering the urinary tract must rapidly adapt to their new environmental niche. To enable this adaptation, UPEC strains express specific genes that encode a class of proteins termed virulence factors. This name originates from the finding that these factors assist in the initiation and progression of the infection. UPEC strains have a larger genome and therefore contain more genes than their nonvirulent ABU or commensal E. coli counterparts. For example, the clinical isolate CFT073 has 590,209 more base pairs in its genome than the K-12 MG1655 strain.[16] Based on recent genetic approaches, it was proposed that UPEC, ABU, and commensal strains may have evolved from the same virulent ancestral parent, with the ABU and commensal strains having lost virulence factors.[17,18] Due to the relatively minor genetic variations between the UPEC, ABU, and a commensal gut E. coli, genetic mutations and differences in expression of certain genes may actually differentiate virulence potential rather than genomic content itself.[17]

The extra genes that confer virulence are commonly located in specific regions of the chromosome, termed pathogenicity-associated islands (PAIs).[19,20] This unique subset of genomic islands has been acquired by horizontal gene transfer.[21] PAIs were originally identified by Hacker and colleagues[22] when they analyzed segments of chromosomal regions that encode multiple, distinct virulence-associated phenotypes in UPEC strain 536.[23] Further characterization has demonstrated that PAIs are present in a wide range of bacterial pathogens, that PAI segments range from 10 to 200 kb in size, and that they are rich in virulence and antibiotic resistance gene insertion sequences or other mobile genetic elements. PAIs are easily identifiable by their unique G+C content,[24] and their location near or within tRNA genes. One bacterium may possess multiple PAIs,[25,26] as exemplified by UPEC strain 536, which contains six well-characterized PAIs.[27–29]

The chromosomes of E. coli appear highly diverse aside from a core genome that is highly homogeneous in G+C content.[24] A large proportion of this diversity arises from a variable pool of mobile genetic elements, conjugative plasmids, bacteriophages, transposons, insertion elements, as well as by the recombination of foreign DNA into host DNA.[24] This has been highlighted in comparative studies of the nonpathogenic E. coli K-12 lab strain MG1655,[28] the enterohemorrhagic O157:H7 strain EDL933,[30] and the UPEC strain CFT073.[16] They were shown to differ significantly in genome size, sharing only 39.2% of proteins in common.[16] The flexibility of bacterial genomes arising from mobile genetic elements may facilitate the timely emergence of new clones,[31,32] which provides new virulence and antibiotic resistance profiles.

However the genetics may look or may have evolved, it remains that pathogenic UPEC strains express proteins that are considered essential for virulence. These virulence factors characterize disease isolates.[33] Although the early definitions of virulence factors came from the basic epidemiology practice of comparing properties of fecal strains from healthy controls with urinary isolates from patients,[34] Falkow[35] introduced a new view in 1988, which he named the molecular Koch's postulates for pathogenesis. These postulates include[36]:

- The phenotype or property under investigation should be associated with the pathogenic members of a genus or pathogenic strains of a species.
- Specific inactivation of the gene(s) associated with the suspected virulence trait should lead to a measurable loss in pathogenicity or virulence, or the gene(s) associated with the supposed virulence trait should be isolated by molecular methods. Specific inactivation or deletion of the gene(s) should lead to loss of function in the clone.

- Reversion or allelic replacement of the mutated gene should lead to restoration of pathogenicity, or the replacement of the modified gene(s) for its allelic counterpart in the strain of origin should lead to a loss of function and a loss of pathogenicity or virulence. Restoration of pathogenicity should accompany the reintroduction of the wild-type gene(s).

Fifteen years later, Falkow[36] reviewed these postulates in light of the new advances in technology available to infection biologists. Here he described how the postulates should be considered as a "working hypothesis for the study of the genetic and molecular basis of pathogenicity" and not a ridged determination of virulence factors. Some virulence factors may play different roles in different model systems and, as technology advances, the definitions of what a virulence factor is may need to evolve. Some of these factors may also be considered as "fitness factors" (i.e., factors that enhance the growth and colonization of the bacteria but may not be absolutely essential for infection). Siderophores, which allow bacteria to sequester iron, have been annotated as fitness factors because their expression is advantageous but not essential to virulence.[37,38] Conversely, the acquisition of certain traits such as antibiotic resistance, which would appear advantageous for virulence, can have a negative effect on bacterial fitness.[39,40]

UROPATHOGENIC ESCHERICHIA COLI ADHESION

The traditionally annotated UPEC virulence factors include adhesion factors, exotoxins, lipopolysaccharides, capsules, proteases, and iron acquisition systems.[41,42] Research on these factors has been carried out in vitro and forms the foundation for their current definition. Thus, the expression of certain adhesion factors is still defined by their in vitro agglutination abilities.[34,42] In UPEC, the best described virulence factors are involved in bacterial adhesion to the uroepithelium, and these proteinaceous structures are referred to as fimbriae or pili.[20] These organelles allow UPEC to bind to the epithelium and help bacteria to withstand the stress of filtrate and urine flow. UPEC express numerous different fimbriae including P, type 1, F1C, S, and Afa/Dr adhesins.[42] The great redundancy in fimbriae expression is further illustrated by the fact that one bacterium contains the genes for many different fimbriae.[43] The current understanding of the roles of the various fimbriae in UTIs is described in detail in the following paragraphs.

Bacteria have many tools aiding their rapid adaptation to changing microenvironments. They contain genes for numerous different fimbriae and it has been shown there is a redundancy between these fimbriae.[43] Phase variation means the bacteria can vary their fimbriae expression, thereby altering their nature of adhesion depending on the microenvironment. This common feature not only allows for rapid adaptation but also, at the same time, allows for the development of a heterogeneous bacterial population.[44] A genetic switch, the so-called *fim* switch, controls the phase variation of type 1 fimbriae expression. This invertible element contains the main promoter for the fimbrial structural subunits.[44] Negative cross-talk between type 1 and P fimbriae has been demonstrated, with PapB being shown to repress the FimB-promoted off-to-on inversion of the *fim* switch.[45] This means that UPEC express either Type 1 or P fimbriae but it is unlikely that they express both simultaneously. A cross-talk between P fimbriae expression and motility has also been reported, showing that the expression of P fimbriae also regulates the synthesis of flagellum, a protein-based extrusion that mediates bacterial mobility. The PapX protein, encoded at the end of the pheV associated pap gene cluster in UPEC strain CFT073, represses motility by binding to the *flh*D promoter, thereby repressing transcription of $FlhD_2C_2$, the master regulator of flagella.[46] These regulatory mechanisms highlight one mechanism by which bacteria can fine-tune their expression to adapt to the challenging microenvironments they encounter upon infection.

Type 1 Fimbriae

Type 1 fimbriae are attachment organelles produced by the vast majority of E. coli strains, both commensal and pathogenic. Initially visualized in 1950,[47] type 1 fimbriae are mannose sensitive adhesion organelles, which means their ability to agglutinate erythrocytes is inhibited by mannose.[48,49] This feature, found in the mid 1950s, is still used today to define type 1 fimbrial expression.

The type 1 fimbriae are made up of 500 to 3,000 repeating subunits of the FimA protein, which is formed into a 7-nm thick right-handed helical rod. At the outermost end of the rod is located a 3-nm thick distal tip that contains several copies of the adapter proteins FimG and FimF as well as the tip adhesin FimH.[50–52] Assembly of the rod-like type 1 fimbriae occurs via the chaperone-usher pathway, which represents a common assembly pathway for fimbriae in gram-negative bacteria.[52] The chaperone and usher proteins required for the formation of type 1 fimbriae are all encoded in the *fim* operon.[51] FimC is the periplasmic chaperone, which delivers bound subunits to the outer membrane usher protein FimD. From here, the subunits are incorporated into the growing fimbriae.[51]

The ability of the tip adhesion FimH to bind mannose-containing glycoproteins means that type 1 fimbriated bacteria can adhere to a wide range of human target cells.[53,54] The crystal structure for FimH was recently resolved.[55,56] Whereas intestinal E. coli express certain variants of the FimH adhesion,[57,58] UPEC express a FimH that has an increased affinity for terminal monomannose (M1) residues[59] and displays a 20-fold higher ability to bind uroepithelium.[57,58,60] The traditional view of type 1–mediated binding is that the mannose moiety is present on cells or structures associated with the mucosal lining, or alternatively, that mannose is bound to abiotic surfaces. Whereas the first situation refers

to bacterial colonization on the mucosal lining, the latter is implicated in bacterial biofilm formation in vitro.[61] Recently, a novel alternative was presented, demonstrating the importance of type 1 fimbriae in mediating interbacterial contact and biofilm formation in vivo, thus providing a means for bacteria to withstand the shear stress from the renal filtrate (see the following text for further details).[62]

In UPEC strains, the role of type 1 fimbriae in cystitis has been extensively described. The uroplakins on the surface of bladder epithelium contain monomannose moieties to which FimH binds.[42,55] Therefore, uroplakins serve as anchoring sites allowing UPEC to gain a foothold in the bladder.[63] Although the kidney lacks mannose moieties, a new hypothesis was recently proposed in which the FimH tip adhesions of type 1 fimbriae facilitate interbacterial binding and, in synergy with P fimbriae, thus enable tubule colonization.[62] This broadens the role for type 1 fimbriae to infectious niches other than those with surface-bound mannose moieties.

The urinary tract represents a compartment continuously exposed to some degree of mechanical flow, primarily in the form of urine. Bacteria entering into this compartment will thus be exposed to shear stress generated by the flow of urine over the epithelial surface. Over recent years, it has become increasingly appreciated that the stress may affect bacterial adhesion. The UPEC FimH protein has been shown to display enhanced binding to mannosylated surfaces in vitro in the presence of shear stress.[64,65] This interaction is reported to operate via a force-enhanced allosteric catch-bond mechanism, functioning via a finger-traplike β sheet twisting mechanism.[64]

In the initial report of FimH shear-dependent binding, it was shown that at a shear of 0.02 dynes per cubic centimeter, the binding strength of FimH was weak, whereas as the shear increased to 0.8 dynes per centimeter, this binding became stronger.[65] The same laboratory also showed that UPEC positively select for a FimH variant that maintains an attachment following a drop in shear, as compared to fecal or vaginal E. coli isolates.[66] This variation in the signal peptide of FimH, which results in expression of less, though longer, fimbriae, may be very relevant under the fluctuating conditions facing UPEC in vivo. The fact that certain bacterial adhesion events are enhanced by tensile force, as opposed to bacteria being washed away, is particularly relevant in an environment such as the urinary tract where bacteria must bind in the face of fluctuating filtrate flow. Thus far, FimH is the best described bacterial adhesin in terms of shear-enhanced adhesion, whereas binding of PapG, the tip adhesion of P fimbriae, has been shown to be shear-independent, being able to mediate binding even under relatively low shear.[67]

Although the shear-mediated adhesion may assist type 1 adhesion to the bladder epithelium via mannose binding, it may also function during UPEC colonization of the renal tubule, albeit via a different mechanism. Within the nephron, FimH may mediate interbacterial binding and help prevent bacterial washout by renal filtrate.[62] Interestingly, FimH is present in all virotypes of E. coli,[60] and a role for FimH in interbacterial binding may explain a general function for these fimbriae in diverse perfused environmental niches.

Type 1 fimbriae have been found to fulfill molecular Koch's postulates. Microarray studies of an in vivo mouse model show high levels of type 1 expression.[68] A mutant unable to make FimH is severely deficient in colonization of the urinary tract in a mouse UTI model, and complementation of the mutant has been shown to restore virulence.[69]

P Fimbriae

P fimbriae were one of the first virulence factors associated with UPEC. In 1976, it was demonstrated that E. coli from patients with acute pyelonephritis adhered in greater numbers to uroepithelial cells in vitro than strains causing asymptomatic bacteriuria.[70] Their adherence was not inhibited by the prototypic type 1 inhibitor mannose, and further investigation led to the identification of P fimbriae. They were designated P because of their ability to agglutinate red blood cells (RBCs) of the P blood group when analyzed in vitro.[34,71] P fimbriae are encoded by the pap (pyelonephritis-associated pilus) operon, which consists of 11 genes located on chromosomal pathogenicity islands. Unlike the *fim* operon, the pap operon is selectively distributed in E. coli.[24,72] The morphology of this fimbriae is extremely similar to type 1 fimbriae.[73] P fimbriae are hetero-polymers consisting of a helical rod of PapA subunits with a tip consisting of the minor pilins PapE and PapF. The tip adhesion PapG mediates attachment to Galα1-4Galβ containing glycolipids, which are often found on the renal epithelium.[73,74] PapG is known to have at least three allele variants: class I, class II, and class III. Class II is primarily linked to human pyelonephritis and class III is linked to cystitis.[71,75,76] Some strains, such as the prototypical UPEC strain CFT073, carries two pap gene clusters, both of which encode for the PapGII allele.[16,71]

Although P fimbriae have long been considered an important virulence factor in UTIs, they do not fulfill the molecular Koch's postulates. P fimbriae are expressed by a majority, but not all, clinical isolates.[34,77] Approximately 80% of UPEC strains express P fimbriae,[78] and a strong relationship exists between the severity of infection and the prevalence of P fimbriae. Indeed, clinical isolates lacking PapG adhesin were observed to cause comparatively less kidney damage than the PapG positive counterpart. Interestingly, despite the known role of P fimbriae in the adhesion colonization capability of strains in both the kidneys and bladder, it was found to be independent of PapG mediated adhesion.[79–82]

Expression of P fimbriae is controlled by phase variation, and varies depending on environmental conditions. The reversible epigenetic switch that controls the initiation of pap operon transcription allows bacteria to fine-tune their P fimbriae expression to suit their changing environment in vivo.[44,83,84]

Recently, P fimbria were shown to facilitate the early stage of UPEC colonization of renal tubules.[62] Using high-resolution live animal imaging, it was shown that strains

expressing P fimbriae were able to bind and initiate colonization in the face of sheer stress from renal filtrate flow. It was also demonstrated that the P fimbriae act in synergy with type 1 fimbriae in a heterogeneous bacterial community to facilitate renal tubule colonization. P fimbriae were shown to mediate bacterial binding to the epithelium, whereas the type 1 fimbriae mediated interbacterial binding as the colony expanded into the tubule and away from the epithelium.[62] It is interesting to note that unlike type 1 fimbriae, only E. coli and not other gram-negative rods carry the genes for P fimbriae.[85]

Dr Adhesin

Aside from type 1 and P fimbriae, several other adhesins are implicated in mediating urinary tract infections, though their roles are not as established. The Dr adhesins family embraces fimbrial and afimbrial structures, which are found on the extracellular surface of E. coli, and have in common that they bind to Dr blood group antigens.[86] The Dr blood group antigen is a component of the decay-accelerating factor (DAF), a membrane protein that prevents host lysis by complement.[87,88] This binding leads to the internalization of Dr^+ E. coli into nonfusogenic intracellular vacuoles where bacteria are shielded from the host immune system.[89] Dr adhesin mediated binding of E. coli to the bladder epithelium has also been correlated with recurrent UTIs in young adults and with pyelonephritis in pregnant women.[90] Among the Dr adhesin family, only Dr fimbria possess the ability to bind both type IV collagen of basement membranes and DAF.[91] The latter is mediated via the subunit DraE.[92] When investigating the significance of DraE-type IV collagen binding, it was shown that disruption of this capability resulted in the inability of E. coli to cause a persistent kidney infection.[93]

F1C Fimbriae and the S Fimbria Family

Although the role of type F1C fimbriae for UTIs has not been fully determined, epidemiologic data suggest this fimbriae to be more prevalent in pyelonephritis and cystitis strains than among fecal strains of E. coli.[94] Data suggest these fimbriae are expressed in vivo and provide bacteria the capacity to adhere to human distal tubular and collecting tubular epithelium, as well as the vascular endothelium on kidney tissue sections.[95,96] The two minor glycosphingolipids, galactosylceramide and globotriaosylceramide, have been identified as target tissue receptors for F1C fimbriae in rats, canines, and humans. Galactosylceramide is found throughout the urinary tract, with the exception of the urethra, whereas globotriaosylceramide is unique to the kidney.[95] The binding of F1C-fimbriated bacteria to renal epithelial cells in vitro was shown to induce similar levels of interleukin (IL)-8 production as compared to those levels produced by the adhesion of type 1- and P-fimbriated bacteria, thus supporting a role for F1C in pyelonephritis.[95]

The S fimbriae bind terminal NeuAcα2, 3-galactose sequences present on glycoproteins. Although shown to bind several host cell types, the occurrence of S fimbriae expressing E. coli strains in UTIs is infrequent.[97,98] The role of S fimbriae in UPEC pathogenesis may thus be minor.

BACTERIAL TOXINS AND VIRULENCE FACTORS

α-Hemolysin

The 107 kDa lipoprotein α-hemolysin (Hly) is considered an important UPEC virulence factor, yet no more than 50% of pyelonephritogenic E. coli organisms express this toxin. The Hly operon is commonly located adjacent to genes encoding P fimbriae,[99,100] which may account for the two- to threefold higher probability of UPEC having hly genes over fecal strains.[101] Hly exerts concentration-dependent, biphasic activities on target cells. The traditional view focuses on Hlys cytotoxic effect. Hly is lytic for numerous cell types, including erythrocytes, polymorphonuclear leukocytes, monocytes, mast cells, basophils, and lymphocytes.[102,103] More recently, the sublytic concentration of Hly was shown to elicit Ca^{2+} signaling in primary proximal tubule cells.[104] Via frequency-modulated activation of the transcription factor nuclear factor-kappa B (NF-κB), Hly activated proinflammatory signaling in epithelial cells. When analyzing a role for Hly in vivo, intravital imaging of the infection process within a nephron of a rat was applied. This showed that the same end result was achieved whether or not UPEC expressed Hly. However, the kinetics of the tissue response was severely influenced.[105]

Cytotoxic Necrotizing Factor

CNF1 is a toxin contributing to UPECs invasion of the epithelium.[106,107] The toxin induces the formation of stress fibers via the deamination-dependent activation of small, actin-regulatory GTPase proteins of the Rho family.[108,109] The gene encoding CNF1 is positioned adjacent to hemolysin,[110,111] and coregulation of their expression is mediated by RfaH.[112,113] Although the role of CNF1 in vivo remains unclear, in vitro studies do suggest a role for the toxin in urinary tract disease.[114,115]

Secreted Autotransporter Toxin

Among the array of toxins studied in UTI models, the 107-kDa Sat protein is more frequently secreted from pyelonephritogenic E. coli strains than fecal isolates, suggesting a possible role of the toxin in pathogenesis.[116] Sat has serine protease activity and shows cytopathic effects (cytoplasmic vacuolization) on human bladder and kidney cell lines, and in the mouse kidney.[117] However, Sat is not required for kidney colonization.[117] Originally isolated from the prototypic UPEC strain CFT073, Sat was found to share homology with various virulence-related proteins from a range of E. coli pathotypes.[118] Among these, Sat possess a high similarity to Pet and EspC, two SPATE (serine protease autotransporters of Enterobacteriaceae) proteins.[118]

Siderophores

Mammals possess efficient systems such as the proteins transferrin and lactoferrin to efficiently scavenge free iron within the host. During an infection, the deprivation of free iron is used as a host defense mechanism as upregulation of iron acquisition and storage mechanisms are up-regulated. Low iron availability limits bacterial viability. To counteract this, bacteria produce low-molecular-weight chelators called siderophores (Greek sideros, iron; and phoros, bearing). Siderophores are secreted into the extracellular environment where they bind ferric iron (Fe^{3+}) and internalize it via receptor-mediated mechanisms. UPEC strains produce four distinct siderophore systems: enterobactin, salmochelin, yersiniabactin, and aerobactin. Among these, enterobactin is conserved in all isolates.[119,120] UPEC also expresses siderophore-associated receptors such as ireA[121] and iroN,[122] and other iron acquisition systems.[16,20] Strains with impaired iron acquisition capability were shown to have decreased fitness and virulence in mouse models.[123]

The precise contribution of each iron uptake mechanism to bacterial virulence is presently unclear. However, a study of coincident urinary and rectal strains from patients with recurrent UTIs suggested UPEC infections are facilitated by yersiniabactin and salmochelin.[119] Some UPEC strains express siderophore receptors but not siderophore. These strains are hypothesized to take advantage of neighboring siderophore-expressing bacteria in a polymicrobial setting by competitively scavenging excreted iron-bound siderophores.[20,124,125]

Lipopolysaccharide

The serotyping of E. coli strains is based on three determinants: the somatic antigen O, the capsular antigen K, and the flagella antigen H.[126] This system, developed by Kauffmann in 1940, has identified more than 50,000 different E. coli serotypes of various combinations of the 173 O, 80 K, and 56 H types, in addition to all nontypable strains. The association of O-antigen serogroups with UTIs is complex. Although studies have observed the presence of certain serogroups (O1, O2, O4, O6–8, O18, O25, and O75) to be more frequent in E. coli isolates in symptomatic UTIs,[127] the pattern of other potential virulence factors confound O-antigen-based epidemiology data.[128,129] Furthermore, the horizontal mobility of antigen determinant clusters obscures the phylogenetic relation of E. coli strains.[130] Employing isogenic mutants of O antigen synthesis, a possible link between UTI pathogenesis and the ability of a strain to synthesize an O antigen was observed.[131] However, there is yet to be clear experimental evidence closely correlating a particular O antigen type with a pathogenic tendency.

ORGANS OF THE URINARY TRACT

Host responses are initiated the very moment a bacterium starts interacting with the host tissue. In response to the accompanying microenvironmental changes, bacteria rapidly adapt their gene expression and alter their physiology to cope with the situation. The net effect of these opposing forces determines the duration of the infection and the end result: bacterial clearance, containment or commensalism, or the death of the host. Depending on the physiology and function of the organ, the nature of such challenges varies.

Urine

Urine is a highly variable and dynamic environment that both prevents and promotes infection. High osmolality, high urea concentrations, and low oxygen tension exhibit bacteriostatic and bactericidal effects.[129–134] On the other hand, thin films of urine retained at the bladder mucosa act as a reservoir of bacteria, allowing repopulation following each voiding event.[131,133–136]

The Bladder

The cascade of events that occur when urinary pathogens and, particularly, UPEC come into contact with the bladder epithelium has been well studied. Type 1 fimbriae bind to mannose residues on the surface of uroepithelia via the FimH adhesion. Once adhered, UPEC can withstand the forces of bladder emptying. However, FimH is believed to act not only as an adhesion, but also as an invasin that promotes bacterial entry into mast cells, macrophages, and the bladder epithelium.[137,138] Lipid raft domains on the host cells have been reported to facilitate type 1 fimbriated bacterial invasion with cholesterol, and caveolin-1 has been shown to cluster around the bacteria upon binding.[50,136,139] Localized rearrangement of host actin cytoskeleton is required for FimH-mediated epithelial invasion and is mediated via phosphoinositide-3-kinase signaling.[140] However, the internalization of bacteria into the bladder epithelium does not appear to be the end of the story; far from it. There are two alternative reported pathways for bacteria once they enter the bladder epithelium.[141] Upon internalization, bacteria multiply within intracellular bacterial communities (IBCs) present in membrane-bound vacuoles.[141,142] Within the IBC, bacteria change from a bacillary shape to a smaller, more coccoid shape[139] that forms pods within the facet cells. At this stage some bacteria on the periphery of the pod regain a bacillary shape and become motile, thus leading to bacterial spread.[143] There have been suggestions that the behavior of bacteria within these pods seems to be "biofilmlike."[139] Some bacteria within IBCs form long (<70 μm) filaments, which move extracellularly and induce reinfection via reseptation, thus leading to further rounds of IBC formation.[144]

Upon infection of the bladder epithelium, one host response includes an exfoliation of the infected cells.[143,145] Exfoliation results in a loss of surface epithelial cells from the underlying transitional layer. Although this functions well to clear many epithelial cells infected with IBCs, the bacteria have an alternative mechanism by which they can establish quiescent intracellular reservoirs (QIRs).[144] In QIRs, bacteria remain within the membrane-bound compartments with no extensive multiplication. When these transitional cells

develop into new facet cells, the QIRs remain intact and are proposed to be a possible source of recurrent UTIs.

The Kidney

Acute pyelonephritis is considered the most serious form of UTI and can lead to renal scarring, kidney damage, kidney failure, hypertension, and sepsis.[71] The study of infection in the kidney has slightly lagged behind the bladder when it comes to high-resolution molecular studies of host–pathogen interaction. Whereas Type 1 fimbriae play a major role in bladder infection, a limited role is implied in the kidney because renal epithelia lack uroplakin. Instead, P fimbriae have been considered a key player in the development of pyelonephritis due to its overrepresentation in pyelonephritogenic isolates.[146] However, experimental data have not yet proven P fimbriae to be essential for disease. Only subtle roles for P fimbriae–mediated adherence have been described in uroepithelial cell culture models,[146,147] and its role in ascending infection models has yielded inconsistent and conflicting results.[81,82,148] Early studies showed that lab strains of E. coli overexpressing P fimbriae persisted longer in mouse kidneys than strains lacking the pap operon.[149] However, bacterial numbers in the tissue never reached the same level as that of clinical strains, and it was accordingly suggested that P fimbriae are not the defining factor in virulence. Years later, when it was possible to genetically introduce a precise deletion of defined pap genes in UPEC isolates, it was demonstrated that a lack of P fimbriae did not significantly affect kidney colonization or pathophysiology 1 week after infection.[82]

Pyelonephritis does show a greater level of inflammation than cystitis, indicating the presence of a sensitive immune response system that detects colonizing bacteria.[150] Toll-like receptor family (TLR) and, particularly, TLR4 play a significant role in UTIs. An experimental ascending UTI model, using mice lacking TLR4 expression, failed to clear the invading pathogens and expressed less proinflammatory mediators.[151] The same group also reported that TLR4 expression on renal medullary collecting duct cells facilitated the translocation of bacteria across this epithelial barrier.[152]

Recently, live animal imaging applied to renal UPEC infection allowed for a high-resolution study of a live infection in real time.[62,153] In this rat model, GFP^+-expressing UPEC bacteria were slowly infused directly into the lumen of a superficial renal tubule to allow for spatial and temporal control of the infection. Fluorescence-based multiphoton microscopy showed that very few bacteria initially adhered to the tubule epithelium in the face of the flowing glomerular filtrate. These few bacteria rapidly adapted to the environment and began colonizing the tubule.[153] In this dynamic study of infection, new roles for the P and type 1 fimbriae in kidney infections were established, functions that had been undetectable in previously used infection models.[146,154] Bacterial P fimbriae expression demonstrated a fitness advantage in withstanding tubular filtrate flow and in mediating early phase adhesion to the epithelium, whereas type 1 was shown to mediate interbacterial binding and biofilm formation in the center of the tubule lumen, away from the epithelium. Synergy between the two fimbriae aids in the efficient colonization of the renal tubule. This work highlighted the narrow nature of the spatial resolution of an infectious niche with the center and periphery of a single tubule lumen exerting different pressures for adaptation.[154]

As an infection progresses, major alterations of the infected organ's physiology occur. One of the first significant findings relates to the rapidity of kidney responses to a local infection, with a majority of events occurring within the first 22 hours (Fig. 21.1A–D). Early tissue changes included vascular coagulation, epithelial breakdown, vascular leakage, immune cell recruitment, and general tissue destruction.[153,154] Coagulation in local peritubular capillaries (Fig. 21.2), and the subsequent vascular shut down, occurred within 5 to 6 hours of the infection, and accompanied a dramatic loss of local tissue oxygen.[155] Subsequent signs of ischemic injury were seen. This response was found to protect the host from urosepsis by keeping the infection site confined. Although extravasation of neutrophils inevitably causes tissue damage, the ischemic response efficiently hindered bacteria from gaining access to the bloodstream, thus giving time for neutrophils to clear the infection (Fig. 21.1E,F). Furthermore, bacterial colonization was shown to affect renal filtration, leading to obstruction.[3] Renal ischemia and obstruction are both well-studied physiologic injuries and both cause inflammation and tissue destruction in their own right.[156,157] Both are multifactorial and can vary in severity. Severe ischemia or obstruction can lead to end-stage renal failure, as can pyelonephritis. Thus, this study revealed that the pathophysiology of pyelonephritis is in fact a combination of infection and physiologic injuries such as ischemia and obstruction.

HOST RESPONSES TO INFECTION

The mammalian urinary tract is protected by numerous defense mechanisms, which together strive to maintain a sterile environment.[3,158] The physical defense of the epithelial barrier is complemented by mechanical defenses, including the sheer stress of urine flow, and chemical defenses, such as the expression of proinflammatory cytokines and antimicrobial peptides.[159,160] The colonization of the urinary tract can either lead to symptomatic disease, such as cystitis and pyelonephritis, or can develop into asymptomatic bacteriuria.[18,160] How the infectious process is developed is defined by the intimate interplay between features specific for the pathogen (i.e., virulence factors) and those defined by the host. One major host defense is the immune response, which usually is divided into the innate and the adaptive immune responses.[160,161] Innate responses are those mechanisms that recognize and respond immediately to the bacterial threat. Innate responses are nonspecific, whereas the adaptive immune response contains a memory that can build a specific immunity to a pathogen. The adaptive response can take days to weeks to develop to its full capacity.[162]

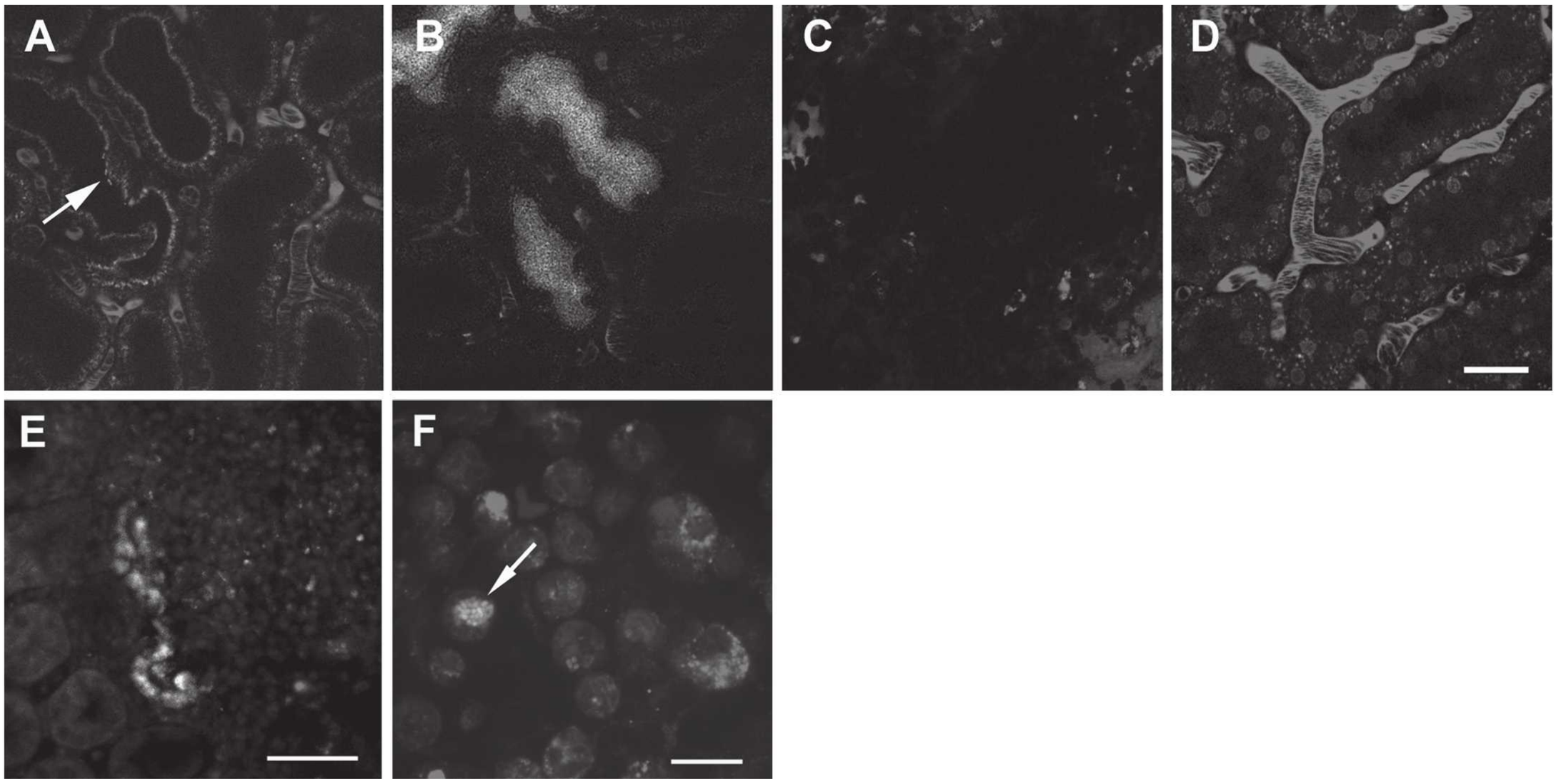

FIGURE 21.1 Real-time imaging using multiphoton microscopy of a uropathogenic *Escherichia coli* (LT004) infection. Labeled dextran outlines the injected tubule *(blue)* and blood flow *(red)*. Bacteria are visualized by their expression of GFP^+ *(green)*. **A:** One hour postinfection. **B:** Five hours postinfection. **C:** 22 hours postinfection. **D:** An adjacent, noninfected nephron of the same kidney 22 hours postinfection. Scale bar = 30 μm. **E:** An ex vivo analysis of image **C** by a confocal microscopy with the addition of nuclear stain Hoechst 33342 *(blue)* and leukocyte marker α-CD18-Cy3 *(red)*. Labeled dextran *(yellow)* outlines the injected tubule. Scale bar = 50 μm. **F:** A magnification of image **E** with an *arrow* indicating neutrophil phagocytosing bacteria. Note the lack of *green* present in images **C** and **E**, signifying bacterial clearance. Scale bar = 10 μm. (Reprinted from Månsson LE, et al. Real-time studies of the progression of bacterial infections and immediate tissue responses in live animals. *Cell Microbiol* 2007;9(2):413–424, with permission.) (See Color Plate.)

Shear Stress as a Natural Defense

One of the first defense mechanisms in the urinary tract is the shear stress caused by the flow of urine. In the bladder, this stress varies dramatically as the bladder fills and then empties upon voiding. In the kidney, this stress may be considered less extreme but it too fluctuates as the body regulates kidney function. Bacterial attachment to the epithelial lining of the urinary tract is considered extremely important to withstand this stress and the relationship between shear stress. Bacterial attachment was discussed in earlier sections of this chapter.

Asymptomatic Bacteriuria

Asymptomatic bacteriuria (ABU), or infection with strains that do not cause clinical symptoms, has also been proposed as a mechanism to protect the urinary tract from more severe infections. Although ABU has been suggested to resemble commensalism due to the apparent lack of host immune response, it differs from the complex commensal flora of the intestine because it is normally a monoculture of only one bacterial strain.[162] The ABU strain E. coli 83972, isolated from a patient with long-term ABU, has been used extensively as a prophylaxis treatment to protect patients from symptomatic UTIs by outcompeting pathogens.

The Immune Response

Epithelia are equipped to rapidly recognize the presence of microbes. Toll-like receptors (TLRs) are closely related to the Drosophila toll protein,[161,162] which is known for its primary role in the innate immune system. Among a family of 10 characterized human TLRs and 12 mouse TLRs, TLR4, TLR5, and TLR11 have been linked to the urinary tract. When TLR recognizes specific bacterial molecules, so-called microbe-associated molecular patterns, activation of TLR signaling via coreceptor engagement leads to the onset of proinflammatory responses.[162] Uroepithelial cells expressing TLR4 are actually as sensitive to LPS, the endotoxin of gram-negative pathogens, as macrophages.[163–167] Though the ligand for TLR11 remains unknown, this receptor is important in a mouse model of UTIs,[168] and so is TLR5, which recognizes bacterial flagellin.[169]

The renal expression of TLR4 has been a matter of some debate, with reports showing that renal cells do[167] or do not[165] express TLR4. This discrepancy may be explained by experimental design, which includes infected as well as noninfected conditions. In uninfected animals, TLR4 is predominantly located at the apical surface of the distal tubule.[170] However, under septicemia, all kidney segments show TLR4 expression,

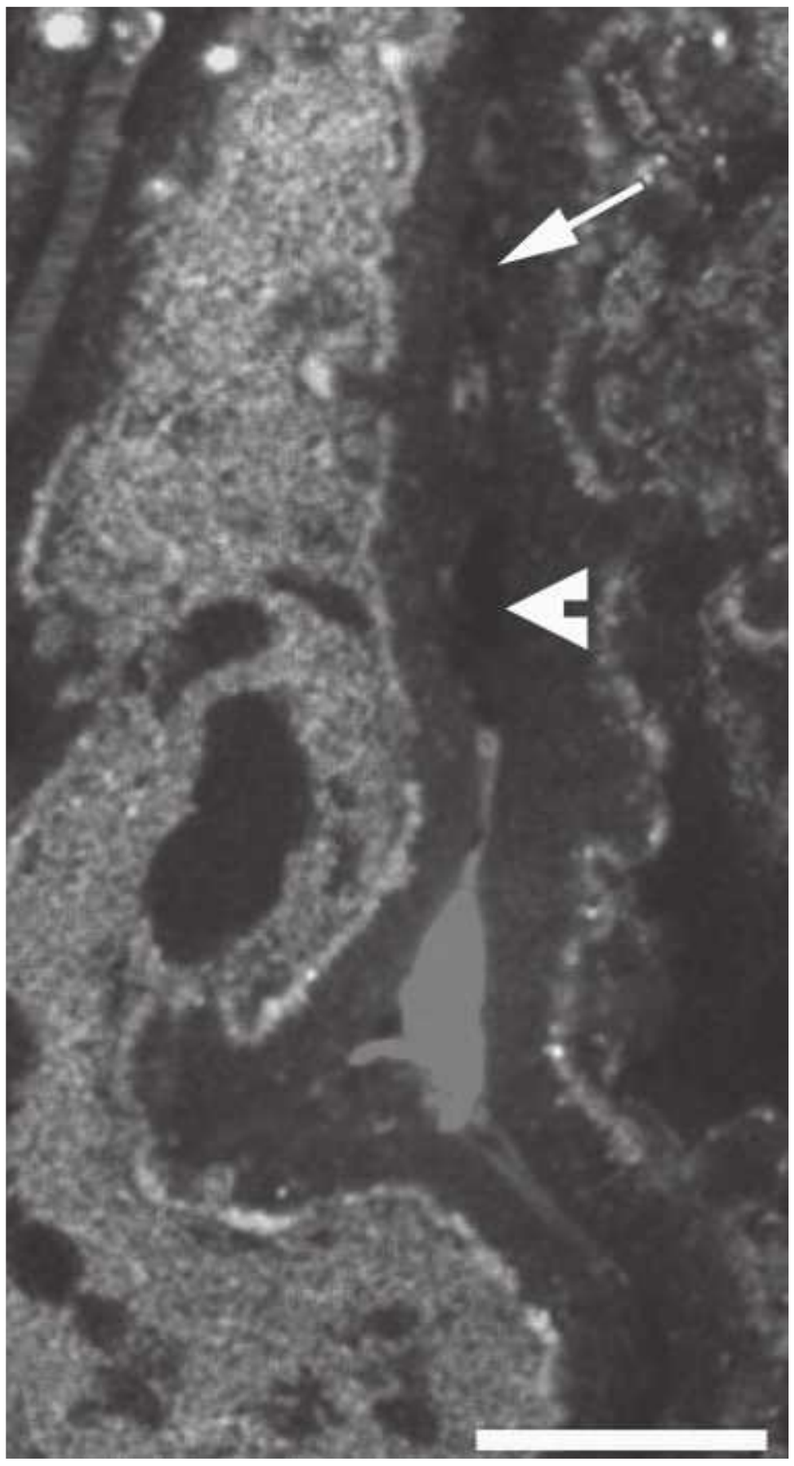

FIGURE 21.2 Blood clotting in mucosal infections. The live multiphoton image shows black silhouettes *(arrow)*, indicative of platelets, within the blood vessels *(red)* surrounding an LT004-infected *(green)* proximal tubule *(blue)* 2.5 hours postinfection. Black masses adhering to the wall of the vessel *(arrowhead)* suggest platelet aggregates. The intense *red* seen in the area represents stagnant flow, indicating a lack of red blood cell movement. Scale bar = 30 μm. (Reprinted from Melican K, et al. Bacterial infection-mediated mucosal signalling induces local renal ischaemia as a defence against sepsis. *Cell Microbiol* 2008;10(10):1987–1998, with permission.) (See Color Plate.)

suggesting TLR4 is upregulated during inflammation. The TLR4 expression pattern is thought to affect an individual's susceptibility to UTIs.[171] Children prone to ABU have reduced TLR4 expression on neutrophils[171,172] and those carrying the TLR4 A(896)G allele are prone to develop recurrent UTIs.[173]

In response to TLR4 signaling, cytokines are produced that orchestrate the immune response. Polymorphonuclear neutrophils (PMNs) and other inflammatory cells extravasate into the tissue as they follow this chemotactic gradient to the infection site. IL-8 is the main human chemokine (MIP-2 in mice) involved in the promotion of transepithelial PMN migration, which involves the IL-8 receptor CXCR1.[174] Studies have identified disease-associated polymorphisms and mutations in the CXCR1 gene of patients prone to pyelonephritis,[175] thus highlighting the importance of this receptor for a proper immune response. Neutrophils are commonly regarded as the primary cell type involved in the eradication of bacteria. Recent studies based on real-time intravital imaging of pyelonephritis in rat kidneys revealed that PMNs started to appear at the infection site as early as 3 to 4 hours postinfection, and by 8 hours, they constituted approximately 20% to 40% of nucleated cells present in the vasculature.[105] Not until hours later were neutrophils the predominant cell type at the infection foci. Although there is little doubt that PMNs are actively engaged in the clearance of bacteria in pyelonephritis, it appears that other cell types of hitherto unknown origin may also be involved in the early inflammatory process. PMNs are expected to kill bacteria via phagocytosis, and their ingestion of pathogens can occur with or without prior opsonization. The latter event is mediated by the main opsonins IgA and IgG, and by antimicrobial peptides.

Neutrophil recruitment is associated with severe tissue damage. The liberation of neutrophil granules containing antimicrobial peptides, proteins, and proteolytic enzymes can lead to the dissolution of extracellular matrix, can harm cell structures or cell function, and can induce acute and potentially irreparable damage.[174] PMN isolates from experimental acute pyelonephritis were observed to kill syngeneic renal epithelial cells in a culture within 24 to 48 hours.[176] In contrast, the suppression of acute suppuration in in vivo models reduced tubular epithelial cell damage and renal scarring despite the greater bacterial burden.[177,178] Furthermore, in IL-8 receptor knockout mice, impaired PMN translocation and unproductive accumulation in the subepithelial space resulted in kidney scarring and abscesses.[179] A picture has started to emerge that tissue damage results from the coherent action of bacteria, the inflammatory response, and physiologic injuries such as ischemia and obstruction.[62]

The antibody response in UTIs occurs both locally and systemically. Elevated levels of IgA, IgG, and occasionally, IgM have been observed in both the urine and the blood of UTI patients.[180,181] Systemic antibody titers vary distinctly between kidney and bladder infections,[182,183] with cystitis patients often showing titers as low as control groups.[180,184] Similarly, bacteria coated with antibodies are less frequently observed in patients with cystitis rather than with pyelonephritis.[182,183] Antibodies in urine play an important role in host protection. They may act as opsonins in opsonophagocytosis for PMNs recruited to the site, or they may target bacterial adhesins and, therefore, are likely to interfere with bacterial attachment to uroepithelial cells.[185] Another possibility is that antibodies trigger the agglutination of bacteria, thereby promoting bacterial clearance by voiding. They may also act to neutralize the detrimental effects of virulence factors.[186–188]

In cystitis and pyelonephritis, cell-mediated immunity is activated a day or more following the acute phase, as observed by an increased number of T cells in the infected organ

of the human. Earlier studies showing a close association between CD4 and plasma cells seemed to demonstrate the involvement of CD4 cells in host responses during repeated infections.[189,190] Experimental evidence now suggests that cell-mediated (T and B cells) immune responses appear to have a larger role in the kidney's response to chronic and repeat infections, rather than against acute infections. This is in contrast to bladder infections where the cell-mediated immune response is implicated in the acute phase. To illustrate this, T-cell depletion or deficiency does not have a significant effect on the outcome of kidneys under experimental bacterial infection.[191,192] γδ T lymphocytes are abundant in the mucosa, where they are known to modulate inflammation in response to various insults.[193] The central importance of γδ T lymphocytes in the clearance of bacteria from the bladder was recently demonstrated because these cells acted as the major source of IL-17A, which is a key mediator for the innate immune response to UTIs.[194]

Antimicrobial Proteins and Peptides

Host defenses against bacteria have been hypothesized to be dependent on epithelial-derived antimicrobial proteins that hinder survival of uropathogenic bacteria. The Tamm-Horsfall protein (THP) is the most abundant protein in human urine. THP is an evolutionarily conserved glycoprotein produced exclusively by epithelial cells of the ascending Henle loop.[195,196] Rich in mannose and sialic acid sequences, THP was shown to bind directly to type 1 fimbriated E. coli, and was therefore initially postulated to alleviate bacterial burden by sequestering bacteria within the urine for voiding.[197,198] This hypothesis was later discarded when the role of THP as a potent activator of innate and adaptive immune responses was revealed.[199,200] Examples include (1) the cell-specific stimulation of granulocyte toward IL-8 production,[199,201] (2) the upregulation of costimulatory molecules and MHC expression on dendritic cells (DCs), (3) cytokine production, and (4) DC maturation via TLR-4 signaling. So potent is the effect of THP that overstimulation of the immune system can lead to interstitial nephritis.[199,200]

Other important antimicrobial peptides in UTIs are the β-defensins and α-defensins, which are secreted from the local renal epithelium and the infiltrating neutrophils, respectively[200,201] Defensins possess a two-pronged effect, showing direct antimicrobial activity on invading bacteria and indirectly via the enhancement of the innate and acquired immune response. In the latter, defensin-induced secondary signaling arising from target cells and tissues have been implicated in acute inflammation regulation, immune cell recruitment, angiogenesis, and wound healing.[200] Examples include mast cell degranulation, the promotion of neutrophil chemotaxis, and naive T-cell and immature dendritic recruitment.[200]

Cathelicidins, such as LL-37, are antimicrobial peptides with direct bactericidal action. LL-37 is produced by neutrophils, myeloid bone marrow cells, epithelial cells of the skin, the gastrointestinal tract, the epididymis, and the lungs.[157,202] In the urinary tract, the peptide is produced from uroepithelial and tubular renal cells with a subsequent release into the lumen.[203] Given the presence of high cathelicidin-related antimicrobial peptides in the early stages of an infection well before leukocyte infiltration, a two-stage process was proposed in which the main source of the peptide shifts from the epithelium to leukocytes as the infection progresses.[203,204] Interestingly, cathelicidins exhibit a greater effect on pathogenic bacteria that cause UTIs as compared to urogenital commensal bacteria.[205] UPEC strains associated with severe UTIs also tend to have higher resistance to the peptide.[206]

Lactoferrin and lipocalin restrict the bacterial availability of essential iron. Lactoferrin is present in the luminal surface of distal collecting tubules where it exhibits its bactericidal activity indirectly by the chelation of iron, and directly by disrupting membrane integrity.[203] Lipocalin, on the other hand, limits iron availability by targeting bacterially expressed siderophores.[207,208]

Genetic Variability in Hosts

Genetic profiles have been observed that influence UTI susceptibility in patients. The genetic variation falls broadly into two groups: (1) factors involving bacterial colonization and (2) components of the host response. Variation in glycolipid receptor expression, which varies with the P blood group, has been shown to be associated with susceptibility to UTIs. Patients prone to UTIs tend to be of the blood group P1 and possess a high density of cell surface receptors for P fimbriae.[209] At present, there is insufficient statistical evidence to substantiate the inverse hypothesis (i.e., that individuals lacking receptors would be resistant to P-fimbriated E. coli).[210] However, treatment with N-butyl-deoxynojirimycin to mimic the previous receptor deficient state showed a protective effect on mice against colonization and inflammation.[211]

Two host factors, TLR4 and CXCR1 (the CXC chemokine receptor for IL-8), have been in focus when linking genetic variation to UTI susceptibility. A deficiency in mouse TLR4 signaling results in an asymptomatic carrier state that can persist for large proportions of the subject's lifespan before the onset of mortality.[33,212] This sequence of events closely resembles untreated ABU patients. CXCR1 is the receptor for the cytokine IL-8, which induces migration and the activation of neutrophils. CXCR1 has been suggested to function in protecting the host against severe infection.[137,213] Subjects with a deficient CXCR1 expression present with symptoms typical of acute pyelonephritis and renal scarring, as both the host's innate defenses and neutrophil migration are disrupted.[143] Children with lower CXCR1 expression and protein levels arising from a single nucleotide polymorphism are prone to pyelonephritis.[140,213] Furthermore, mice unable to express this IL-8 receptor have a higher titer of bacteria, a more rapid progression to bacteriuria, and renal scarring during an infection.

Intracellular Bacterial Reservoirs for Persistent Colonization

Recurrent infections are generally associated with repeated infections by the same bacterial strain. To persistently colonize the host UPEC have been proposed to enter into cells, thus generating a refuge by protecting bacteria from host defense systems. However, the significance of such intracellular reservoirs (IR) in UPEC persistence is not ubiquitous across the urinary tract.

Within the bladder, IRs appear to confer great advantage to UPEC survival. IRs are established when UPEC interacts with integral membrane proteins via FimH-mediated binding to uroplakin, which in turn triggers host cell signaling cascades that results in bacterial internalization.[131,190] The luminal surface of the mammalian bladder is lined by thick cell layers of pseudostratified transitional epithelia. Several studies have observed an intracellular transition of bacteria 12 hours postinfection,[139,141,154,214] after which IRs are established as bacteria rapidly multiply. Once an IR has become established in one cell, neighboring cells are invaded as UPEC progenies exit the host cell and invade other surrounding cells.[215,216] Although an exfoliation of superficial facet cells occurs, it does not appear to significantly deter UPEC persistence. While substantial IRs are removed with sloughed cells, the inadvertent exposure of the underlying cell layer allows UPEC to establish new IRs.

In the kidney the situation is different. Here, sloughing of the proximal tubule epithelium arising from ensued ischemia appears to negate the survival advantage of bacterial internalization.[217] During early periods of infection, ischemia-induced actin rearrangement and the associated relocalization of membrane-bound intergrins breaks the epithelial barrier function,[154,214] and detachment of epithelial cells from the tubular basement membrane occurs.[218] Loss of the epithelial barrier, however, did not compromise the host. The naked tubular basement membrane hinders the immediate bacterial dissemination into the interstitium.[139,154] while giving time for host responses to occur, such as the cessation of blood flow and PMN recruitment. Moreover, loss of the tubular integrity does not appear to solely benefit the invading pathogen because paracellular movement of UPEC enhances the neutrophil's accessibility to the tubular lumen.

The formation of IRs within the bladder is a highly effective strategy for UPEC persistence in which IRs or bacteria have been observed months after the initial infection, albeit with antibiotic treatment.[215–217] Yet, because of distinct histologic differences of the kidney from the bladder, IR establishment does not provide UPEC the same survival advantage within the kidney.[102,107,154,219]

Acute Kidney Damage

Infection and subsequent injury to the kidney causes extensive damage, which may affect kidney function and which may lead to kidney failure. Over the years, a number of proposed mechanisms for this injury have been suggested. A direct damaging effect of infecting bacteria is possible for those UPEC strains that express tissue-damaging toxins.[176,178,220,221] The strong inflammatory response to pyelonephritis has also implicated a role for collateral damage. Suppression of the immune response in experimental models has shown reduced kidney scarring despite a high bacterial load.[222,223] It has also been shown that neutrophils isolated from acute pyelonephritis can kill syngeneic kidney cells in vitro,[154] and neutrophil-mediated oxidative injury of kidney cells has been confirmed in vivo.[154]

Though physiologic alterations that accompany ischemia–reperfusion injury previously have been implicated in renal scarring,[224] recent data show renal infections do in fact cause ischemia.[154] Ischemia is a restriction in blood supply to a tissue or an organ that is closely associated with tissue oxygen delivery and tension (pO_2). Each kidney nephron has an intertwined peritubular vasculature. During the first hours (4 to 5 hours) of a UPEC infection in the proximal convoluted tubule, epithelia-endothelia signaling initiates the clotting cascade in peritubular capillaries (Fig. 21.2). This clotting leads to localized ischemia that manifests prior to the major infiltration of immune cells. Tissue pO_2 drops significantly, reaching 0 mm Hg within 3 to 4 hours.[154] This infection-mediated ischemia was demonstrated to act as an innate defense mechanism, preventing the systemic dissemination of the pathogen because anticoagulant therapy to prevent this response led to fatal sepsis within a few hours.[154] Although a massive engagement of neutrophils cleared the bacteria (Fig. 21.1E,F), no indications of reperfusion or repair were seen within the first 24 hours. Renal scarring thus appeared to be the end result of infection.

Another physiologic injury that is known to cause tissue damage in its own right is kidney obstruction. Real-time monitoring of renal filtrate flow in a live animal model of pyelonephritis showed how bacterial colonization of the renal tubules affects the flow of filtrate, with complete stoppage occurring within 8 hours. Kidney ischemia and obstruction are both well-studied physiologic injuries and both can cause inflammation and tissue destruction in their own right.[154,155,216,225–232] Both are multifactorial and can vary in severity. Severe ischemia or obstruction can lead to end-stage kidney failure, as can pyelonephritis. Thus, the emerging view is that the pathophysiology of pyelonephritis is in fact a combination of infection and physiologic injuries.[154]

Treatment

A UTI is most commonly treated by antibiotics. Wagenlehner et al.[233] present a comprehensive statistic of clinically prescribed antibiotics and their respective efficacies. The group describes UTIs as broadly divided into uncomplicated and complicated cases, against which treatment is tailored.[233,234]

The following paragraph is adapted from Wagenlehner et al.[233]

> Uncomplicated UTI denotes UTI without relevant structural and functional abnormalities arising from the urinary tract (uropathies), without relevant kidney diseases (nephropathies) and without relevant comorbidities. Conversely, complicated UTI is a complex condition of the following conditions: (1) Anatomical, structural or functional alterations of the urinary tract. (2) Impaired renal function by parenchymal and renal nephropathies. (3) Accompanying diseases or conditions that impair the patients' immune status.

In the treatment of uncomplicated UTIs, the choice of prescription is made based on five considerations[233]: (1) the individual risk to antibiotic treatment; (2) the bactericidal spectrum of the antibiotic and the known susceptibility of the bacterium to the antibiotic; (3) the clinical data of the effectiveness of the antibiotic; (4) the effect of the antibiotic on commensal microbial flora; and (5) side effects.

Complex UTIs require a two-pronged treatment strategy directed at the treatment of complicating factors and the invading pathogen.[234] In complex UTIs, pathogens can be a heterogeneous population of gram-positive and gram-negative strains with a wide range of antibiotic susceptibility and resistance.[233] Very often, the devised treatment must account for the possibility of the resistance development and cross-resistance among antibiotics of the same family.[233]

In general, the treatments of uncomplicated and complicated UTIs share two fundamental aims, namely, to use rapid acting therapy with high efficacy for recurrent infections within a patient, and to prevent the generation of pathogens resistant to the treatment.[233] When treatment is delayed or ineffective, uncomplicated UTIs can progress to sepsis and severe sepsis, requiring specific sepsis therapy. At this point, treatment of urosepsis becomes a combination of (1) eradicating the pathogen, (2) resolving the cause of the infection and the complications (e.g., obstruction), (3) providing life-supportive care, and (4) providing appropriate antimicrobial therapy.[233]

Aside from clinical treatments, folk remedies are commonly used in the treatment of UTIs. Although there are a variety in existence, cranberry juice is by far one of the most common. Cranberry juice and tablets have been used extensively as a remedy for infections of the urinary tract. Originally thought to be due to the bactericidal acidification of the urine by hippuric acid, recent studies have dispelled this mechanism of action. Cranberries contain proanthocyanidins, which inhibit P fimbriae–mediated bacterial adherence,[235,236] and fructose, which inhibits type 1–mediated adherence.[237] Despite the apparent positive effect of cranberries, no definitive findings have been determined in clinical trials.

CONCLUSION

Data presented in this chapter reveal that our current knowledge of UPEC and host–pathogen interactions in UTIs primarily originate from molecularly well-defined in vitro systems. The simplicity of such systems zooms in on specific reactions, controlling for and filtering out the myriad of relevant but confounding interactions that occur simultaneously in the host during an infection. Applying this wealth of information as a foundation and coupled with advanced imaging platforms for real-time studies in the live animal, researchers can now advance from "cellular microbiology" to "tissue microbiology," in their attempt to generate an integrated view on host–pathogen interactions. Such coherent models will help to not only identify both the cross-talk between host and pathogen, but also the dynamic changes occurring in the immediate environment in response to an infection. Murine intravital models of pyelonephritis have shown these changes include tissue oxygen tension and the cessation of blood flow to the infected site. These are but a few of the many factors that need to be tracked during an infection. Across the globe, interdisciplinary research coupling all fields of science is blooming and working to create new technologies. And as our understanding of host–pathogen interactions advances, so will clinical diagnostics, treatment, and patient care.

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CHAPTER

22 Cystitis and Urethritis

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Bacteriuria, which is bacteria in the urine, is often (but not necessarily) a sign of infection. Therefore, it is important to put the results from bacterial urine culture into the context of the patient, including clinical symptoms, results of other laboratory tests, as well as methodology used.

Urine samples can be obtained in different ways. In adults, the most common sampling is clean-catch voided urine. In infants up to 1 year of age, however, suprapubic bladder aspiration is recommended, whereas for toddlers it is more common to collect with a urine collecting bag. From patients with an indwelling urinary catheter the urine sample has to be obtained through the catheter. These differences in modes of collection of urine samples inevitably result in differences in the type and number of bacteria isolated.

DEFINITIONS

Urinary tract infections (UTIs) in general can be symptomatic or asymptomatic. Symptomatic UTI can be divided into infections restricted to the lower urinary tract (bladder and urethra) to the upper urinary tract (kidney) or infections with systemic involvement, which is urosepticemia. The focus of this chapter is on infections of the lower urinary tract.

Symptomatic Urinary Tract Infection

Acute Cystitis and Urethritis

Cystitis is defined as an inflammation of the urinary bladder. Urethritis is an inflammation of the urethra. Both are most commonly caused by a bacterial infection; in which case, they are also referred to as lower UTIs.

Classic symptoms of lower UTIs are dysuria, urinary frequency, and suprapubic pain sometimes in combination with hematuria, but normally without fever. The extent of symptoms varies between different patients and can be very mild to severe. Other diseases can mimic lower urinary tract bacterial infections like vaginitis, interstitial cystitis, and pelvic inflammatory disease.

Asymptomatic Bacteriuria

Asymptomatic bacteriuria (ABU) refers to bacteriuria in patients with no clinical UTI symptoms. For women $\geq 10^5$ colony forming units (CFU) per mL in two consecutive clean-catch urine samples is required for the diagnosis of asymptomatic bacteriuria, whereas for men only one clean-catch urine sample with $\geq 10^5$ CFU per mL is required—or a single catheterized urine specimen with one single bacterial strain of $\geq 10^2$ CFU per mL in women or men.[1]

CLASSIFICATION

Acute cystitis and urethritis can be classified as uncomplicated verus complicated UTI, nosocomial versus community-acquired UTI, and sporadic versus recurrent UTI.

Uncomplicated UTI occurs in persons with normal urinary tract, whereas complicated UTI occurs in individuals with functional or structural changes, implying deteriorated voiding predisposing for bacteriuria.

Nosocomial UTI are infections that occur 48 hours or more after admission to the hospital or as a result of health care, whereas community-acquired UTI are UTIs not included in the previous group.

Sporadic UTI include a single UTI treated with antibiotics during 6 months or maximum two UTIs needing antibiotics during 1 year, whereas recurrent UTI comprise at least two antibiotic treated UTIs during 6 months or three or more antibiotic treated UTI during 1 year.

Recurrent UTI can be further divided into relapse or reinfection. Relapse infection includes a recurrent infection with the same bacteria as the previous UTI, whereas a reinfection is caused by different bacteria than in the previous infection. Superinfection is a new infection during antibiotic treatment and where the new bacterial strain is resistant to the used antibiotic.

ETIOLOGY

By far the most common bacterial uropathogen is Escherichia coli, causing more than 80% of UTIs among female ambulatory patients. In men and hospitalized patients, E. coli is

still the most commonly isolated bacteria, but with a lower frequency. Other common uropathogenic bacteria include Klebsiella pneumoniae, Proteus mirabilis, enterococci, Streptococcus agalactiae, and Staphylococcus saprophyticus.[2,3] S. saprophyticus is the only urinary pathogen with a seasonal variation, being most common during the late summer and early autumn months.[4]

In patients with long-term indwelling catheters, bacteriuria is almost inevitably found after about 14 days. Initially, a single species of bacteria is found but later a polymicrobial flora is common, with a wide variety of infecting microorganisms found. In patients with long-term catheters Proteus mirabilis, Providencia stuartii, Morganella morganii, Klebsiella pneumoniae, E. coli, and Pseudomonas aeruginosa are most commonly isolated. In patients with short-term catheters, staphylococci are also common. Although staphylococci seldom cause symptomatic UTIs, they contribute to bacterial biofilm formation.[5,6]

Unusual causes of urethritis and cystitis include urethritis caused by Neisseria gonorrhoeae and Chlamydia trachomatis. They have a distinct pathogenesis and symptoms that can be similar to acute cystitis. They are usually described among sexually transmitted diseases. Adenoviruses (mainly type 11) cause epidemic hemorrhagic cystitis in children, especially in boys, but may cause endemic cystitis as well.[7] Other infectious causes, which are much less common, include Herpes simplex virus; atypical bacteria, like Mycoplasma genitalium and Ureaplasma urealyticum; Mycobacteria; fungi and parasites; Trichomonas spp.; and Schistosoma haematobium. There are also noninfectious forms of cystitis and urethritis—for example, traumatic cystitis, interstitial cystitis, eosinophilic cystitis, and hemorrhagic cystitis—which are described elsewhere.

EPIDEMIOLOGY

UTIs are among the most common bacterial infections.[3,8] Prevalence is different depending on the age and gender of the patient. The overall risk in childhood before puberty is 3% to 5% in girls and 1% to 2% in boys.[9,10] In the neonatal period, the incidence of bacteriuria is about 1%, and this is also the only period when UTI is more prevalent in males than females.[11] After 1 year of age the incidence of UTIs reaches 2% in boys and 8% in girls.[12] During the reproductive period, the gender difference becomes even more pronounced and UTIs are some 50-fold more common in women as compared to men. Approximately 20% of women between 24 and 64 years old have at least one episode of dysuria each year, most of these being caused by bacterial infections.[13] Almost half of all women will experience at least one episode of UTI during their lifetime[8] and about 25% of these have recurrent infections. In the United States alone, UTIs account for approximately 11 million office visits and 1.7 million emergency room visits each year, resulting in almost half a million hospitalizations at a cost of $3.5 billion.[8,14,15] This figure probably underestimates the true incidence because many lower tract UTIs resolve without medical attention.

ROUTE OF INFECTION

UTIs are most commonly ascending infections caused by bacteria mainly from the intestinal tract or vagina. Hematogenous spread of infections to the urinary tract is rare and restricted to a few pathogens, such as S. aureus and Mycobacterium tuberculosis, which cause primary infections elsewhere in the body. Lymphatic spread from sites of infection elsewhere, bacterial spread through a fistula from the bowel, and retrograde infection from the prostate or kidney are very unusual but may also occur.

The perineum is a couple of square centimeters area of skin between the anal and urogenital regions. This is also a place that is highly colonized by bacteria from the gastrointestinal tract and vagina, and from where the bacteria may reach the urinary tract.[16] In the majority of cases, the bacteria are cleared off from the bladder. Sometimes, they persist and colonize the urinary tract causing asymptomatic bacteriuria or even symptomatic UTI.[16] The short female urethra is an insufficient anatomic barrier to the entry of bacteria, which may be massaged easily into the bladder. This may explain the association of UTIs and bacteriuria with sexual activity.[17] Bacteria may also be introduced into the bladder during catheterization of the urethra. Single catheterization of the bladder in ambulatory patients results in a 1% incidence of subsequent UTI.[18] Finally, voiding dysfunction in children is also clearly associated with recurrent UTI[19] and complex treatment of the dysfunction resulted in a substantial decrease of the frequency of UTI attacks.[20] All these observations suggest that ascendance of bacteria from perineum to the urethra and urinary bladder is the most common route of UTI.

PATHOGENESIS

Bacterial Factors in the Pathogenesis of Urinary Tract Infection

How do harmless commensal bacteria from the perineum become urinary pathogens? It is believed that some bacterial clones from the gut can acquire specific virulence characteristics, which increase their ability to adapt to new niches. These virulence and fitness properties are frequently encoded in specific genetic elements called pathogenicity islands. Virulence factors are here defined as proteins or macromolecular structures that contribute to causing disease, whereas fitness factors offer a competitive advantage during infection, but are not required for virulence. A combination of virulence and fitness factors form a specific type that could be called uropathogenic bacterium. However, despite many common features, there is no single profile that would cause UTI.[21,22] Uropathogenic bacteria use a multistep scheme of pathogenesis that consists of adhesion, colonization, invasion, survival, and host damage.[23] Bacterial factors can accordingly be described as adhesion and colonization factors, survival and immune escape factors, and toxins (for details, see Chapter 21).

Host Factors in the Pathogenesis of Urinary Tract Infection

The urinary tract is located in a fragile region in close proximity to the outside environment. Around 2 L of urine a day, produced by the kidneys, are emptied close to the rectum, the area highly colonized by bacteria. Still, the urinary tract is very resistant against infection. It was observed by urologists more than 100 years ago that, despite extensive instrumentation (e.g., frequent catheterization), and even without aseptic precautions, some individuals never or very seldom developed UTI.[24,25] This observation has later been confirmed experimentally, showing that bacteria introduced to the urinary bladders of healthy volunteers were rapidly eliminated.[26] In accordance, only very high concentrations of bacteria, or a combination of bacteria and an irritant substance such as paraffin or turpentine, were needed to establish UTI in experimental animals.[27,28]

In order to successfully colonize the urinary tract and cause infection, bacteria must overpower the specific anatomic organization of the urinary tract as well as chemical defense components of urine and the urinary tract mucosa. Moreover, recognition of uropathogens by human urinary epithelium leads to a strong inflammatory response.

Anatomic Properties of the Urinary Tract

The draining system of the urinary tract is covered with urothelium, a firm carpet of epithelial cells. Urothelium is transitional epithelium consisting of three to seven layers of cells: the basal layer of stem cells, one or more intermediate layer(s), and the superficial layer, usually referred to as umbrella or facet cells. Normal human bladder urothelium is arranged in an increasing complexity from base to surface. Urothelial cells of all layers are connected by interdigitations of cytoplasmic processes and by desmosomes. Adjacent surface cells, in addition, are linked by tight junctions. This organization enables the urothelium to withstand frequent changes in bladder volume with changes in pressure on the bladder wall.[29]

The urothelium is exposed to large changes in hydrostatic pressure with the surface superficial cells and is in contact with urine varying in pH, osmolality, and containing a number of cytotoxic substances. Therefore, cell membranes of umbrella cells need a unique lipid and protein composition that contributes to the low permeability of the membrane and that controls the passage of water, ions, solutes, and large macromolecules across the mucosal surface of the cell into the underlying tissue. The apical surface of umbrella cells is folded and contains specialized uroplakin membrane domains, which undergo active reorganization.[30,31] During the initial phase of bladder filling, the apical membrane unfolds. In the latter phase of filling, the cytoplasmic vesicles are inserted to the apical membrane to accommodate the increasing bladder volume. Emptying of the bladder is then accompanied by endocytosis of the cytoplasmic vesicles and folding of the apical membrane.[32,33] In addition to the highly specialized urothelium, a mucous layer on the surface of the urinary bladder has been described.[34,35] This layer seems to be very thin, and substantially differs from the mucus in the gastrointestinal tract. It consists mainly of glycosaminoglycans and is most likely membrane-bound rather than secreted.[36]

Urine flow, regular bladder emptying, and the valve mechanisms of the urinary tract have traditionally been considered the most important protective mechanisms maintaining this area free of microbes. Accordingly, studies in patients with anomalies of the urinary tract indicate their importance in the protection against bacteria.[37] Functional abnormalities of the lower urinary tract directly influence the entry of uropathogens into the urinary tract and may lead to recurrent UTIs, mainly in children.[19,20] For premenopausal women a new sexual partner, increased frequency of intercourse, and use of spermicides are recognized as risk factors.[38] In postmenopausal women the loss of estrogen results in change of the vaginal flora, with decreased growth of lactobacilli, as well as thinning of the vaginal epithelium and decreased amounts of glycogen which contribute to the risk of recurrent UTIs.

Mucosal Antimicrobial Mechanisms

Although regular urine flow and valve mechanisms of the urinary tract protect the urinary tract against the excessive growth of bacteria, they are not enough to completely eliminate pathogens. This has been demonstrated using an in vitro model of the urinary bladder[26] as well as mathematical simulation.[39] In accordance, the mucosa of the urinary bladder has been shown to possess antimicrobial properties in vitro.[40] Only a combination of mechanical and chemical antimicrobial factors may explain the high efficiency of the urinary tract in eliminating bacteria. Although chemical antimicrobial mechanisms of the urinary tract mucosa have so far not been systematically analyzed, a number of molecules inhibiting the growth of bacteria in the urinary tract or killing bacteria have been identified: Tamm-Horsfall protein, secretory IgA, antimicrobial proteins, and peptides, namely lactoferrin, β-defensin 1 and 2, and cathelicidin.

Recognition of the Presence of Bacteria

The first cell layer in contact with invading bacteria is the urothelium. The presence of uropathogenic bacterium induces a robust immune response already after a short contact with urothelial cells. After sensing the presence of uropathogenic bacteria, epithelial cells react in different ways. They produce substances toxic to bacteria, like nitric oxide, cathelicidin, and β-defensin-2.[41–43] Exfoliation of superficial umbrella cells is also an important protective mechanism, which helps clearing bacteria from the bladder.[44] Despite the effective first line of epithelial defence, uropathogens may sometimes persist or even multiply and invade the host. Therefore, the epithelium possesses efficient tools in order to engage the help of professional

immune system cells. Epithelial cells in the urinary tract and kidney, in response to pathogens, produce a number of chemokines and proinflammatory cytokines.[45] Chemokines attract professional immune system cells, and cytokines activate them. Out of a number of chemokines, interleukin 8 seems to be of crucial importance because of its chemoattraction of neutrophils.[46,47] Cytokine-mediated upregulation of adhesion molecules and cytokine receptors facilitates the process of migration of immune cells. Amongst them, the CXCR1 receptor on renal epithelial cells has been shown to facilitate transepithelial migration of neutrophil granulocytes and bacterial clearance during UTI.[48] Neutrophils accumulate in the inflamed tissue and kill bacteria by different mechanisms: either phagocytosis or the release of the toxic content of their granules.[49] The influx of neutrophils is followed by an influx of other professional immune cells, namely monocytes/macrophages and lymphocytes, which are predominantly important in later stages of infection.

SYMPTOMS

The symptoms of a UTI substantially differ depending on age and type of infection. The symptoms in infants and young children are very nonspecific and UTIs are usually diagnosed first in the stage of upper urinary tract involvement, or septicemia. The signs may involve tachypnea, dyspnea, as well as icterus in neonates and poor feeding, fever, and vomiting in infancy. Therefore, UTIs must always be excluded in unwell children or children with unexplained fever. After infancy, the classic symptoms of lower UTIs—dysuria, urgency, and frequency—are more usual. Adults with urethritis and cystitis typically have frequent and urgent voiding of small volumes of urine and dysuria and nocturia is common. Sensation of lower abdominal discomfort also is a frequent symptom. The urine may be turbid or even bloody in one third of cases.[50] Some infections may progress after 1 or 2 days to develop a clinical picture of upper UTI, including flank or abdominal pain, fever, and vomiting, but acute cystitis very seldom progresses to cause septicemia. On the contrary, the infection may resolve spontaneously even without antimicrobial therapy. Still it cannot be justified to withhold antimicrobial therapy.[51,52]

Studies localizing bacteria by laboratory or imaging techniques have demonstrated a poor correlation between clinical manifestations and localization results. Moreover, UTIs often are asymptomatic in the elderly[53]; and other diseases may also manifest by frequency, urgency, nocturia, and incontinence in this age group. Likewise, patients with neurogenic bladders, a long-term indwelling catheter, or intermittent catheterization usually have unspecific or no symptoms referable to the bladder when a UTI develops.[5] Therefore, there should be a low threshold for microbiologic examination of the urine in these patient groups.

DIAGNOSIS

Examination of urine specimens for bacteriuria and leukocyturia are the primary laboratory investigations in suspected UTI.

Diagnostic Pitfalls

Diagnosis of cystitis and urethritis, as well as other types of UTIs, relies on the detection of bacteria and leucocytes in urine. There are two major pitfalls in the bacteriuria assessment: contamination of urine and confusion with asymptomatic bacteriuria. These problems are more obvious in certain groups of patients—namely children, the elderly, and patients with indwelling catheters—because of nonspecificity of symptoms and difficulty of collection of clean urine samples.

Studies comparing clean-voided urine samples with samples obtained by suprapubic bladder puncture showed contamination rate of 25%.[54] In infants where alternative urine sampling, mainly urine bags and catheterization, are common, the situation is even more difficult. Contamination rates reach 63% in bag specimens and 9% in catheter specimens.[55] Clean-catch sampling of infants is time- and skill-demanding and prepuce flushing in boys may lead to paraphimosis. Therefore, bladder puncture is often the recommended method to collect urine samples in infants younger than 1 year of age when the correct diagnosis is essential. In older children and adults, urine samples should if possible be cultured from clean-catch, midstream urine to avoid contamination.

Another problem is confusion of a true UTI with asymptomatic bacteriuria. Asymptomatic bacteriuria is a common condition[16,56] and, in the majority of cases, no treatment is recommended because antibiotic treatment may increase the risk for development of symptomatic infection.[57] However, in an infant presenting with high fever and bacteriuria, the differential diagnosis between pyelonephritis and asymptomatic bacteriuria with another systemic infection (e.g., adenovirus infection) is very difficult.

Similarly, in the elderly, diseases that are not related to the urinary tract may also manifest by frequency, urgency, nocturia, and incontinence,[53] therefore making the differential diagnosis between lower UTI and asymptomatic bacteriuria troublesome.

The present cut-off levels of significant bacteriuria were defined in a series of classic studies in the 1950s by Ed Kass.[58,59] As in many biologic systems, the numbers of bacteria in urine of patients with UTIs are a continuum and any cut-off is accompanied with a given sensitivity and specificity. The number of bacteria in urine may be influenced by incubation time in the urinary bladder as well as the doubling time of the bacteria. Therefore, a small bladder (e.g., low age), low incubation time (e.g., frequency), and slowly growing bacteria may negatively influence the bacterial numbers in urine specimens. Kass suggested that a threshold of $\geq 10^5$ bacteria per mL of urine reliably distinguished contaminated specimens from true bacteriuria in asymptomatic women, and accurately diagnosed women with acute pyelonephritis. Many clinicians subsequently adopted this single criterion to diagnose cystitis, although Kass had not, in fact, studied women

with lower tract symptoms.[50] Later, it has become apparent that cystitis with significant bacteriuria and cystitis with lower bacterial counts have a similar pathogenesis and may represent different stages of the same disease.[60,61] Approximately 40% of women who experience symptoms of cystitis have midstream urine cultures containing less than 10^5 bacteria per mL.[50,62] Similarly, a pediatric study of 366 infants found that 20% of children with proven symptomatic UTI had less than 10^5 bacteria per mL urine from both suprapubic aspiration and bag specimen.[63] Handling of the urine sample may also significantly influence the result of urine cultures. Only a few hours of room temperature incubation can result in significant multiplication of bacteria and false-positive results.

Nowadays, bacterial counts of 10^4 or sometimes even 10^3 CFU per mL, depending of the infecting microorganism, are regarded as significant in patients with clinical UTI symptoms. Therefore, the number of bacteria in urine must be interpreted in a complex view together with other laboratory tests and in the whole clinical context.

Another laboratory sign of UTI is leukocyturia or pyuria.[64] The finding of pyuria is unfortunately not specific for urethritis and cystitis. Both systemic inflammation and asymptomatic bacteriuria may be accompanied by the presence of leukocytes in urine. Despite its limitations, pyuria together with other tests serves as one of the indications of infection of the urinary tract.[65]

Urine Culture

Conventional microbiologic quantification of bacteriuria is performed by inoculating a predefined urine volume, mostly 1 or 10 μl, onto appropriate agar plates, incubating at 37°C overnight, then identifying the bacterial species and estimating the number of bacterial colonies.[66] Susceptibility testing can be performed either directly from the urine or by subculturing from bacterial colonies. Bacterial species and recommendation of appropriate antibiotic usage can therefore be presented within 24 hours. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a novel technique for rapid identification of bacterial pathogens, possibly also directly from infected urine. Although it gives high accuracy, conventional culture is still needed for bacterial susceptibility testing.[66a]

Another way of monitoring bacterial growth, especially in the outpatient clinical settings, is the dip slide test. The dip slide consists of a plastic paddle with culture agar on each side that is immersed in urine and then incubated at 37°C overnight. The method allows semiquantitative measurements; however, the accuracy is limited, mainly because of the relatively high inoculums and the small agar surfaces. There are several drawbacks with the method, one being the absence of antibiotic susceptibility testing. Another limitation is the risk of over-interpretation of mixed bacterial cultures, especially gram-negative bacteria and, conversely, the risk of not being able to see and evaluate weak bacterial growth like that often seen for group B streptococci.[67,68]

Microscopic Examination of Urine

Urine can be examined microscopically for the presence of bacteria and leukocytes. Microscopic examination is simple and faster than urine culture. The Gram staining is useful both for analysis of bacterial type, gram-negatives or -positives, morphology, rod or cocci, as well as for quantitative analysis. The presence of one or more bacteria per oil immersion field in uncentrifuged urine correlates with $\geq 10^5$ bacteria per mL on culture with a sensitivity and specificity over 90%.[69] However, this method does not detect low count infections, nor does it give possibility for species identification or antibiotic resistance.

More than 95% of patients with symptomatic UTIs have significant leukocyturia.[70] However, pyuria is also found in several other diseases not related to UTI. Because examination of centrifuged urine sediment is not reproducible, quantitative analysis of a fresh, uncentrifuged specimen of urine is recommended.[64] The most common method is counting under the microscope using a hemocytometer chamber. A count of 10 or more leukocytes per mL is considered abnormal. Most women with symptomatic lower UTI have more than 60 leukocytes per mL.[71] As mentioned before, leukocyturia must be carefully evaluated together with other symptoms and signs because it lacks specificity for symptomatic UTIs. In addition, the accuracy of microscopic evaluation of the urine for leukocytes is no better than that of rapid esterase test on a dipstick. Moreover, laboratory facilities are needed, and results are delayed. Therefore, the role of microscopy for white cells for the diagnosis of UTI was recently questioned.[72]

Rapid Diagnostic Tests

Several methods are being used for the rapid detection of bacteriuria and leukocyturia. Such methods may be very useful in a clinical setting when a fast diagnosis is essential. Optimally, rapid tests have low cost, high sensitivity, and high specificity. Therefore, they are useful as screening methods for groups at risk, such as pregnant women. Moreover, in the laboratory these tests may also help select which specimens require further microbiologic investigation. Two biochemical tests have been devised: the nitrite and leukocyte esterase tests.

The nitrite test is based on the bacteria's ability to reduce nitrate to nitrite. It is rapid to perform and evaluate and easy to interpret.[72,73] A test strip is immersed in urine and a color change is observed within 2 minutes, if positive. The test has high specificity, but low sensitivity, which implies that positive results indicate prevalence of bacteria, whereas negative results do not rule out bacteriuria because high bacterial concentrations are needed. Likewise, some common uropathogenic bacteria will not be positive in the test. S. saprophyticus and enterococci do not reduce nitrate to nitrite and Pseudomonas reduce nitrite further to ammonia and nitrogen—therefore, none of these bacteria will be positive. False-positive results can be obtained after having

eaten phenazopyridine, whereas high levels of vitamin C can give false-negative test results. The Leukocyte esterase test is a simple and rapid test for leukocytes.[74] It has high specificity and sensitivity and gives similar results as microscopy of urine sediment. When granulocytes are available, the test strip rapidly turns purple with intensity corresponding to the leukocyte concentration. It is important to remember that negative results in either or both tests do not exclude bacteriuria and that especially a positive leukocyte esterase test can be due to reasons other than bacterial infection.

TREATMENT

Many countries have guidelines for the treatment of cystitis and asymptomatic bacteriuria. These guidelines take into consideration the local bacterial susceptibility pattern in the respective countries and may therefore vary between different areas.

Asymptomatic Bacteriuria in Adults

Only few patient groups run an increased risk for adverse outcomes due to asymptomatic bacteriuria, mainly pregnant women and patients undergoing urologic intervention. Pregnant women have an increased risk of developing acute pyelonephritis in the early phase of pregnancy and are also likely to experience premature delivery.[75,76] Therefore, screening during the early stage is recommended and pregnant women should be treated with antibiotics if urine culture shows significant bacterial growth.[1]

In the majority of patients who undergo urologic interventions, bacteriemia occurs, with clinical evidence of septicemia in up to 10%.[77] Therefore, in patients with urologic intervention, where mucosal bleeding is expected, antibiotic treatment should be initiated prior to such intervention.[1] Although an increased risk of symptomatic UTIs may be observed in remaining cases, screening for or treatment of asymptomatic bacteriuria is not indicated.

Cystitis in Adult Women

Uncomplicated acute sporadic cystitis in previously healthy nonpregnant women should be treated with antibiotics if the patients present themselves with typical symptoms. Recommendations differ regarding the need of verification with rapid tests or urine culture. Most countries have reached consensus that nonpregnant women with uncomplicated sporadic cystitis presenting with typical symptoms can be treated without previous urine culture or dipstick. The treatment strategy is to cover the infecting microorganism but not disturb the normal bacterial flora in the gut. The antibiotic concentrations in the urine should be high with a narrow antibacterial spectrum. If left untreated about 30% have no symptoms after about 1 week. There are currently new guidelines from the Infectious Disease Society of America (IDSA) and European Society for Microbiology and Infectious Diseases (ESCMID),[78] which will be referred to in the text. Variations may of course exist in other countries.

For women with symptoms of acute uncomplicated cystitis and absence of flank pain, fever, or other suspicions of acute pyelonephritis, and where the patient is able to take oral medication, recommended treatment is with one of the following antimicrobial agents: nitrofurantoin, trimethoprim-sulfamethoxazole, fosfomycin, or pivmecillinam. Pivmecillinam is only available in some European countries and not licensed/available in North America.[78] Trimethoprim-sulfamethoxazole is the only of these drugs that can be used if early acute pyelonephritis is suspected. The duration of treatment depends on the choice of therapy; for nitrofurantoin and pivmecillinam 5 days is proposed whereas only 3 days for trimethoprim-sulfamethoxazole. For fosfomycin a single dose is sufficient; however, lower efficacy than some other recommended agents has been observed.[78] Both nitrofurantoin and pivmecillinam are rapidly eliminated through the urine and therapeutic concentrations are only found in the urine up to 1 day after therapy is ended. Trimethoprim on the other hand can be detected in the urine up to 2 or 3 days after the medication is stopped. Patients who are treated with nitrofurantoian should be advised not to take alkalizing agents, such as potassium citrate, because the effect of higher pH correlates to higher minimal inhibitory concentration (MIC).[79] Moreover, women with renal impairment should not be treated with nitrofurantoin because effective concentration of antibiotics in the urine is not possible to reach and toxic concentrations of antibiotics can occur in the plasma.

Quinolones should be avoided for empirical treatment of cystitis and instead used for acute pyelonephritis. Moreover, the high prevalence of resistance against fluoroquinolones has been reported in some areas. Ampicillin and amoxicillin alone should be avoided because they require close follow-up and have lower efficacy than other available agents. When the resistance prevalence to trimethoprim exceeds 20%, or if used for UTI within the previous 3 months, alternative therapy should be considered.[78]

Recurrent Cystitis in Women

The main risk factors for recurrent cystitis differ between premenopausal and postmenopausal women. In premenopausal women an association with sexual intercourse, the use of spermicides, and age of first UTI has been demonstrated. In postmenopausal women vaginal prolapse, cystocele, postvoid residue, changes in vaginal flora, and urinary incontinence are the main risk factors.[80,81]

Nitrofurantoin, trimethoprim-sulfamethoxazole (avoid if resistance prevalence >20%, or if used for UTI during the last 3 months), and pivmecillinam (if in a country where it is available) can be used for treatment.[78] However, it is important to establish a correct diagnosis and, therefore, urine culture with susceptibility testing should be performed and antibiotic treatment adjusted according to the susceptibility results. Patients with hematuria or persistent bacterial growth in spite of appropriate antibiotic treatment should undergo cystoscopy and imaging of the upper urinary tract.

Prevention of Recurrent Cystitis in Adult Women

Recurrent UTI is a significant problem for the individual patient and a need for prophylaxis is often called upon. A Cochrane Review demonstrated that the use of antibiotics is beneficial to reduce the number of clinical as well as microbiologic recurrent UTIs in pre- and postmenopausal women.[82] There are several possibilities for prophylaxis. Most commonly, antibiotics can be continued daily for between 4 and 12 months. However, prophylaxis should not be initiated until 1 to 2 weeks after treatment, when urine culture is negative, ensuring bacterial eradication. Low doses of nitrofurantoin, cephalexin, trimethoprim, and trimethoprim-sulfamethoxazole have been recommended.[83] Women who experience recurrent UTIs, in association with sexual activity, can be offered postcoital prophylaxis. A major advantage with single dose therapy is that it produces fewer side effects because the women only take one third of the antibiotic otherwise used, and the outcome is still similar to daily prophylaxis.[83,84] Postmenopausal women could also benefit from vaginal estrogen, because it has been demonstrated to reduce the number of UTIs.[85,86] Cranberries might reduce the incidence of UTIs in women with recurrent infections,[87] but there is no evidence on concentrations that must be used. In studies performed the withdrawal rate has been high, suggesting that cranberry products are not acceptable over long periods. Adverse events like gastrointestinal intolerance, weight gain, and drug–cranberry interactions have been reported.[88]

Acute Cystitis in Men

Bacteriuria in men is rare and, when it occurs, it is mostly associated with predisposing factors like prostate hyperplasia, prostate cancer, or urethral stricture. At least 50% of men with recurrent UTIs and more than 90% of those with febrile UTIs have prostate involvement,[89,90] which may lead to complications like chronic bacterial prostatitis or prostate abscesses. Therefore, it is important to examine the prostate to rule out prostatitis or other concurrent pathologic changes of the prostate. Ciprofloxacin or trimethoprim-sulfamethoxazole are generally recommended as first choice. For infections caused by enterococcus, with a natural resistance to trimethoprim, amoxicillin is recommended. Due to the frequent prostate engagement treatment duration should be 2 weeks.[91]

Asymptomatic Bacteriuria in Children

Children with asymptomatic bacteriuria do not present with symptoms but bacteriuria is detected by screening. There is relatively good evidence of long-term outcomes and influence of treatment from randomized controlled trials.[92–94] The conclusion from these trials was that treatment of asymptomatic bacteriuria does not influence the emergence of symptomatic UTIs, clearance of vesicoureteric reflux, kidney growth, or the progression of kidney scars. Therefore, in contrast with previous recommendations,[50] the screening for and the treatment of asymptomatic bacteriuria in children is not currently recommended.

Cystitis and Urethritis in Children

The aim of the treatment is to effectively eradicate the infection, relieve symptoms, and minimize the development of complications after a UTI. Currently, there is consensus that symptomatic cystitis/urethritis in children should be treated with antibiotics. There are many studies addressing questions regarding type and duration of antibiotic treatment.[95–97] In summary, there is no difference in outcomes between short duration (2–4 days) and longer duration (7–14 days) of treatment, so short-duration antibiotic treatment should be used for children with lower UTIs. The evidence does not provide guidance as to which antibiotic is most useful but a choice from trimethoprim, nitrofurantoin, a first-generation cephalosporin, or amoxicillin would be supported by current clinical practice. It is recommended to follow local bacterial susceptibility patterns and recommendations in the respective countries and regions.

It is also important to emphasize that infants and children with high risk of serious illness should be referred immediately to a pediatric nephrologist. This also applies for all infants younger than 3 months with a possible UTI, who are initially treated as upper UTI with parenteral antibiotics. Urine analysis including urine culture is essential in the diagnostic algorithm in both of the above-mentioned groups. For children 3 years and older, with typical lower UTIs and positive nitrite and leukocyte esterase tests, urine culture is not necessary. Oral 3- to 5-day courses of antibiotic treatment are recommended for children 3 months and older with uncomplicated lower UTI.[98] The parents or caregivers should also be advised to bring the child for reassessment if he/she is still unwell after 24 to 48 hours. If an alternative diagnosis is not made, a urine sample should be sent for culture and antibiotic sensitivity test if that has not already been carried out. If an infant or child is receiving prophylactic antibiotic medication and develops symptomatic UTI, treatment should be with a different antibiotic. The urine culture and the antibiotic resistance pattern are then especially important.

IMAGING AND FOLLOW-UP

In contrast to upper UTIs, investigations after a single cystitis/urethritis are only recommended in infants younger than 6 months of age when distinction between lower and upper UTI may be difficult. Ultrasound examination of the kidneys and urinary tract within 6 weeks is the recommended method. Further investigation (99m-technetium dimercaptosuccinic acid [DMSA] scintigraphy, micturating cystourethrography [MCUG]) is only required in cases of abnormal ultrasound finding. In infants 6 months and older, and in children, no investigations and no follow-up are required.[98,99]

In children with recurrent lower UTIs, defined as three or more episodes of cystitis/urethritis, or one episode of pyelonephritis and one or more episodes of cystitis/urethritis, much more extensive investigation should be done in order to identify and treat the underlying risk factors. For infants younger than 6 months, ultrasound during the acute infection, DMSA scintigraphy 4 to 6 months following the acute infection, and eventually MCUG are recommended. In infants and children older than 6 months with recurrent UTIs, an ultrasound within 6 weeks and DMSA 4 to 6 months following acute infection are recommended as the initial investigation.[98] Further investigation is indicated in the case of abnormal ultrasound. A careful patient history, with focus on volume and frequency of micturition and toilet habits, is highly recommended because it may reveal functional abnormalities of the urinary tract and may lead to further examinations, uroflowmetry, or more detailed urodynamic investigation.

PREVENTION OF RECURRENCE IN CHILDREN

Recurrent UTIs are associated with considerable suffering and substantially influence quality of life. Therefore, all opportunities to prevent recurrences should be explored. All underlying anatomic and functional anomalies of the urinary tract should be treated. Moreover, clinical studies have identified the following risk factors for recurrent infections in children: age under 6 months at the first infection, family history of UTI, high-grade vesicoureteric reflux, infrequent voiding, poor fluid intake, and functional stool retention, but evidence is limited.[19,100,101] Although no prospective randomized studies have, to our knowledge, been carried out to investigate strategies to influence the risk factors in order to prevent recurrent UTIs, consensus recommendations were made.[98] According to them, children who have had a UTI should be examined with focus on dysfunctional elimination syndromes and constipation. Children should be encouraged to drink an adequate amount, and they should have ready access to clean toilets when required and should not be expected to delay voiding.

Antibiotic Prophylaxis

In many national guidelines it is currently recommended to use a low-dose antibiotic treatment after a UTI until imaging of the urinary tract has been completed. Moreover, long-term low-dose antibiotic treatment has been used as a prophylaxis of recurrent UTIs regardless of the presence of the anatomic or functional abnormality in the urinary tract. Putting together infections of the upper and lower urinary tract, prophylactic antibiotic treatment reduced bacteriuria based on a meta-analysis of eight studies including 1,103 patients.[98] There is, however, no evidence of a reduction in the incidence of symptomatic UTI based on meta-analysis of five studies including 539 patients.[98] Therefore, antibiotic prophylaxis should not be routinely recommended in infants and children following a first-time lower UTI. However, antibiotic prophylaxis may be considered in infants and children with recurrent UTIs.

CATHETER-ASSOCIATED URINARY TRACT INFECTIONS

At the time of catheter insertion, microorganisms belonging to the patient´s flora can gain access to the urinary bladder. Chronic catheterization leads almost inevitably to bacteriuria. The preferred mechanism of bladder entry is extraluminal. It is speculated that bacteria migrate within the mucopurulent space between the urethra and the catheter. However, organisms can also enter the bladder intraluminally, where the bacteria migrate into the bladder as a result of manipulation of the catheter system.[102–104] Bacteria adhere to the indwelling catheter and form biofilm. Catheter-associated UTI in patients with urethral, indwelling suprapubic, or intermittent catheterization is defined by the presence of symptoms or signs compatible with UTI combined with $\geq 10^3$ cfu/mL of ≥ 1 single bacterial species in a single catheter urine specimen or in a midstream voided urine sample from a patient where the urethral catheter, suprapubic, or condom catheter has been removed. Importantly, no other source of infection should be identified.[5] Due to the variety of infecting microorganisms, with various antibiotic sensitivity patterns, liberal attitude to urine culture including susceptibility testing is advocated. To prevent catheter-associated UTIs, guidelines from many countries recommend antimicrobial coated or impregnated urinary catheters. The most common antimicrobial compounds are silver and nitrofurazone. In a recent Cochrane Review from 2008 it was concluded that silver alloy catheters significantly decreased the incidence of asymptomatic bacteriuria during the first week of catheterization, and with less effect thereafter.[102] A similar effect was observed for nitrofurazone impregnated catheters, but only during the first week of catheterization.[102] However, the effect on bacterial adhesion and persistence has recently been questioned.[103]

When a symptomatic infection occurs, a change of catheter combined with urine culture and antibiotic therapy is appropriate. But as long as the person is asymptomatic, urine culture is not needed and routine treatment is not recommended.

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CHAPTER

23 Infections of the Upper Urinary Tract

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In the normal urinary tract, ascending upper tract infections are prevented or delayed by the dynamics of urine flow and the interference of the vesicoureteral junction. The microbial etiology of urinary tract infections (UTIs) is similar throughout the urinary system, but the infection site determines the clinical features, the response to therapy, complications, and the ultimate prognosis; therefore, it is appropriate to identify upper tract infections in this chapter as a unique and significant illness. Parasitic, fungal, and mycobacterial infections of the upper tract are discussed in other chapters. Viruses are commonly excreted in the urine, but with the exception of the syndrome of hemorrhagic fever with renal insufficiency, and parvovirus B19 and other infections in renal transplant patients, the role in renal disease is ill defined; therefore, this chapter focuses primarily on bacterial infections. An infection of the upper urinary tract is not readily diagnosed in the absence of the classic clinical features of acute pyelonephritis. As a result, much of the information available is derived from studies in which the diagnosis of renal infection was imprecise. Complicated urinary infections are reviewed in Chapter 25. Uncomplicated upper tract infections in adults is the principal focus of this review, with a discussion of renal infection in patients with complicated urinary infections where relevant.

DEFINITIONS

Acute Pyelonephritis

Acute pyelonephritis is a clinical syndrome of flank pain, renal tenderness, fever, and chills, and is accompanied by bacteriuria. It may be associated with bacteremia and can progress to the systemic inflammatory response syndrome, septic shock, and, rarely, death. Acute nonobstructive pyelonephritis, also called acute uncomplicated pyelonephritis, occurs in women with a normal genitourinary tract.[1] Pyelonephritis may also occur as one clinical presentation of a complicated urinary infection in persons with structural or functional abnormalities of the genitourinary tract.

Chronic Pyelonephritis

Chronic pyelonephritis is a radiologic diagnosis characterized by renal scarring and destructive changes in the calyceal system, presumed to be caused by bacterial infection, vesicoureteral reflux, or both. Although the classic histologic features of chronic pyelonephritis have included interstitial inflammation and fibrosis, this description lacks specificity for bacterial infection and is now recognized as common to a variety of pathologic processes.

Bacteriuria

Bacteriuria is the presence of bacteria in the urine. Kass[2] introduced the term significant bacteriuria to differentiate contaminated from infected urine using quantitative urinary bacterial counts. Significant bacteriuria is the presence of at least 10^5 colony-forming units (CFU) per milliliter or at least 10^8 CFU per liter (International System of Units). Patients with symptomatic upper tract urinary infections occasionally demonstrate lower bacterial counts in their urine.

Asymptomatic Bacteriuria

Asymptomatic bacteriuria is bacteriuria without accompanying signs or symptoms attributed to urinary infections.[3] Pyuria or a serologic response to the infecting organism often accompanies asymptomatic bacteriuria, suggesting the patient is infected and not simply colonized. In females, two voided urine samples with at least 10^5 CFU per milliliter of the same organism are required to diagnose asymptomatic bacteriuria. In men, only one urine culture is necessary. Any quantitative count of a single specimen obtained by catheterization or suprapubic aspiration in men or women is also sufficient.

Recurrent Urinary Infection

Persons who experience one episode of a UTI have a greatly increased risk for a subsequent infection. A recurrent urinary infection is considered a reinfection when a new bacterial strain is isolated in the subsequent infection and is considered a relapse if the strain isolated posttherapy is similar to the

pretherapy isolate. When a relapse occurs, the infecting strain is presumed to have persisted within the genitourinary tract.

Pyuria

Pyuria is the presence of more than 5 polymorphonuclear leukocytes per high-power field (HPF) on a microscopic examination of spun urine sediment, or the presence of more than 10 polymorphonuclear leukocytes per microliter of unspun urine.[4]

HISTORICAL PERSPECTIVES

The initial description of pyelonephritis is attributed to a 9th century Arabic physician. By the mid-19th century, the associations of pyelonephritis with pregnancy and chronic pyelonephritis with a history of recurrent urinary infection were described. In 1881, Roberts[5] noted bacteria in the urine of patients with urinary symptoms, and in 1894, Escherich[6] identified "Bacillus coli" in the urine of children with urinary infections. During the preantibiotic era, recurrent urinary infection was identified as a cause of progressive renal impairment, bilateral contracted kidneys and death from uremia in girls,[7] of long-term complications of hypertension and atrophic pyelonephritis in women,[8] and of hypertension and progression to end-stage renal disease following acute pyelonephritis of pregnancy.[9] Kass,[2] in 1956, revolutionized the study of urinary infection by introducing the quantitative assessment of bacteriuria, and also identified the importance of asymptomatic bacteriuria during pregnancy. The introduction of effective antimicrobial therapy in the middle of the last century has subsequently profoundly altered the impact and adverse outcomes associated with renal infection.

PATHOGENESIS

Bacteriology

Escherichia coli is responsible for 80% or more of 16 cases of acute nonobstructive pyelonephritis (Table 23.1). Strains isolated from renal infection belong to a restricted number of O:K:H serotypes characterized by an array of virulence factors that include toxins, such as hemolysin; iron binding proteins, such as aerobactin; and specific adhesin proteins that bind to receptors on uroepithelial cells.[17,18] These and other virulence genes are often clustered within the genome in pathogenicity islands.[17] Over 95% of strains of E. coli isolated from patients with acute nonobstructive pyelonephritis contain genes for the pap G class II allele of the P fimbria adhesin.[17] The P fimbriae family is one of the mannose resistant adhesins, with a binding specificity for the gal (α1–4) gal-β disaccharide galabiose, which mediates adherence to uroepithelial cells. Specific clonal groups of E. coli, some of which are multidrug resistant, have caused global

23.1 Distribution of Bacterial Species Isolated in Patients with Acute Uncomplicated Pyelonephritis

Number of Isolates (%)						
	Urine Culture				Blood Culture	
Organism	Safrin et al.[10]	Pinson et al.[11]	Schloes et al.[12]	Talan et al.[13]	Stamm et al.[14]	Valesco et al.[15]
Escherichia coli	140 (80.9)	63 (77)	199 (85)	136 (85)	54 (85)	133 (90.5)
Klebsiella spp.	5 (2.9)	(6)	4 (1.7)	3 (1.9)	2 (3.1)	
Citrobacter spp.	-	-		2 (1.3)		1
Enterobacter spp.	-	3 (4.0)	3 (1.3)	10 (6.3)	-	1
Proteus mirabilis	2 (1.2)	2 (2.0)	2 (1)	3 (1.9)	1 (1.6)	1
Pseudomonas spp.	-	4 (5.0)				
Staphylococcus aureus	-	1 (1.0)		1 (0.6)	-	
Staphylococcus saprophyticus	8 (4.6)	4 (5.0)	8 (3)	5 (3.1)	3 (4.7)	
Enterococcus faecalis	1	-			-	1
Other	-	6		-	4 (6.3)	

outbreaks of uncomplicated urinary infection, including pyelonephritis.[19] For instance, Prats and colleagues[20] described a uropathogenic clone (E. coli 015:K52:H1) isolated during a 1-year period in Barcelona, Spain, which was overrepresented in patients with acute pyelonephritis.

Other gram-negative rods, including Klebsiella spp., Proteus mirabilis, and Enterobacter species, are isolated in a few patients with community-acquired renal infections but are much more common in patients with complicated infections. P. mirabilis accounts for more than 40% of infections in infant boys.[21] P. mirabilis is particularly significant as a renal pathogen because of its propensity to promote struvite calculi.[18] Coagulase-negative staphylococci and Enterococcus faecalis each cause 2% to 3% of invasive renal infections. The latter is a more important pathogen in elderly men. Group B streptococci are isolated in less than 1% of urinary infections, but appear to have a propensity for patients with diabetes and pregnant women.[22] Group A streptococci, Streptococcus pneumoniae, and Neisseria spp. are rare upper tract pathogens. Some patients with Staphylococcus aureus bacteremia from other sites will have S. aureus bacteriuria, with or without associated renal infection.[23] Although Staphylococcus saprophyticus is an important cause of acute cystitis in women, its role in invasive upper tract infections is uncertain. Both Mycoplasma hominis and Ureaplasma urealyticum have infrequently been isolated as the sole pathogen in patients with classic acute pyelonephritis; increases in specific antibody titers to these agents support a role for infection.[24]

Some relatively uncommon organisms isolated include Leptospira spp., Brucella spp., and Salmonella spp. A leptospiral infection usually involves the kidneys and has pathologic changes of interstitial inflammation, hemorrhage, and tubuloepithelial damage.[25] The pathogenesis of these lesions is attributed to leptospiral proteins together with hypotension, hypovolemia, and hyperbilirubinemia. Although renal insufficiency is common, localized renal symptoms are unusual; plasma creatinine and blood urea nitrogen (BUN) usually normalize in the second week of illness. A culture of urine on special leptospiral media is usually positive during the acute illness; a polymerase chain reaction or serology are also useful diagnostic tests, if available. Brucella spp. infection may, rarely, be associated with bacterial pyelonephritis and may also present as glomerulonephritis or interstitial nephritis.[26] Salmonella spp. are also a rare cause of pyelonephritis, although renal dysfunction is reported to occur in up to 36% of infected adults, usually due to dehydration and rhabdomyolysis.[27,28]

The microbiology of pyelonephritis in patients with a complicated urinary infection, including catheter-associated hospital-acquired urinary infection, is substantially different. E. coli, usually arising from the patient's own gastrointestinal tract, remains the most common urinary pathogen, but many other species are frequently isolated, and bacteria are likely to be of increased resistance. The spectrum of infecting organisms in a patient will be influenced by exposures to the health care environment as well as current or recent antimicrobial exposure. E. coli isolated from pyelonephritis in patients with complicated urinary infection have a much lower prevalence of potential virulence factors, including the P fimbria, consistent with host rather than organism factors being the principal determinants of infection.[29–31] More resistant gram-negative rods, including Pseudomonas aeruginosa and Serratia marcescens, account for 10% to 15% of hospital-acquired invasive upper tract infections in some reports, and may occur in outbreaks.[32] Corynebacterium group D2 has been identified as a unique etiologic agent of nosocomial urinary infection, particularly in catheterized patients.[33] These organisms are urease producers and may be isolated from persistent infections including bladder and renal calculi, pyelonephritis, and bacteremia. They are slow growing and sometimes missed if routine cultures are discarded after 24 hours.

Organisms that are unable to use urine as a nutrient source only rarely cause pyelonephritis. These include most species of obligate anaerobes, for which the relatively high oxygen tension in normal urine also likely inhibits growth. In a prospective study of 5,781 urine specimens, Segura et al.[34] identified only 10 patients with positive urine Gram stains and negative aerobic cultures from which anaerobic bacteria were isolated—an overall prevalence of 1.2% of bacteriuric specimens. All but 1 of these 10 patients had complicated urologic problems. Uropathogens surviving in the kidney without cell walls, also called L forms or protoplasts, have been suggested to contribute to relapsing pyelonephritis by enabling organisms to persist and cause chronic disease in the hypertonic environment of the renal medulla.[35] However, studies have not yet confirmed a role for these bacterial forms in urinary infections.

Host Factors and Host Response

Host factors contributing to pyelonephritis and the host immune and inflammatory response are described in detail in Chapter 21. Behavioral factors associated with acute nonobstructive pyelonephritis in premenopausal women are similar to those for acute uncomplicated cystitis—most importantly sexual intercourse and spermicide use for birth control.[12] There is also a genetic predisposition, as evidenced by a twofold to sixfold increased prevalence of urinary infections in the mothers and female siblings of girls and women with recurrent urinary infections.[36] Women who are nonsecretors of the blood group substance have an increased risk of recurrent acute uncomplicated urinary infections,[37] and selected blood group antigens are associated with an increased frequency of urinary infections in girls without vesicoureteral reflux.[21] Epithelial cell receptors necessary for E. coli binding are glycolipids of the globoseries, the antigens of the P blood group system. Recent studies exploring polymorphisms of effector molecules of the innate immune response have described other potential genetic determinants. In children without vesicoureteral reflux, pyelonephritis is reported to be associated with polymorphisms and mutations of the CXCR1 receptors for interleukin (IL)-8 and GCP-2 in some,[38,39] but not all studies.[40] A single nucleotide polymorphism for IL-8 was associated with severity of pyelonephritis among

children.[40] Genetic variation in the Toll-like receptor (TLR) promoter TLR-4 may also influence susceptibility to pyelonephritis in children,[41] whereas a TLR-1 receptor polymorphism has been reported to be associated with protection from pyelonephritis in women.[42]

Individuals with structural or functional abnormalities of the urinary tract have a greatly increased risk of pyelonephritis, apparently independent of behavioral or genetic factors (Table 23.2). Obstruction at the level of the kidney or ureter may directly inhibit urine flow from the upper tract, allowing bacteria to establish infection behind the obstruction. In addition, voiding abnormalities, such as neurogenic bladders, are frequently associated with reflux, which promotes upper tract infections.

Acute pyelonephritis is characterized by an intense local and systemic inflammatory and immune response.[17] Following the stimulation of epithelial receptors within the urinary tract by bacteria or bacterial products, there is activation of the innate immune response through TLRs. The Pap G adhesin appears to be important in stimulating the epithelial cytokine response.[43] Pyuria occurs early, and urinary and serum cytokines and chemokines including IL-6, IL-8, and others are elevated.[42–45] Alterations in the expression of antimicrobial peptides may be another element of the innate response, but there is a limited evaluation to date of the role of these molecules within the urinary tract.[46] The intensity of the inflammatory response correlates with the severity of symptoms. Pyelonephritis is associated with a greater inflammatory response than a lower tract infection, and urinary cytokines have increased levels in symptomatic compared with asymptomatic UTIs. This inflammatory response resolves promptly with the institution of effective antimicrobial therapy.[47] Renal infection is also associated with both a local and a systemic humoral immune response. An immunoglobulin (Ig)M antibody predominates with the initial infection but subsequent infections are dominated by an IgG response.[17,18] The primary antibody response develops about 7 to 10 days following the initial infection. Local urine antibody includes both an IgA and an IgG response to the infecting bacteria. Whether this humoral immune response has any protective role for subsequent infection remains controversial.

23.2 Abnormalities Associated with Complicated Renal Infections

Associated Systemic Diseases
Renal transplantation
Chronic renal failure
Diabetes mellitus with complications
Neutropenia
Immunosuppression
Underlying Structural Abnormalities
Congenital abnormalities (obstruction and/or stasis)
Acquired obstruction (pelvicalyceal, ureteral, urethral)
Neurogenic bladder
Catheter associated (indwelling or intermittent)
Cystocele
Bladder diverticulae
Renal cyst
Renal calculus
Atrophic or malfunctioning kidney
Less Susceptible Pathogens
Pseudomonas aeruginosa
Proteus mirabilis
Candida albicans

PATHOLOGY

Acute Pyelonephritis

A renal biopsy is contraindicated for patients with acute pyelonephritis. When pathology specimens are available, the histologic hallmarks of acute pyelonephritis include abscess formation and edema in the renal parenchyma with an accumulation of polymorphonuclear leukocytes in and around the tubules. Bacteria are often demonstrable in the foci of acute renal suppuration. In general, glomeruli are spared, although small abscesses may surround them. Areas of infection are characteristically wedge-shaped with the apex in the medulla resembling an infarct. Although tissue destruction is greater in the cortex than in the medulla, the relative smaller size of the medulla means the inflammatory response appears to have a greater effect on medullary anatomy and function. The distribution of wedge-shaped areas of suppuration is characteristically focal, usually corresponding to renal lobes, and sharply demarcated from areas of uninvolved renal parenchyma. In adults, the kidney is unlikely to be uniformly affected unless concomitant obstruction is present.

Chronic Pyelonephritis

Chronic pyelonephritis is a focal parenchymal disease with associated changes of the renal collecting system owing to inflammation and deformation. Fibrosis with atrophy of overlying renal tissue leads to surface depression or scars. Neighboring unaffected renal tissue often undergoes hypertrophy and may appear to be a mass lesion. A sharply defined border between normal and diseased tissue is characteristic of chronic pyelonephritis. The capsule is adherent and the cortical surface is irregular. Calyceal clubbing results from a papillary retraction into the scar. Dilatation, muscular hypertrophy, and fibrotic inflammation causing a thickening of the calyceal system all occur to a variable extent. The two kidneys are usually markedly asymmetrically involved, whereas other diseases that cause interstitial inflammation usually affect both kidneys equally.

The histology of chronic pyelonephritis is the pathology of interstitial or tubulointerstitial nephritis and is characterized by a pleomorphic infiltrate of lymphocytes, plasma cells, and macrophages in the interstitium of the kidney. Polymorphonuclear leukocytes and eosinophils may also be present. The vessels in zones of the normal kidney may be normal or may demonstrate hyaline intimal changes. Histologic features of acute and chronic pyelonephritis can overlap. The relative degrees of edema and fibrosis rather than the interstitial cellular response are the most useful criteria to delineate these entities. Previously, many end-stage kidneys were referred to pathologically by the term chronic pyelonephritis. This is now recognized as being a common end stage for many renal diseases, and is seldom attributable to infection.

PREVALENCE AND INCIDENCE

Symptomatic Pyelonephritis

Bacteriuria occurs in 0.7% of full-term infants, and clinically evident urinary infections occur in 0.3%.[21] Males predominate in the first 3 months of life, and account for almost 80% of neonatal urinary infections. Most of these infections appear to involve the upper tracts. In a retrospective review of 11,655 children born at a Stockholm hospital between January 1, 1979 and June 30, 1982, the annual incidence of pyelonephritis during the first 2 years of life was 34 per 10,000.[48] Acute pyelonephritis occurred after a mean of 5.6 months following discharge, with a range of 1 week to 17 months; only one infant had an underlying malformation of the urinary tract. A more recent report from a Seattle group health cooperative reported hospitalization rates for pyelonephritis were 17/10,000 for girls and 6/10,000 for boys age 0 to 4 years.[49] It is estimated 1% to 3% of all girls 1 to 5 years of age experience an episode of pyelonephritis.[21]

About 250,000 episodes of acute pyelonephritis with over 160,000 hospitalizations occur each year in adult women in the United States.[50] The highest rates of acute nonobstructive pyelonephritis are in young women, many of whom also experience acute uncomplicated cystitis (Table 23.3). Ikaheimo and associates[51] reported an incidence of 2.7 episodes of pyelonephritis per 100 patient years during a 1-year follow-up of women in a family practice in Finland who originally presented with an episode of acute cystitis. The ratio of episodes of cystitis to pyelonephritis was 29:1. Stamm and associates[52] followed 51 American women with recurrent uncomplicated urinary infections for a median of 9 years. The mean infection rate was 2.6 per patient year with the ratio of cystitis to pyelonephritis episodes of 18:1 for women not receiving prophylactic antimicrobials.

Hospitalization for treatment of acute pyelonephritis reported from a Canadian province was 11/10,000 women.[54] Pyelonephritis accounted for 0.4% of all hospital admissions.

23.3 Reported Frequency of Pyelonephritis in Selected Populations

Study Design (with Reference)	Population	Incidence
Retrospective,[48] Sweden	Children <2 years	34/10,000 population
Prospective, 12 months, Finland[51]	179 women initially presenting to GP with acute cystitis	2.7/100 patient years
Retrospective,[52] United States 1969–1985	51 women with recurrent cystitis	0.1 ± 0.3/patient year
Prospective,[53] Canada	Diabetic women with bacteriuria	7.2/100 patient years
Administrative data,[54] Canada, hospitalized	Women—all Women >60 years Men	10.9/10,000 population 14.0/10,000 population 3.3/10,000 population
Administrative data,[50] United States, hospitalized	Women <60 years Women >60 years Men <60 years Men >60 years	7.8–15.0/10,000 population 13.5–23.3/10,000 population 1.1–2.4/10,000 population 6.3–12.9/10,000 population
Administrative data, United States[49]	Hospitalized women men Outpatient women men	12–13/10,000 population 2–3/10,000 population 3–4/10,000 population 1–2/10,000 population

The frequency of hospitalization underestimates the incidence of pyelonephritis because many patients with pyelonephritis, especially healthy young women, are not admitted for treatment. Peak pyelonephritis hospitalization rates occurred in women 20 to 29 years old, and men and women older than 50 years. From 0.3% to 0.7% of all pregnancies required hospitalization for pyelonephritis. Women with diabetes were six to 24 times more likely to be admitted with pyelonephritis than nondiabetic women, stratified by age. An American study reported a similar hospitalization rate of 11.7/10,000 for women,[50] but hospitalization rates were not increased in patients with diabetes. The highest rates were observed in younger women aged 20 to 39 years, with 15 hospitalizations/10,000, and women over 80 years, with 23.3/10,000. For women enrolled in a group health cooperative in Seattle, estimated annual rates of outpatient and inpatient pyelonephritis were 12 to 13/10,000 and 3 to 4/10,000, and the highest incidence of 18 to 20/10,000 occurred in young women.[49]

Pyelonephritis in men usually occurs in the context of complicated urinary infections, but acute nonobstructive pyelonephritis may rarely occur. Krieger and colleagues[55] reported an incidence of uncomplicated symptomatic urinary infection of 4.9/10,000 men per year in a 6-year study of male university students, but most of these were likely lower tract infections. Rates of hospitalization for acute pyelonephritis in men, most with complicated urinary infections, were reported to be 3.3/10,000 per year in Canada,[54] and 2.4/10,000[50] and 1 to 2/10,000[49] in the United States. In selected populations with complicated urinary infections, Waites and associates[56] reported 1.8 episodes per person year of urinary infections presenting with fever and chills, presumably upper tract infections, in 64 spinal cord–injured patients managed with intermittent catheterization or condom drainage. A prospective study of residents in long-term care facilities reported 1.1 episodes of febrile urinary infection per 10,000 resident days.[57] The rate was 0.8/10,000 resident days for individuals without chronic indwelling catheters, and 4.6/10,000 for those with chronic indwelling catheters.

Asymptomatic Upper Tract Infections

Asymptomatic bacteriuria is common in many populations,[3] and frequently involves the upper urinary tract (Table 23.4). Presumably, factors such as the duration of bacteriuria, infecting organisms, associated medical illnesses, the presence of

23.4 Localization of Infection in Bacteriuric Populations

Reference	Method	Population Investigated	Symptoms	Site of Infection		
				Renal	Bladder	Equivocal
58	Ureteral catheterization	95 Women 26 Males	Variable	67	54	—
59	Bladder washout	125 Women	52 Asymptomatic 30 Upper 43 Lower	63	52	10
60	Bladder washout	133 Women	90 Asymptomatic 15 Upper 28 Lower	56	54	23
61	Bladder washout	50 Women	25 Upper 25 Lower	21	22	7
62	Bladder washout	105 Women	60 Asymptomatic 24 Lower 20 Upper	65	39	—
63	Bladder washout	50 Women Mean 80 yr	Asymptomatic	17	14	19
64	Bladder washout	51 Women Mean 80.5 yr	Asymptomatic	34	17	—

vesicoureteral reflux, and urinary obstructions influence the likelihood of a UTI in patients with asymptomatic bacteriuria. At least 50% of institutionalized elderly women with bacteriuria have upper tract localization.[63,64] Bacteriuria in these populations often persists and remains asymptomatic for months to years. Bacteriuria is likely attributable to the same biologic variables as recurrent uncomplicated or complicated symptomatic infection, but bacterial isolates causing bacteriuria are rarely a direct cause of subsequent symptomatic infection in the absence of uroepithelial trauma or obstruction.

NATURAL HISTORY AND CONSEQUENCES

Infection during Infancy and Childhood

The short-term morbidity of acute pyelonephritis in children may include hospitalization, severe sepsis and septic shock, metastatic infection, and, rarely, acute renal failure. Following the introduction of antimicrobial therapy, the outcome for adequately diagnosed and treated children is excellent. However, there remain concerns about the potential for long-term renal damage following acute pyelonephritis in childhood.

Renal scarring is observed in 10% to 30% of children following acute pyelonephritis.[21] Established risk factors for the development of renal parenchymal scarring include vesicoureteric reflux, recurrent infection, delayed treatment, and a young age at the time of initial infection.[65] Parvex et al.[66] observed 88 scars in 50 children at 6 months after acute pyelonephritis; 3 years later, 27% were unchanged, 63% were partially resolved, and 9% were completely disappeared. The number of scars was the most important variable associated with decreased renal growth. Increased serum and urine markers of the inflammatory response at presentation with acute pyelonephritis, including IL-8,[67] IL-6,[68] procalcitonin,[69] and C-reactive protein,[69] are associated with subsequent increased occurrence of renal scarring, regardless of vesicoureteral reflux. Recent studies have suggested that cytokine gene polymorphisms may partly explain the differential inflammatory response.[65,70,71] Studies do not support a role for bacterial virulence factors as predictors of subsequent scarring.[72]

The relative importance of vesicoureteral reflux and pyelonephritis in the development of renal scars and impaired renal function in childhood remains controversial. It is now accepted that congenital vesicoureteral reflux, primarily occurring in males and associated with higher levels of reflux and renal dysplasia, is most likely to progress to renal failure, regardless of infection.[73] Postnatal renal damage may also occur with vesicoureteral reflux associated with an infection, or with acute inflammation from an infection of the renal parenchyma without reflux. Scarring after pyelonephritis in infancy is associated with renal growth arrest in the involved kidney and may be associated with progressive kidney damage.[67,73] The risk of renal failure is significantly greater with high-grade reflux (grade IV through V) and with multifocal or global scarring. A careful follow-up with an immediate diagnosis and the adequate treatment of all recurrent episodes of infection, particularly during infancy and early childhood, are considered necessary to prevent progressive renal impairment.[73]

Asymptomatic bacteriuria occurring in girls 5 years or older with normal kidneys on a study entry is benign.[74] It is not associated with renal scar development regardless of antimicrobial treatment. Thus, programs to screen for bacteriuria in school-age girls and to treat infections, if found, are not worthwhile.

Adult-Onset Infection

Short-Term Morbidity

Potential negative short-term outcomes of acute pyelonephritis are lost days of work, hospitalization, septic shock, requirements for urologic intervention, and sequelae of metastatic infection. For patients with complicated urinary infections, additional morbidity may be attributable to the underlying abnormality, which promotes infection. Women with diabetes have been reported to have more prolonged fever and a higher rate of mortality.[75] Acute renal failure occurring with acute nonobstructive pyelonephritis caused by E. coli is rare, but well described.[76,77] This is usually reversible, may be more common in diabetic patients, and, for some of the reported cases, concomitant nonsteroidal antiinflammatory drugs likely contributed to renal failure.

Mortality

A comprehensive population-based study of the incidence and prevalence of bacteremic acute pyelonephritis from 1977 to 1981 in an urban population of 400,000 reported 22% of community-acquired bacteremias were attributed to invasive urinary infections, with an annual incidence of 15.7 per 100,000.[78] The attributable mortality for bacteremic urinary infections was 4.8%, but all 15 deaths occurred in patients with a significant underlying illness. During the same period, these investigators observed 1,520 episodes of hospital-acquired bacteremia, of which 221 (14.5%) originated from the urinary tract (71% in catheterized patients), thus yielding a rate of 7.3 per 10,000 hospitalized persons.[79] The mortality rate attributed directly to infections in these patients with bacteremic nosocomial pyelonephritis, virtually all with complicated urinary infection, was 12.7%. This mortality rate is one-third that of gram-negative bacteremia originating from other sites. A review of 542 episodes of bacteremic gram-negative urinary infection in Olmsted County, Minnesota, from 1998 to 2007 included 57% that were community acquired, 36% that were health care associated, and 7% that were nosocomial.[80] All cause mortality was 4.9% at 28 days and 15.6% at 1 year. The only independent predictor of increased mortality was increasing age, whereas a lower mortality was associated with community-acquired infections and an isolation of E. coli. In critical care units,

14.6% of patients admitted with septic shock had a urinary source.[81] The 28-day mortality when the urinary tract was the origin of the septic shock was only 18%, compared with 36% for all other sites. Berger et al.[82] described a case series of 65 patients admitted from 1994 to 2007 at one Australian center who required emergency nephrectomies due to severe urosepsis; the mortality in these individuals was 20%. Thus, mortality attributable to pyelonephritis occurs, but virtually only in patients with complicated infections.

Metastatic Infections

Genitourinary sepsis accompanied by bacteremia may be complicated by metastatic infections at other sites. Siroky and colleagues[83] identified 175 patients in whom metastatic infections developed from a primary source in the genitourinary tract. Most patients (86%) were men and the mean age was 57 years. A primary prostatic focus was considered the site for dissemination in many of the men, but in 46 patients the upper urinary tract was the likely source of the bacteremia. One hundred six patients (59%) had infections of the skeletal system; 51 patients (28%) had endocarditis; and 13% involved miscellaneous sites, including the eye and the central nervous system. Almost 70% of the skeletal infections were caused by gram-negative rods, with the vertebral column the most common metastatic site, identified in 83 patients (78%) with skeletal infections. In patients with endocarditis, gram-positive organisms were responsible for two-thirds of the infections, and these patients usually had preexisting heart disease. About one-third of these patients had undergone a manipulation of the upper urinary tract prior to the development of metastatic infections. Underlying host factors that would impair resistance to infections were unusual. In a Danish review of cases of vertebral osteomyelitis from 1978 to 1982, the urinary tract was the most common identified source, with the mean latent period separating an episode of acute urinary infection from the onset of symptoms of vertebral osteomyelitis being 54 days.[84]

Renal Impairment

Adult onset pyelonephritis rarely contributes to chronic renal failure. Abnormalities previously attributed to pyelonephritis in autopsy studies are now recognized as being an end stage of other processes such as vascular disease, papillary necrosis, or medullary cysts, with little contribution from infections. In one autopsy study of patients with renal failure, 13% of subjects were considered to have pyelonephritis, but all had vesicoureteral reflux, analgesic abuse, nephrolithiasis, or obstruction as underlying contributory factors for pyelonephritis.[85]

The Bristol Pyelonephritis Registry followed 375 women for 1 to 13 years after a clinical diagnosis of recurrent pyelonephritis. Only one patient had a radiographic progression of renal scars.[86] Other long-term studies that have reported patients with progression to renal failure also invariably identify alternate diagnoses to explain renal functional deterioration in affected patients. Parker and Kunin[87] retrospectively reviewed 74 cases among 163 women hospitalized for acute pyelonephritis 10 to 20 years previously, in the early antimicrobial era. Continuing clinical illness following the index hospitalization had occurred in more than 40% of patients; 28% had had an operative urologic procedure, and 23% had renal stones. Seventeen percent were bacteriuric at the follow-up examination. One patient died of complications of pyelonephritis, one required a transplantation for end-stage renal disease, and two others had significant renal impairment. Seven patients had undergone unilateral nephrectomy for pyelonephritis. Gower[88] followed 62 adult women with treated infections and a radiologic diagnosis of chronic pyelonephritis for a mean follow-up of almost 5 years. Serial studies demonstrated radiographic progression in 11 women, but persisting infection and analgesic ingestion contributed to progressive radiologic damage in all these cases. Two of the 36 patients with bilateral pyelonephritis had renal failure or died during the follow-up. Alwall[89] described a selected series of 29 women with an initial normal-appearing intravenous pyelogram (IVP) who developed contracted kidneys from 1 to 15 years following acute pyelonephritis. Several went on to end-stage renal disease, but for all these cases, analgesic abuse was a concomitant factor that likely accounted for disease progression.

Raz and colleagues[90] described long-term outcomes for women admitted with acute pyelonephritis to a hospital in Israel between 1982 and 1992. Only 31% of the patients were available for a 10-year follow-up, likely a group representing those with more serious or persistent disease. With technetium ^{99}Tcm–labeled dimercaptosuccinic acid (^{99}Tcm-DMSA) scanning, 46% of these women had evidence of renal scarring. Pregnancy and hypoalbuminemia at hospitalization for pyelonephritis were independent risk factors correlated with the finding of renal scars at a 10-year follow-up. Although four women with scars had a glomerular filtration rate (GFR) of less than 75 mL per minute, none had developed renal impairment. Despite the high proportion of this selected group of women who had renal scarring, there were no clinically relevant adverse outcomes.

These long-term prospective studies support the observation that recurrent UTIs in adult women usually have a benign natural history. Adults with recurrent UTIs and no other complicating illness seldom experience clinically significant renal damage directly attributable to an infection. Persons with infection and renal impairment invariably have significant underlying urologic abnormalities or associated renal diseases.

Hypertension

The long-term follow-up of cohorts that have enrolled large numbers of women have consistently reported no significant differences in blood pressure between patients with bacteriuria and those without bacteriuria.[3] Patients entered into the Bristol Pyelonephritis Registry and followed for up to 13 years developed hypertension at the same rate as the

general population.[86] Raz et al.[90] reported a similar frequency of hypertension in women with and without renal scarring 10 to 20 years after hospitalization for acute pyelonephritis. Thus, evidence does not suggest acute or recurrent pyelonephritis contributes directly to development of hypertension.

Pregnant Women

Acute pyelonephritis occurs in 1% to 2% of all obstetric patients in the absence of screening and treatment programs for bacteriuria.[91] It is the most common medical complication requiring hospitalization during pregnancy. Women with asymptomatic bacteriuria early in pregnancy have a 20- to 30-fold increased risk of acute pyelonephritis in later trimesters.[92] This is attributed to ureteral dilation and urinary stasis resulting from progesterone-induced smooth muscle relaxation, together with mechanical compression by the enlarging uterus. Acute pyelonephritis in pregnancy occurs primarily in the second and third trimesters. Case series have reported 52% of episodes occurring in the second trimester, 46% in the third, and 2% in the first,[93] and 11% occurring in the first trimester and the remainder in the second or third trimester.[94]

Acute pyelonephritis, as with any febrile bacterial illness in late pregnancy, is associated with an increased risk for premature labor, presumably due to systemic inflammation.[95] The attributable risk of acute pyelonephritis for maternal toxemia, prematurity, and perinatal mortality remains controversial.[91] Sever and associates,[96] in data collected from more than 55,000 pregnant women, reported a higher incidence of low-birth–weight infants and stillbirths in the 3.5% of women with documented UTIs. Naeye[97] reported a combined perinatal mortality rate of 42 per 10,000 births in bacteriuric women as opposed to 21 per 10,000 births in nonbacteriuric women. McGrady and colleagues,[98] using birth certificate data from Washington State, showed that the fetal mortality rate was 2.4 times higher for UTI-associated pregnancies. Romero and colleagues,[95] in a meta-analysis, documented an increased occurrence of low-birth–weight and preterm delivery with asymptomatic bacteriuria. Smaill (in a Cochrane review)[99] also showed that antibiotic treatment significantly reduced the risk of low birth weight. Thus, asymptomatic bacteriuria is associated with prematurity and low birth weight, but it is not clear whether acute pyelonephritis is also a risk, or whether any presentation of urinary infection is causative for these outcomes.

Pregnant patients have a reduced GFR following acute pyelonephritis, which reverts to normal within 8 weeks of effective treatment.[100] Long-term follow-up studies of women known to have previously experienced bacteriuria during pregnancy report a benign course for the majority of patients.[101,102] In a follow-up period of 10 to 14 years, almost 40% of 134 women with bacteriuria during pregnancy also had bacteriuria on follow-up cultures. Although pyelography showed renal scarring in 28% of these patients, creatinine clearance was normal for both the bacteriuric and the nonbacteriuric women.[102] Raz et al.[90] reported that pregnancy at hospitalization for acute pyelonephritis was an independent risk factor for the presence of renal scars 10 to 20 years later. Subtle differences in renal function were found in long-term follow-ups between women with and without urinary infection during pregnancy, but these were not clinically significant.

Neurogenic Bladder

Following a traumatic spinal cord injury, patients managed with permanent indwelling catheters have substantial subsequent morbidity and mortality attributable to renal infections. The mortality rate from renal failure was 20% within 3 decades from injury in survivors of World War II spinal injuries. This was a consequence of obstruction, nephrolithiasis, suppurative renal disease, and progressive nonobstructive chronic pyelonephritis.[103] More than one-half of the deaths during the 2nd decade after spinal cord injury were caused by renal failure.[104] The introduction and widespread practice of voiding management to maintain a low-pressure system within the genitourinary tract, including the use of intermittent catheterization, have dramatically altered the occurrence of and mortality from complications of urinary infections in this population. Spinal cord injured patients, however, remain at an increased risk of urosepsis and other complications of infection including urethritis, periurethral abscesses, bladder and renal calculi, vesicoureteral reflux, and renal or perinephric abscesses.

LABORATORY DIAGNOSIS

Urine Culture

Quantitative counts of bacteria isolated from urine collected before the initiation of antimicrobial therapy in patients with acute nonobstructive pyelonephritis exceed 10^5 CFU per milliliter for 95% of patients.[2,105–107] In patients with renal infections, ureteral urine bacterial counts vary between 10^1 and 10^6 CFU per milliliter.[58] The maximum stationary phase growth is reached in the bladder after a period without emptying; thus, whenever possible, urine cultures should be obtained following an overnight "incubation" of urine within the urinary bladder. A number of variables, including diuresis, frequency of voiding, partially effective antibacterial chemotherapy, infection owing to fastidious organisms, obstruction, and extraluminal infection, may reduce bacterial counts to lower levels.

The diagnosis of asymptomatic bacteriuria in women is made with 95% assurance if two consecutive urine cultures are positive for the same organism in counts equal to or greater than 10^5 CFU per milliliter.[2,3] In men, a single positive urine culture establishes the diagnosis of bacteriuria.[3] Urine cultures with more than one organism isolated may be difficult to interpret. Such "mixed cultures" usually reflect contamination or, when urinary devices are in place, biofilm colonization. A true multiple organism infection of the urinary tract is uncommon in patients with acute uncomplicated pyelonephritis, but frequent for some patients with complicated urinary infection, particularly in

patients with foreign bodies or renal stones. Occasionally, different organisms may be present in each kidney. Differential growth rates in the bladder or suppression of one organism by the other may result in a report of a single organism isolated in voided urine despite different infecting organisms in ureteral urine cultures, or even the bloodstream.

Pyuria

Demonstrating the presence of pyuria is the most readily available means of establishing the evidence of a host response, presumably differentiating colonization from infection. Renal infection is usually characterized by a higher urinary leukocyte count than bladder infection.[64,108] However, the sensitivity, specificity, and predictive value of pyuria as a diagnostic test for acute pyelonephritis have not been determined. Leukocytes disintegrate at alkaline pH, potentially leading to false-negative findings for pyuria in patients with infections with urease-producing organisms. In addition, neutropenic patients with symptomatic urinary infections may fail to demonstrate pyuria.

Pyuria is usually identified by the presence of leukocyte esterase on a dipstick screening of urine. The more traditional measurement of pyuria by counting the number of cells in centrifuged urine per high power field is imprecise, with many sources of error.[4] Leukocyte excretion rates are more reproducible, but are not applicable to routine patient care. Excretion rates do correlate well with absolute leukocyte counts of random, unspun urine counted in a hemocytometer; counts in excess of 10/mm^3 represent an abnormal host response.[4]

Other Urinalysis Characteristics

Leukocyte casts indicate intrarenal inflammation. They are present in about two-thirds of patients with invasive renal infection but are nonspecific, also being present in many interstitial and glomerular renal diseases. Microscopic hematuria is common in patients with renal infection but has no documented clinical significance. It usually resolves with adequate treatment. Red blood cell casts are unusual. Persistent hematuria after antimicrobial treatment may require a urologic investigation to exclude other causes. Quantitative proteinuria with a urinary protein excretion rate exceeding 100 mg per 24 hours is unusual in either acute or chronic renal infection unless a second renal disease associated with proteinuria is present.

Bacteremia

Between 15% and 30% of hospitalized patients with acute pyelonephritis have a positive blood culture at presentation and, presumably, are at greater risk of metastatic infection to other sites.[10,11,109] Elderly women, patients with diabetes, and individuals with obstruction are more likely to be bacteremic.[75,110]

The routine collection of blood cultures from all patients presenting to the emergency department with acute pyelonephritis is not clinically useful. McMurray et al.[111] reported that 56 (18%) of 307 patients hospitalized with acute pyelonephritis from 1990 to 1992 had positive blood cultures; 32% of positive blood cultures were coagulase negative staphylococci and were presumed to be contaminants. Only one blood culture grew a pathogenic organism that was not also isolated from urine culture, and clinical management was not altered for any patient by positive blood cultures. Velasco et al.[15] obtained routine blood cultures from outpatients presenting with a diagnosis of acute pyelonephritis at a Spanish hospital. Positive blood cultures were found in 147 (25%) of 583 patients, with only coagulase negative staphylococci isolated in 17 (12%) of these. E. coli grew from 91% of positive blood cultures, and 23% of women with E. coli pyelonephritis had positive blood cultures. The blood culture was consistent with the urine culture result in 98% of cases. Wing et al.[16] pooled data from three randomized controlled trials in pregnant women with acute pyelonephritis, from whom routine blood cultures were obtained at study entry. Among 391 women, 94 (24%) had positive blood cultures and 5 (5.3%) grew only S. epidermidis. Smith et al.,[112] however, retrospectively reviewed results from nonpregnant women admitted with acute pyelonephritis through the emergency department of an urban county teaching hospital where blood cultures were obtained selectively rather than routinely. Bacteremia occurred in 36% of 64 women, and was more frequent in black women, women with genitourinary abnormalities, with higher pulse rates on admission, higher levels of pyuria, and more prolonged fever. Blood cultures were positive in 2 patients with negative urine cultures. van Nieuwkoop and colleagues[113] reported that 23% of patients presenting to emergency departments in the Netherlands with both uncomplicated and complicated febrile UTIs had bacteremia; 95% had concordant blood and urine cultures. Factors independently associated with positive blood cultures but negative urine cultures included the presence of a urinary catheter, any malignancy, and already receiving antimicrobial treatment for urinary infection when the urine culture was obtained.

These studies suggest routine blood cultures obtained from clinically stable women presenting with mild or moderately severe symptoms of acute pyelonephritis are not useful and should not be requested. However, patients who are more severely ill, in whom the diagnosis is uncertain, or when an underlying abnormality is present or suspected, should have blood cultures obtained prior to the institution of antimicrobial therapy. This is particularly the case for pregnant women, where failure to initiate appropriate antimicrobial therapy may have adverse fetal outcomes.

C-reactive Protein and Procalcitonin

C-reactive protein and procalcitonin are elevated in children and adults with acute pyelonephritis.[114,115] These are, however, nonspecific markers for inflammation with limited diagnostic use for differentiating pyelonephritis from other febrile illnesses. Procalcitonin has been suggested to be useful

to identify upper tract involvement in children who present to the emergency department with urinary infections.[114] C-reactive protein and procalcitonin have also been evaluated as prognostic markers. Pratt et al.[115] showed that elevated procalcitonin (using a level of 1 ng per milliliter) correlated with the development of renal scars in children, and was more specific for predicting subsequent scarring than C-reactive protein or leukocyte counts. In adults, a higher C-reactive protein level at admission correlated with prolonged hospitalization, whereas an elevated C-reactive protein at discharge predicted recurrence.[116] Other studies in adults reported that the procalcitonin level at admission in patients with urosepsis predicted bacteremia and bacterial load,[117] but did not predict adverse outcomes at 28 days.[118] The clinical use of these or other inflammatory markers for either the diagnosis or prognosis of acute pyelonephritis remains uncertain, and routine testing is currently not recommended.

INFECTION LOCALIZATION

The clinical presentation of acute pyelonephritis is often straightforward, and a diagnosis of renal infection can be made on the basis of clinical signs and symptoms. However, patients with asymptomatic bacteriuria or only lower tract symptoms may also have a renal infection. Table 23.4 summarizes selected studies performed to localize infections in adults with variable symptom presentations.[58–62] In these studies, as many as one-half of women with asymptomatic infections and a substantial minority of women with only bladder symptoms have a renal infection. However, no epidemiologic studies and few therapeutic studies of bacteriuria and its complications have attempted to prospectively localize renal bacteriuria so the clinical implications, if any, of upper tract localization are not clear. Currently, the localization of the site of infection has a clinical use only for the infrequent patient with relapsing infection where localizing the site of infection may influence clinical management. A large number and variety of different approaches for infection localization have been described (Table 23.5), but most of these methods are now of only historical interest.

Cystoscopy with ureteral catheterization is a direct approach to localization and continues to be the only definitive method for confirming renal localization. It also permits the localization of infection to one kidney and can identify different infecting organisms in each collecting system.[119] In practice, however, this procedure has limited usefulness. Careful urologic manipulation with meticulous attention to avoid contamination is required. A cystoscopy and ureteral catheter insertion are performed in an infected urinary tract because antibacterial therapy is withheld until the urine collections are complete. Infected bladder urine must be removed by repeated washing with sterile irrigating fluid before ureteral catheters are introduced through the bladder.[58] Otherwise, positive ureteral urine cultures may result from the carriage of infected bladder urine into the ureters during catheterization.

23.5 Methods Proposed to Identify Urinary Infection Localized to the Kidney

- Ureteral catheterization with differential urine cultures
- Intravenous pyelography/CT scanning
- Nuclear medicine imaging: ^{67}Ga citrate scanning/^{99}Tcm-DMSA scanning
- Bladder washout
- Renal biopsy with culture
- Fluorescent examination of renal tissue for bacterial antigen
- Serum antibodies to lipopolysaccharide antigen
- Urinary enzymes, cytokines
- C-reactive protein
- Serum procalcitonin
- Tamm-Horsfall protein antibodies
- Maximal renal concentrating ability
- Antibody-coated bacteria (ACB)
- Relapse following short course antimicrobial therapy

CT, computed tomography; Tcm-DMSA, technetium-dimercaptosuccinic acid.

The histopathologic examination of renal tissue obtained by a biopsy or at necropsy is another direct approach to the diagnosis of a renal infection. However, a kidney biopsy is contraindicated in the presence of an acute infection. In addition, pyelonephritis is a focal disease, and a random biopsy may not provide either a pathologic or bacteriologic diagnosis.[120] Percutaneous renal biopsies are seldom used to diagnose chronic pyelonephritis, and bacteriologic studies of renal tissue are rarely helpful. The nonspecificity of the histologic findings and their focal nature further limit the diagnostic use of biopsies because concomitant diseases may distort the gross and histologic features and may therefore make it impossible to attribute changes specifically to bacterial inflammation.

Imaging of the kidneys with ^{67}Ga citrate has been proposed to identify a renal infection. Hurwitz and associates[61] compared imaging with ^{67}Ga citrate to cultures obtained by ureteral catheterization or bladder washout in 47 patients and found a false-positive rate of 15% and a false-negative rate of 13% for the imaging procedure. Radioisotopic localization, particularly with ^{99}Tcm-DMSA, may be a more sensitive procedure for diagnosing a renal infection and assessing renal scarring.[121–123] These tests are often positive in patients with invasive upper tract infections, but are less reliable in those with asymptomatic infection.

The measurement of C-reactive protein has been used as a test to localize renal infections in symptomatic children,[124] but the specific level to differentiate upper from lower tract infections has varied among different studies and is not standardized.[125,126] Serum procalcitonin has also been studied to

diagnose pyelonephritis and UTIs in febrile infants and children. When compared to a DMSA scan, procalcitonin levels >0.5 to 0.6 pg per milliliter[127] had an average sensitivity of 85% and specificity of 76%. Urinary IL-6 is increased in children with acute pyelonephritis, with >15 pg per milliliter reported to have a specificity of 94% and a positive predictive value of 87.5%[128] for upper tract infections. None of these parameters, however, have yet been shown to be of use for routine clinical application.

Measurements of urinary enzymes, including urinary dehydrogenase, leucine aminopeptidase, α-glucuronidase, catalase, lactic dehydrogenase, lysozyme, and urinary α1 microglobulin, have all been reported to be useful for localization in at least one study, but not confirmed in subsequent studies to have adequate sensitivity or specificity to be a reliable diagnostic test. Other localizing methods used in previous studies have included the Fairley bladder washout technique,[60] a measurement of serum or urine antibodies to the infecting bacteria, and the fluorescent antibody-coated bacteria test.[129] None of these has proved to be of sufficient ease of use or reliability for clinical applications. Maximal urinary concentrating ability is reduced in many patients with renal bacteriuria. Fluid deprivation for 24 hours with the administration of antidiuretic hormone achieves maximum urinary concentration of more than 800 mOsm per kilogram of water in more than 80% of patients with bladder bacteriuria, but at least 70% of patients with renal infections are unable to concentrate urine to this level following maximal stimulation.[130] Urinary concentration, however, is altered in a number of pathologic states, and the test is not sufficiently sensitive or specific to be of value for an individual patient.

In practice, clinical presentations are used to assess the site of infection. Smeets and Gower[131] screened 43 symptomatic women with the bladder washout technique. Among these women, only fever higher than 38°C correlated with renal involvement. Two-thirds of the patients with upper tract infections were febrile at the time of localization compared to one-third of patients with lower tract infections. These studies and others suggest that no clinical criteria are uniformly reliable to localize the site of the infection.[132]

A short course of effective antimicrobial therapy will cure most uncomplicated bladder infections in women. It has been proposed that differential outcomes with short-course therapy may localize the site of the infection. Comparisons of single-dose therapy outcomes with other localization methods have documented an association between renal infections and failures of single-dose therapy.[133–135] However, single-dose therapy will fail in 10% to 20% of individuals with lower tract infections, and a small proportion of individuals with covert upper tract infections will be cured by short course therapy. For women with a normal genitourinary tract and repeated relapses following nitrofurantoin therapy, however, a renal localization of infections may be presumed because this agent is effective for sterilizing bladder urine, but it will not eradicate renal infection.

DIAGNOSTIC IMAGING

Acute Pyelonephritis

A plain abdominal film of the kidneys provides limited information in patients with presumed acute pyelonephritis. Renal calculi may be visualized, one or both kidneys may be enlarged, or gross changes such as hydronephrosis or renal atrophy may be apparent from the renal outline. Occasionally, perinephric gas or other changes in the retroperitoneal space provide diagnostic clues.[136] Other findings recognized on abdominal films may lead to diagnoses other than acute pyelonephritis, such as a perforated viscus.

Renal ultrasonography and computerized tomographic (CT) scanning have largely replaced IVP for the initial screening for genitourinary obstruction in patients with acute pyelonephritis.[137,138] In addition to identifying anatomic abnormalities and focal complications such as intrarenal or perinephric abscess, ultrasonography usually shows swollen kidneys, although this may only be appreciated by repeat scanning following antimicrobial therapy. The degree of renal enlargement on ultrasonography has been correlated with prolonged pretherapy symptoms, higher leukocyte counts, and prolonged hospitalizations.[137] However, ultrasonography is less sensitive or specific than CT or magnetic resonance imaging (MRI).[138]

An unenhanced CT scan is usually sufficient and will detect most calculi, gas forming infections, hemorrhage, parenchymal calcifications, obstruction, and inflammatory masses. A contrast-enhanced CT scan may be indicated in acute pyelonephritis if the differential diagnosis includes other intra-abdominal or retroperitoneal pathology, or if there is a delayed response to therapy and the ultrasound is normal or equivocal. With an unenhanced CT scan, global swelling of the infected kidney is present.[138] With contrast-enhanced CT scans, either an enlarged kidney with a uniformly homogeneous nephrogram, a striated parenchymal nephrogram, or wedge-shaped segmental defects are seen.[138] The striated pattern is caused by the localization of inflammatory cells and fluid within the collecting ducts. The wedge-shaped low-attenuation areas include renal parenchyma with impaired function caused by vascular spasm, tubular obstruction, or interstitial edema. Renal enlargement, delayed visualization, and a poor definition of calyceal architecture in the involved kidney are also common findings. Renal parenchymal volume increases by 25% to 50% during an episode of acute pyelonephritis and can take 4 to 6 weeks to regress.[139] For atrophic or chronic pyelonephritis, the involved kidney is irregular in outline and below average size unless hypertrophy has occurred owing to compensatory enlargement. A contrast-enhanced study is necessary to fully define changes in renal excretion that occur as a result of inflammation. A helical (or spiral) CT

scan can provide information addressing specific phases of contrast media excretion.[140,141]

An MRI is also used for the imaging of acute pyelonephritis.[141] Features are consistent with those observed with CT scans, including renal enlargement, perinephric stranding, focal decreased enhancement, and abscess cavities. Typically, an infected area has a low signal intensity on T1-weighted images and increased signal intensity on T2-weighted images with a loss of normal corticomedullary differentiation. The use of gadolinium is essential to correctly identify areas of renal involvement. An MRI may also differentiate acute infections from chronic scars. An MRI may be preferred for some patients because it does not require radiation or iodinated contrast material. A limitation of MRI, however, is poor discrimination for the interpretation of gas-forming collections or calculi.

In children, ^{99}Tcm-DMSA scintigraphy is more sensitive then ultrasound for detecting acute pyelonephritis, cortical lesions, and renal scarring.[140,143] Sattari et al.,[144] however, found contrast-enhanced CT scanning more accurate for identifying acute pyelonephritis than DMSA scintigraphy in adult populations. Small lesions and those localized to the inner layer of the renal cortex were present on the CT scan but were not appreciated with the lower resolution of scintigraphic images. Scintigraphy has also been evaluated in children as a predictor of outcomes at a longer term follow-up. Wallin et al.[145] reported that scintigraphy defects present at the time of an acute infection persist at 6 months in 34% of kidneys, and Agras et al.[146] reported that 38.2% of initial cortical lesions persisted at 6 months, and 17.6% at 12 months. Hitzel et al.[147] evaluated the quantitative analysis of DMSA scintigraphy to predict long-term renal scarring following acute pyelonephritis in children and reported the intensity of abnormality with DMSA scintigraphy at the time that an acute presentation was predictive of subsequent scarring.

The term nephronia refers to a renal mass confined to a single lobe, representing localized inflammation but without suppuration. It is thought to be an intermediate phase in the progression from inflammation to abscess.[148] Gallium scanning demonstrates an increased uptake in the area of the mass, which is usually accompanied by generalized increased activity elsewhere in the same or opposite kidney. Ultrasonography shows a sonolucent ovoid mass that disrupts the normal corticomedullary definition and produces low-level echoes. These findings permit differentiation of nephronia from renal abscess or tumor. In adults, this finding may occur more commonly in diabetics. In children, the presence of acute lobar nephronia was reported to be associated with an increased incidence of renal scarring.[149] Identification of this abnormality on imaging studies, however, does not alter therapeutic approaches.[138]

Radiologic changes induced by acute inflammation are usually reversible with antimicrobial treatment.[61,137,149,150] However, a progressive reduction in renal size or the development of a scar may follow an episode of acute pyelonephritis.[149,150] Scars may involve an entire pole of the kidney or, in patients with atrophic pyelonephritis, the entire kidney. The upper pole is the most common site for scars. In serial studies, the initial abnormality was the loss of the renal cortex, with the renal parenchyma becoming thinned. Calyceal clubbing then developed as the renal papilla is retracted into the scar. The cup of the calyx is no longer a "cup," because the papilla does not project into it. In adults with acute pyelonephritis on a CT scan or an MRI at presentation, a focal lesion with peripheral ring enhancement and without central contrast uptake correlated with subsequent scar development.[150] In men and women with acute nonobstructive pyelonephritis and hypodense images on a CT scan at presentation, abnormalities persisted at the follow-up CT scan in 10 of 44 people.[151] In another study, persistent abnormalities were reported at follow-up in 29% of 55 adult women hospitalized with acute pyelonephritis.[150] Early scar formation was present in two patients, whereas two patients with atrophy had renal biopsies that showed chronic interstitial nephritis.

Indications for Radiologic Investigation

Imaging studies of the genitourinary tract for patients presenting with acute pyelonephritis should be selective.[140] Indications for imaging in individuals with suspected acute pyelonephritis include: (1) to assist with the diagnosis of acute pyelonephritis; (2) to assess whether there are underlying abnormalities present that may require intervention; (3) to assess the severity of infection, including identifying abscesses or emphysematous infections; or (4) in follow-up, to determine the extent of persistent damage such as renal scarring. Patients with atypical presentations, with severe sepsis or septic shock in whom obstruction must be excluded, or with a delayed response to therapy should have prompt imaging studies. Radiologic investigations should also be considered in patients who relapse shortly after discontinuing an adequate course of antimicrobial therapy.

Among 170 patients (163 women, 7 men) with acute pyelonephritis for whom an intravenous pyelogram was routinely obtained, 85 had normal pyelograms and 75 had structural or functional abnormalities that were attributable to the acute infection or represented a risk for relapse, but only 10 (5.9%) had specific disorders identified that resulted in a change of management.[152] In an Australian report, only 1 of 74 patients with acute pyelonephritis who had imaging with an ultrasound or a CT scan at admission had an abnormality identified that required an immediate intervention.[153] van Nieuwkoop et al.[154] prospectively identified patients presenting with febrile urinary infection to eight emergency departments in the Netherlands. There were 346 episodes, 140 (41%) in men, and 138 of these episodes (40%) were considered complicated. For all cases, 245 had an ultrasound or a CT scan; 6% of these showed urologic disorders requiring urgent intervention (pyonephrosis), 32 (13%) had nonurgent urologic disorders (nonobstructive renal stones, urologic malignancy, ureteropelvic junction stenosis, enterovesicle fistula), 175 (71%) had clinically irrelevant or normal results, and 24 (10%) had incidental nonurologic

disorders. They reported that a history of urolithiasis, urine pH ≥7.0, and renal insufficiency were the only variables predicting an abnormality on imaging.

Although contrast-enhanced CT scanning is the preferred imaging test for upper tract infection in adults, renal ultrasonography is rapid and noninvasive and may be more accessible. The intravascular injection of radiologic contrast media has risks of hypersensitivity or contrast media–induced renal failure. The concomitant presence of diabetes mellitus, particularly with renal impairment, is a relative contraindication. In children, the initial screening with ultrasonography or DMSA scintigraphy detects scarring and other renal abnormalities.[121,123] Investigations should be performed in any male infant or boy with a proven bacterial urinary infection, and in girls with a recurrent or complicated infection.[155] A voiding cystourethrogram is added for young children or if there is evidence of upper tract disease.

Unless specific new indications emerge in a given patient, serial periodic imaging studies are redundant in adults with recurring infection. Even among patients with a prior radiologic evidence of chronic pyelonephritis, the development of new findings is unusual.[88] Upper tract pathology rarely develops after the age of 1 year in adequately treated children, and repeated examinations are seldom indicated.[156]

CLINICAL PRESENTATION

Infants and Children

In the neonatal period, UTIs usually present as sepsis. The clinical picture, however, can vary from life-threatening septic shock in association with pyelonephritis to asymptomatic bacteriuria.[21] Nonspecific symptoms potentially associated with urinary infections in infants include fever; inadequate weight gain; gastrointestinal symptoms such as anorexia, emesis, diarrhea, and paralytic ileus; meningitis; seizures; lethargy; irritability; hypotonicity; respiratory irregularity; pallor; cyanosis; abdominal distention; gray skin color; and jaundice. Studies have identified jaundice as the hallmark of ongoing infection in the neonate. The frequent occurrence of generalized sepsis, premonitory symptoms prior to the onset of bacteriuria, and necropsy findings demonstrating cortical infection in the presence of a normal renal pelvis support a hematogenous route of renal infection in neonates. The predominance of males is unexplained. The intestinal tract is the presumptive source.

In older children, the clinical features of urinary infection more closely approximate those in adults.[21] As girls mature, abdominal tenderness, vaginal discharge, vomiting, and anorexia become less common features and fever and flank pain predominate. Asymptomatic UTIs in childhood occur almost exclusively in females.

Adults

The classic clinical presentation of acute upper UTIs in adults include fever, often over 38.5°C, chills, unilateral or bilateral pain in the lumbar flank region, and variable systemic symptoms, including malaise, anorexia, nausea, emesis, diarrhea, myalgia, and headache. The illness may progress rapidly, and many patients seek care within 24 hours of the onset of symptoms. Between 15% and 30% of patients experience concomitant symptoms consistent with a lower UTI, including dysuria, frequency, urgency, and suprapubic discomfort. Renal pain may radiate to the epigastrium or the lower abdominal quadrants. Severe flank pain with radiation to the groin is unusual and suggests a ureteral obstruction. Gastrointestinal symptoms, primarily nausea, vomiting, and diarrhea, occur frequently and predominate in about 10% of patients. Although patients presenting with acute pyelonephritis may be severely ill, the spectrum of disease also includes individuals with low-grade or no fever or only mild flank discomfort. Flank pain or discomfort on palpation or fist percussion is usually present. The physical findings are variable, however, ranging from mild discomfort to severe pain or systemic symptoms including septic shock.

Other diseases, both above and below the diaphragm, can mimic the pain of acute pyelonephritis. The differential diagnosis includes acute bacterial pneumonia, appendicitis, cholecystitis, a perforated viscus, diverticulitis, splenic infarction, acute pancreatitis, and aortic dissection. Acute pelvic inflammatory disease may be misdiagnosed as acute pyelonephritis and should be excluded by a pelvic examination in women at risk of sexually transmitted infections. Varicella-zoster virus reactivation (shingles) in an appropriate dermatome can also mimic renal pain. Renal infarction, acute renal vein thrombosis, obstructive uropathy, and acute glomerulonephritis may each have a clinical presentation that can be confused with acute pyelonephritis.

Pinson and colleagues[105] assessed the use of fever in differentiating acute pyelonephritis from other potential diagnoses. In a retrospective chart review, 93% of women who presented to the emergency department with pyuria and other findings consistent with acute pyelonephritis including leukocytosis, costovertebral angle tenderness, and two or more of abdominal pain or tenderness, back pain, and history of nausea and vomiting, were ultimately diagnosed with acute pyelonephritis when fever was also present. When fever was not present, 35% of hospitalized women with this constellation of symptoms ultimately had an alternate diagnosis. All nonhospitalized patients with fever were ultimately diagnosed with pyelonephritis, but 13% without fever had an alternate diagnosis. They concluded that when patients present with findings compatible with acute pyelonephritis but without fever there should be a high index of suspicion for an alternate diagnosis.

The presenting clinical features of pregnant women are similar to those in nonpregnant women. Septic shock may occur, and acute respiratory distress syndrome is reported in 1% to 8% of these women.[92] Patients with diabetes mellitus may present with deteriorating glycemic control. Renal infections in patients with diabetes mellitus and hyperglycemia may, rarely, present as emphysematous pyelonephritis

owing to carbon dioxide production from fermentation of glucose by gram-negative rods.[136,157] Acute pyelonephritis in patients with diabetes may also be accompanied by papillary necrosis. Fragments of the renal papillae can block the ureter, producing colic and hydronephrosis, often accompanied by gross hematuria. In elderly patients, symptoms of a urinary infection may be more difficult to ascertain and chronic symptoms of dysuria, frequency, urgency, and incontinence often occur unrelated to infection. Flank tenderness is less common in elderly patients, and acute confusion, sometimes with other neurologic symptoms or signs, is more common relative to younger age groups. Despite this, classic features of upper tract infections do occur in most elderly patients with acute pyelonephritis.

The clinical manifestations of chronic pyelonephritis are usually nonspecific. Some patients have recurrent acute symptomatic exacerbations of renal infection. Others have no clear-cut symptoms of infection despite persistent bacteriuria. Sometimes, patients may complain of vague flank discomfort, abdominal pain, or intermittent low-grade pyrexia. The improvement of symptoms after a trial of antimicrobial therapy may clarify an association between mild symptoms and a persistent renal infection.

TREATMENT OF UPPER TRACT INFECTIONS

Therapeutic Principles

The following should be considered whenever an upper urinary infection is diagnosed and treated:

1. A laboratory confirmation of infection is essential. The clinical findings of an invasive upper tract infection can be mimicked by many illnesses and, for patients with pyelonephritis, the antimicrobial susceptibility of infecting organisms must be determined to facilitate optimal therapy.
2. Bacteriuria by itself is a nonspecific finding. Patients with asymptomatic bacteriuria, particularly elderly patients and patients with indwelling catheters, frequently have bacteriuria but without renal infection being present or as a cause for acute clinical deterioration.[3]
3. The initial antimicrobial selection is based on known or presumed organism susceptibility and patient tolerance. The clinical evidence base to support any specific regimen is limited. Many clinical studies enroll patients presenting with both acute nonobstructive pyelonephritis and complicated urinary infection, and the relevance of reported outcomes to acute pyelonephritis alone is difficult to assess.
4. An antimicrobial that provides adequate blood and tissue levels as well as a high urinary level is preferred for the treatment of invasive renal infection. Agents that provide high sustained medullary antimicrobial levels are more effective for upper tract infections.[158]
5. Suppurative and obstructive complications frequently complicate acute pyelonephritis, occurring in 5% to 15% of cases. Patients who present with life-threatening infection, who do not respond within 72 hours of antimicrobial therapy, who deteriorate after therapy is started, or who rapidly recur after therapy is discontinued must be investigated urgently to exclude an obstruction or abscesses that require surgical intervention.
6. If the urine culture subsequently reports growth of an organism resistant to the empiric antimicrobial therapy, the antimicrobial should be changed to a susceptible agent even if there has been a clinical response. Despite apparent improvement, these patients will usually rapidly relapse following the discontinuation of antimicrobial therapy.[13,159]
7. Clinical improvement may not equate with the permanent eradication of bacteriuria. A urinary infection is usually a recurrent disease and patients should be aware of this possibility. However, follow-up urine cultures are not necessary to document a bacteriologic cure unless symptoms recur or if the patient is pregnant.

Acute Pyelonephritis

A suspected clinical diagnosis of acute pyelonephritis requires an urgent assessment and the prompt institution of therapy. Appropriate diagnostic tests, including a urine culture prior to antimicrobial therapy, complete blood count (CBC) and creatinine for all cases, and blood cultures for subjects with more severe presentations, should be obtained. In addition to the prompt institution of empiric antimicrobial therapy and appropriate supportive measures, initial therapeutic decisions include whether hospitalization is required and whether early diagnostic imaging studies are indicated to exclude an obstruction or other complicating factors (Fig. 23.1).

Hospitalization

Only 10% to 30% of nonpregnant women with acute uncomplicated pyelonephritis will require hospitalization[160]; a higher proportion of individuals with a complicated urinary infection presenting as pyelonephritis require hospitalization. Patients with severe clinical presentations, including severe costovertebral angle pain, rigors, high fever (>38.5°C), severe nausea or vomiting, and hemodynamic instability should be hospitalized for the initial investigation and parenteral antibacterial treatment. Intravenous fluids, analgesics and antiemetics, and bed rest are usually prescribed during the initial 24 to 48 hours. Hypotension and diminished urine output should be identified early and managed appropriately. Patients with severe sepsis or septic shock should be cared for in a critical care unit. Women who are pregnant or for whom there is diagnostic uncertainty require a more careful follow-up, and hospital admission is recommended in these cases. Where it is unclear whether hospitalization is necessary, an initial dose of parenteral therapy with observation for 12 to 24 hours is an alternate approach. If the

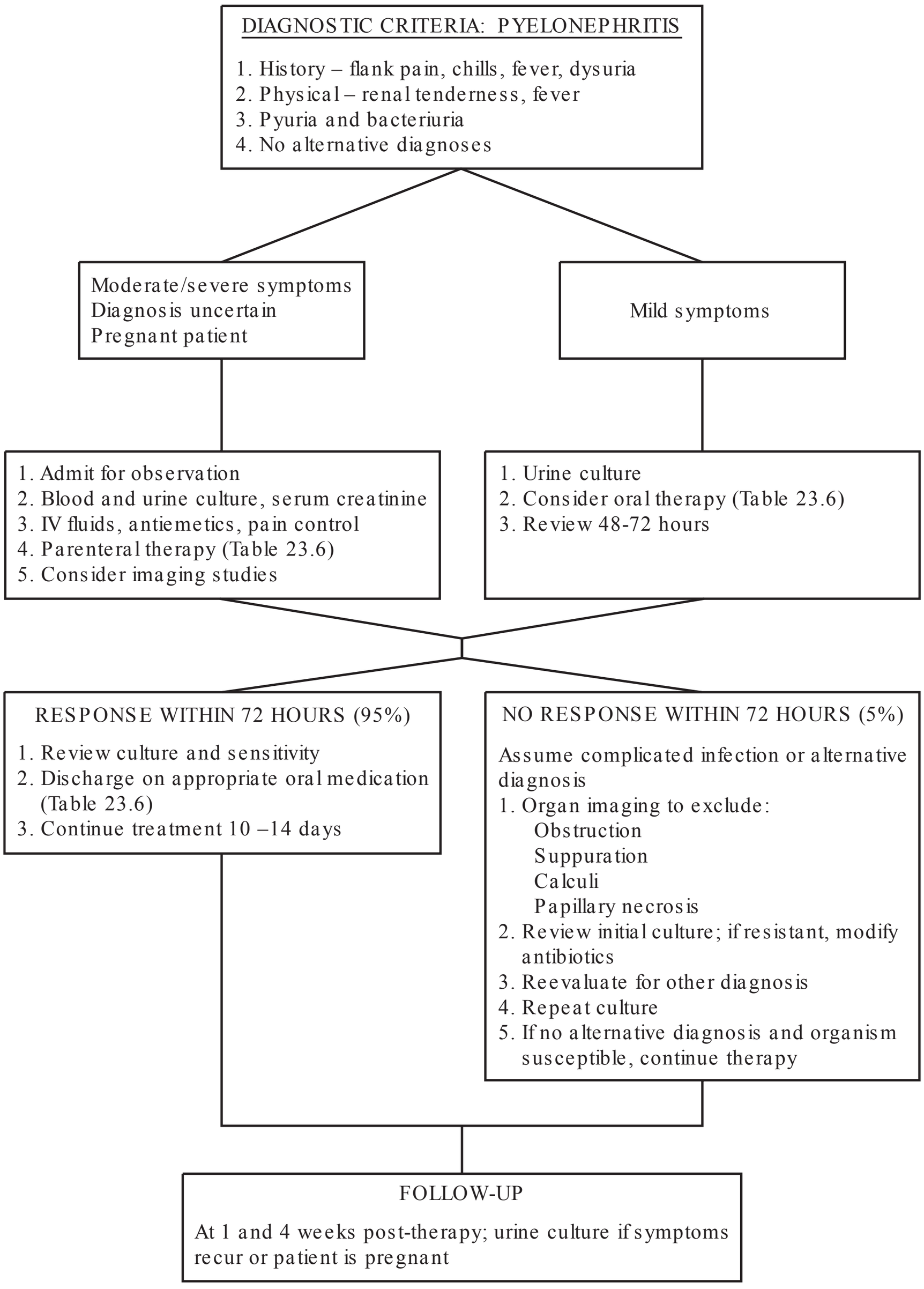

FIGURE 23.1 The management of pyelonephritis.

patient is stable without nausea or vomiting after the initial parenteral therapy and management of systemic symptoms, a discharge home to complete oral therapy is appropriate.

Patients with acute pyelonephritis may be only mildly or moderately symptomatic with low-grade fever, mild flank pain, and few constitutional symptoms. These patients may be managed as outpatients with an oral antimicrobial regimen (Fig. 23.1).[160,161] Specific criteria to identify patients for whom outpatient therapy is appropriate include those who are hemodynamically stable, those who are able to tolerate oral medications, those who are anticipated to be compliant, and those with absent or low-grade fever (38°C or less).

Several studies report similar outcomes for parenteral and oral therapy, with unsatisfactory outcomes in 10% of either group.[10,160,161] Pinson and associates[11] reviewed management and outcomes of 111 febrile women presenting to an emergency room with acute pyelonephritis. Eighty three (75%) were not hospitalized. Women hospitalized for management were older, more frequently had diabetes or known genitourinary abnormalities, and were more ill, as evidenced by vomiting and a higher temperature. Management of nonhospitalized patients usually included a single parenteral antimicrobial dose followed by oral therapy.[11] Nine (12%) of 75 outpatients returned in the follow-up with continuing symptoms of acute pyelonephritis, usually within 24 hours, and 7 of these subsequently required admission but were ultimately cured. The authors concluded that most febrile women with acute pyelonephritis could be treated as outpatients, but an early follow-up after initiation of therapy was recommended. Elkharrat et al.[161] used an algorithmic approach for women presenting to the outpatient department with acute pyelonephritis. All patients received a single intravenous dose of a fluoroquinolone after cultures were obtained, and had an ultrasound examination of the abdomen and pelvis. Patients with normal ultrasonography could be discharged. Of 68 patients, 10 were discharged directly from the emergency ward, 48 after a 12 to 24 hour stay in the observation unit, and 10 were hospitalized. Favorable outcomes occurred in all but one woman.

Parenteral Antimicrobial Therapy

Initial empiric parenteral therapy is indicated for patients with severe symptoms or who cannot tolerate oral therapy. The goal is to step down to oral therapy as soon as the patient is clinically stable and urine culture results are available, usually 48 to 72 hours after the initiation of therapy. A full course of parenteral therapy is necessary only for patients who continue to be unable to tolerate oral therapy or who are infected with a resistant organism for which no oral therapy is available.[162] The Infectious Diseases Society of America (IDSA) guidelines provide evidence-based recommendations for empiric antimicrobial therapy.[163] An aminoglycoside with or without ampicillin, parenteral fluoroquinolone, or extended spectrum cephalosporin or penicillin are recommended as first-line parenteral antimicrobial options. Any empiric regimen for initial treatment of patients with acute pyelonephritis should include in its antibacterial spectrum at least 95% of the potential organisms in the population treated. The selection of a "standard" antimicrobial regimen for empiric therapy requires knowledge of antimicrobial susceptibilities in the community or the individual facility.

A standard approach for initial therapy, pending culture and susceptibility results, is an aminoglycoside, such as gentamicin or tobramycin, in a dose of 3 to 5 mg per kilogram every 24 hours, combined with ampicillin 1 g every 4 hours (Table 23.6). The ampicillin therapy provides coverage for E. faecalis, an uncommon pathogen in younger populations. For the penicillin-allergic patient, an aminoglycoside alone may be adequate empiric parenteral therapy. Aminoglycoside-related nephrotoxicity and ototoxicity are unusual if high sustained trough levels are avoided and the duration of therapy is 4 days or less. Aminoglycoside levels should be monitored if the patient has

23.6 Selected Therapeutic Regimens Appropriate for the Treatment of Pyelonephritis in Patients with Normal Renal Function

Parenteral Therapy
Recommended
Ampicillin,[a] 1 g q4–6h plus aminoglycoside (gentamicin, tobramycin, or netilmicin at 3–5 mg/kg q24h, or amikacin 15 mg/kg q24h)
Ceftriaxone, 1–2 g q24h
Ciprofloxacin, 400 mg q12h
Levofloxacin, 500–750 mg OD
Alternatives
Cefotaxime, 1 g q8h
Ceftazidime, 1 g q8–12h
Ceftizoxime, 1 g q8–12h
Aztreonam, 1 g q8h
Piperacillin-tazobactam, 3.375 g q8h
Ticarcillin-clavulanate, 3 g/100 mg q4h
Ampicillin-sulbactam, 2 g/0.5–1.0 g q6h
Piperacillin, 2 g q6h plus aminoglycoside
Ofloxacin, 200–400 mg q12h
Meropenem, 500 mg–1g q8h
Ertapenem, 1g OD
Oral Therapy
Recommended
Norfloxacin, 400 mg b.i.d.
Ciprofloxacin, 500 mg b.i.d.
Levofloxacin, 500 mg or 750 mg OD
Alternatives
Amoxicillin, 500 mg t.i.d.[a]
Amoxicillin-clavulanate, 500 t.i.d. or 875 mg b.i.d.
Cefuroxime axetil, 500 mg b.i.d.
Cefixime, 500 mg OD
Cephalexin,[b] 500 mg q.i.d.
Ofloxacin, 400 mg b.i.d.
Trimethoprim-sulfamethoxazole,[b] 160/800 mg b.i.d.
Trimethoprim,[b] 100 mg b.i.d.

[a] For Enterococcus spp. or Streptococcus spp.
[b] If the organism is known to be susceptible.
q, every; h, hour; b.i.d., twice a day; t.i.d., three times a day; OD, once daily; q.i.d., four times a day.

renal impairment or will be continued on therapy longer than 5 days. Trough levels should not exceed 2 μg per milliliter for gentamicin and tobramycin or 5 μg per milliliter for amikacin. If a high prevalence of resistance to gentamicin or tobramycin is present, amikacin may be the aminoglycoside of choice for empiric treatment, especially for nosocomial pyelonephritis where more resistant isolates may be anticipated.

Parenteral fluoroquinolones such as ciprofloxacin, ofloxacin, and levofloxacin are also useful agents for the treatment of acute pyelonephritis.[163] The extended spectrum cephalosporins, including ceftriaxone, cefotaxime, ceftizoxime, and ceftazidime, have each been studied as single-agent alternatives to the combination of ampicillin and an aminoglycoside, with cure rates for acute pyelonephritis of about 90% in patients with normal renal function and a normal urinary tract.[163–165] The addition of an aminoglycoside to extended spectrum therapy with cefotaxime did not improve outcomes in women with acute pyelonephritis.[166] Cefazolin should not be used alone for the empiric treatment of invasive renal infection owing to the relatively high prevalence of resistance to first-line cephalosporins among community-acquired and nosocomial pathogens.[163]

Extended spectrum penicillin derivatives have been prospectively compared to the aminoglycoside with ampicillin regimen (Table 23.4).[163–165] In clinical studies reported to date, all regimens appear equivalent. Piperacillin is preferred over the third-generation cephalosporins if Pseudomonas aeruginosa and Enterococcus faecalis are probable etiologic agents. Piperacillin should generally be used in combination with an aminoglycoside but piperacillin/tazobactam may be used alone. Aztreonam is a monobactam β-lactam antibiotic, with an antibacterial spectrum limited to aerobic gram-negative rods, including P. aeruginosa.[167] It has been used successfully to treat patients with acute pyelonephritis. The β-lactam inhibitors in combination with a β-lactam antibiotic are also effective and equivalent to other regimens, and may be drugs of choice for hospital-acquired infections where resistant organisms are a concern. Piperacillin-tazobactam, ampicillin-clavulanic acid, and ticarcillin-clavulanic acid are all effective regimens. The prevalence of infections with an extended spectrum β-lactamase producing E. coli is increasing globally. These strains, if suspected, require empiric treatment with a carbapenem: meropenem, ertapenem, or dorapenem.[168,169] Vancomycin may be required to treat enterococci or staphylococci resistant to the β-lactam antibiotics. Newer parenteral antimicrobial agents are more expensive than the standard therapy of ampicillin plus aminoglycoside and, for susceptible organisms, have not been shown to have improved outcomes. Potential therapeutic advantages must be weighed against the increased expense.

Oral Antimicrobial Therapy

The IDSA guidelines recommend a fluoroquinolone for the initial oral empiric treatment of pyelonephritis.[163] Ciprofloxacin, norfloxacin, ofloxacin, and levofloxacin are all efficacious for the treatment of acute pyelonephritis; fluoroquinolones with limited renal excretion, such as moxifloxacin, should be avoided. Clinical trials document the effectiveness of ciprofloxacin 500 mg twice daily or 1,000 mg extended release daily[13] or levofloxacin 500 to 750 mg daily for acute, uncomplicated pyelonephritis.[170]

A randomized clinical trial compared oral trimethoprim/sulfamethoxazole (TMP/SMX) for 14 days or oral ciprofloxacin for 7 days in 378 women with acute pyelonephritis.[13] For either regimen, an initial parenteral dose of ceftriaxone or ciprofloxacin, respectively, could be given. Therapy was not modified once antimicrobial susceptibilities were obtained (i.e., therapy with TMP/SMX was continued even if the infecting isolate was found to be resistant). The regimens were equally effective when the infecting organism was susceptible to the antimicrobial given. However, TMP/SMX therapy was not effective when given by itself to patients with TMP/SMX-resistant organisms so a higher rate of resistance of E. coli to trimethoprim/sulfamethoxazole resulted in the ciprofloxacin arm being superior overall, with a 96% cure rate at 4 to 11 days and 85% at 22 to 44 days posttherapy. Thus, TMP/SMX is effective therapy but, given the relatively high prevalence of resistance of community-acquired E. coli, this agent is recommended only when the infecting organism is known to be susceptible.

Stamm and colleagues[14] compared 2- and 6-week oral regimens of TMP/SMX or ampicillin for the outpatient management of women with pyelonephritis. They found TMP/SMX to be superior to ampicillin for both treatment durations; the 6-week regimen did not improve cure rates. For TMP/SMX, 83% to 90% of women remained cured at 6 weeks following the discontinuation of antibiotics. Amoxicillin by itself should not be used for nonenterococcal or nonstreptococcal infection because of high community rates of resistance to gram-negative organisms and a tendency to promote colonization with resistant organisms that may cause subsequent infections.[163] However, amoxicillin in combination with the β-lactamase inhibitor clavulanic acid can be prescribed as an oral regimen for selected resistant organisms.

Cronberg et al.[171] enrolled 171 patients, about 60% of whom were women with acute pyelonephritis, into a comparative study of initial cefuroxime for 2 to 3 days followed by ceftibuten 200 mg twice a day (b.i.d.) or norfloxacin 400 mg b.i.d. to complete 10 days once the fever had subsided and the culture results were available. The norfloxacin treatment arm was superior for the eradication of bacteriuria. Sanchez et al.[172] enrolled 105 women into a study comparing ceftriaxone 1 g daily for 10 days or a single initial dose of ceftriaxone followed by oral cefixime for 9 days. The cure rate was 91% for both groups.

Pregnant Women

It is recommended that pregnant women with acute pyelonephritis should be admitted to the hospital, at least for the first several days, to ensure an adequate response to antimicrobial therapy and pending culture results confirming the

infecting organism and susceptibilities.[92] Parenteral outpatient therapy with close monitoring is an option for selected patients in the first and early second trimester.[92] Recommended empiric parenteral regimens are ceftriaxone or an aminoglycoside with or without ampicillin. A prospective, randomized trial of intravenous ampicillin and gentamicin, intravenous cefazolin, or intramuscular ceftriaxone found all three to be equally effective for the parenteral treatment of acute pyelonephritis in pregnancy.[173] Gentamicin has been widely used in pregnant women with no evidence for congenital complications.[92] Extended spectrum penicillins may also be used. Ampicillin or cefazolin, by themselves, are not currently recommended because of the high prevalence of antimicrobial resistance in community isolates. Fluoroquinolones are avoided in pregnancy because of adverse fetal effects, and TMP/SMX should be avoided in the first trimester.

Children

Approaches to the antimicrobial management of acute pyelonephritis in children have recently been critically reviewed in a Cochrane collaboration report.[174] This concluded that initial oral compared with parenteral antimicrobial therapy had similar short-term outcomes of the duration of fever and long-term outcomes of persistent kidney damage at 6 or 12 months. In addition, there was no difference in persistent kidney damage when intravenous was followed by oral therapy or only intravenous therapy was given, each for 7 to 14 days. There was also no difference in outcomes with once or thrice daily aminoglycoside dosing. Thus, the selection of parenteral or oral therapy, or whether to initiate a short initial course of intravenous therapy and complete with oral therapy or treat with oral therapy alone, should be based on clinical evaluation. Infants aged 1 month or less require hospitalization for treatment and investigation because of the very high prevalence of concomitant bacteremia and urologic abnormalities.

For parenteral therapy, aminoglycosides or a third-generation cephalosporin are preferred, with TMP/SMX or amoxicillin/clavulanic acid as first-line agents for oral therapy.[175] Fluoroquinolones are avoided because of potential adverse effects on cartilage. Outpatient parenteral therapy is an option.[176] Although the early treatment of infants and young children will limit the duration of acute symptoms, the earlier institution of antimicrobial therapy has not been shown to decrease the subsequent development of renal scars.[177,178]

The Duration of Therapy and Follow-Up

The appropriate duration of therapy to achieve optimal cure rates in patients with acute pyelonephritis is uncertain, and may differ depending on the antimicrobial used. A minimum of 2 weeks of therapy has been routinely recommended, but 10 days is clearly sufficient in many clinical studies[172] and 7 days of ciprofloxacin[13] or 5 days of levofloxacin[170] have been as effective as longer courses of treatment in controlled clinical trials.

Behr et al.[179] reported, in a series of patients admitted with both complicated and uncomplicated acute pyelonephritis, that 26% remained febrile at 48 hours and 13% remained febrile at 72 hours. The median duration of fever was 34 hours. Johnson et al.[106] reported that 30% of women with acute uncomplicated pyelonephritis remained febrile at 2 days, but virtually all were afebrile by 72 hours. Thus, most patients become afebrile within 72 hours following the initiation of therapy; other clinical findings, including renal-angle tenderness, also rapidly improve.

Treatment in the Presence of Impaired Renal Function

In the management of patients with impaired renal function, antimicrobials, which are effective despite decreased renal perfusion, should be selected, whereas agents that might further compromise function should be avoided if possible. Antimicrobial therapy may ameliorate symptoms but frequently will not cure the infection in the presence of moderate-to-severe renal impairment, presumably because of the failure of antimicrobials to access the site of bacterial infection. Long-term suppressive therapy with an oral antimicrobial may be required in selected cases for the management of recurrent symptomatic episodes.

The β-lactam antibacterials have little dose-related toxicity and are relatively safe in patients with renal failure. Patients with impaired renal function are at increased risk of seizures with imipenem and encephalopathy with cefepime. Interstitial nephritis occurs rarely in patients receiving β-lactam antibacterial agents, but the risk of this complication is not increased by coexisting renal impairment. A dose adjustment for renal impairment is required for most β-lactam agents. No untoward consequences of trimethoprim prescribed without sulfonamide have been reported in patients with moderately advanced renal impairment. The fluoroquinolone antimicrobials—ciprofloxacin, ofloxacin, and levofloxacin—are also effective in renal failure. Dosage adjustments are required for patients with creatinine clearance rates less than 30 mL per minute.

Aminoglycosides should be avoided in patients with renal impairment. In the presence of unstable renal function, a rise in blood urea or creatinine levels may be incorrectly attributed to an aminoglycoside. Doxycycline is relatively safe in renal impairment but usually fails to achieve adequate urine levels. Other tetracyclines are contraindicated. Sulfonamides have been associated with a further loss of renal function and should not be used. Methenamine mandelate or other organic salts also are contraindicated in patients with renal impairment.

Recurrent Upper Tract Infection

In prospective studies, about 5% to 10% of women with acute pyelonephritis have a relapse with the initial infecting organism within 4 weeks of completing therapy.[13,14,170,171] Women with acute nonobstructive pyelonephritis are also

at increased risk of both upper and lower tract urinary reinfection, especially within the first year following the initial episode.[51,52] About 50% of patients with urinary infections in the setting of structural or functional genitourinary abnormalities (Table 23.2) recur with either upper or lower tract infection by 6 weeks after therapy, although most recurrences are asymptomatic. The pattern of recurrence is a predictor of infection site, with relapses predictive of renal infections in women and renal or prostate infections in men. For women with a symptomatic relapse, renal infection is presumed and retreatment with a 2-week course of antimicrobial therapy results in cure for most of those with a normal genitourinary tract. Longer courses of antimicrobial therapy (4 weeks or more) may be considered in patients with repeated relapsing symptomatic infections and chronic renal failure or where progressive renal damage is a concern. Men without upper tract abnormalities and with relapses should receive retreatment for 4 to 6 weeks for presumed prostate infection,[180] as prostate localization is the more likely source of relapsing infections in men.

Several principles should be considered in all patients with recurrent infections:

1. The urine culture becomes negative shortly after the institution of effective chemotherapy.[181] The isolation of any quantitative count of the initial infecting organism while antibiotics are being taken is a failure of therapy. Bacterial persistence with positive urine cultures occurs due to inadequate levels of the antimicrobial agent in the urine, the presence of resistant organisms, or patient noncompliance. Continuing the same antimicrobial regimen, even with a clinical response, is inappropriate if urine cultures remain positive with the same pretherapy isolate and if the patient has been compliant.
2. Relapse is frequently associated with urologic abnormalities. Infected renal cysts, calculi, indwelling devices, prostate infection, and a nonfunctioning kidney allow organisms to persist in sites where effective antimicrobial levels are not achieved.[180,182] Patients with relapses require a careful reevaluation including radiologic and urologic investigation. In the absence of urologic or radiologic abnormalities, many women who have a relapse can be cured with a sufficiently prolonged course of therapy. On the other hand, infection recurs in patients with calculi in the kidneys, prostate infection, or a nonfunctioning kidney, even following prolonged treatment courses of several months or years.
3. Clinical trials of the treatment of urinary infections in men have not generally localized infection to the prostate or kidneys; some may have infections at both sites. Owing to the frequency of complicating urologic abnormalities in men with urinary infection, attempts to define a homogeneous group with renal infections and to determine treatment responses have generally been unsuccessful. Treatment should be undertaken only if recurring symptoms or complicating disease suggest these men are at risk of morbidity from infection. A very prolonged course of therapy with TMP/SMX or a fluoroquinolone for 4 to 12 weeks should be prescribed to eradicate a persisting but curable renal or prostate focus. Even with prolonged fluoroquinolone treatment, however, a cure is obtained in 70% or less of men with chronic prostate infection.[180]
4. Antimicrobial concentrations in renal tissue and urine may be markedly diminished in patients with impaired renal function caused by acute or chronic parenchymal renal disease or obstruction. In patients with unilateral renal impairment, the antimicrobial concentration of some agents may be inadequate to inhibit bacterial growth or sterilize urine originating from the diseased kidney.[183] The contralateral normal or hypertrophied kidney may be excreting the antibacterial agent effectively, so the drug does not accumulate in the serum. Excretion of the antimicrobial agent in the urine from the healthy kidney may be adequate to sterilize the bladder urine and make it appear that the bacteriologic outcome is satisfactory despite persistent or continuing bacterial multiplication in the diseased kidney.
5. Patients with frequent reinfections have altered bacterial flora, reflecting the impact of repeated courses of antibacterial agents on the fecal reservoir.[163] Sulfonamides, penicillins, cephalosporins, and fluoroquinolones eradicate susceptible gram-negative organisms within the intestinal tract, which may be replaced by resistant Enterobacteriaceae or Pseudomonas spp. Broad-spectrum antimicrobial agents preferentially excreted in the bile have a greater impact on the fecal flora than do agents preferentially excreted in the urinary tract. The "next infection," if it occurs within a few months, often will be caused by an organism that is resistant to the therapeutic regimen previously prescribed. With frequent reinfections, particularly in patients with devices such as ureteric stents or an indwelling catheter, the pathogens become progressively resistant to antimicrobial therapy.
6. The treatment of asymptomatic bacteriuria should be avoided except for bacteriuria in pregnancy or prior to an invasive urologic procedure likely to be associated with trauma to the genitourinary mucosa.[3] Following from this, posttherapy urine cultures should only be obtained if there is a symptomatic recurrence.
7. Patients with long-term indwelling suprapubic or urethral catheters are always bacteriuric. Although transient suppression may occasionally yield a negative urine culture, bacteriologic failure is predictable. These patients should only be treated for symptomatic episodes, as efforts to prevent infection with continuous suppressive antibacterial regimens select multiply resistant organisms and have not been shown to prevent morbidity.[184]

8. The successful treatment of struvite stones requires the complete eradication of all stone material. Antimicrobial therapy is an adjunct to maintain sterile urine while a complete stone dissolution is achieved. Advances in the endourologic treatment of infected renal calculi make it possible for most patients with renal stones to be treated with complete removal of all calculous material. The necessary duration of antimicrobial therapy following lithotripsy, however, is controversial.

THE PREVENTION OF PYELONEPHRITIS

Some women with recurrent acute uncomplicated urinary infections may present as recurrent pyelonephritis, although this pattern is uncommon. Subsequent infections in these women can be effectively prevented by chemoprophylactic regimens. The most important intervention to prevent infections in patients with complicated urinary infections is to correct or optimize management of the underlying abnormality that is promoting infection. Recurrent symptomatic infections from a renal or prostate focus in selected patients with abnormalities that cannot be corrected may be suppressed indefinitely without "cure" by long-term treatment with suppressive antimicrobial regimens. Immunization has also been explored for the prevention of recurrent urinary infections. Animal studies have reported some success for the prevention of both cystitis and pyelonephritis through vaccination with E. coli antigens, but effectiveness in humans is not yet documented.[17,185]

Antimicrobial Prophylaxis

Prophylaxis of symptomatic acute uncomplicated cystitis or nonobstructive pyelonephritis in women is highly effective (see Chapter 22). Prophylactic antimicrobial therapy is an option for children[186] or women[187] who experience three or more episodes of symptomatic infections, either cystitis or pyelonephritis, within 1 year. Effective and widely used regimens include nitrofurantoin 50 mg monohydrate or 100 mg monohydrate/macrocrystals, TMP/SMX 0.5 tablet, or trimethoprim 100 mg, all taken once daily at bedtime, or postintercourse. TMP/SMX is also effective taken as a 0.5 tablet every second day. Both symptomatic and asymptomatic urinary infections are prevented with current strategies of TMP/SMX prophylaxis in renal transplant recipients.[188]

The need for and the efficacy of antimicrobial prophylaxis for children with vesicoureteral reflux is controversial. Although some guidelines have recommended continuous prophylactic antimicrobials for these patients, there remains considerable controversy whether this approach prevents the development of new or progressive renal scars, or renal impairment.[186,189,190] In an open randomized trial of Swedish boys and girls with reflux, prophylactic antimicrobial therapy significantly decreased subsequent febrile urinary infection, for girls only, during 2 years of follow-up.[191] Additional prospective, randomized clinical trials to answer the question of whether long-term prophylactic antimicrobial therapy prevents renal scarring and preserves renal function for children with vesicoureteral reflux compared with an optimal management of symptomatic episodes alone are ongoing.[190]

Prevention in Pregnancy

The prevention of acute pyelonephritis in pregnant women should be a part of the antenatal care of all patients.[91] The prevalence of asymptomatic bacteriuria during pregnancy has varied from 2% to 7%, with an incidence of acquiring bacteriuria about 1%—similar to the incidence of new infections in age matched women who are not pregnant.[91] In the absence of a screening and treatment program, acute pyelonephritis occurs in 20 to 30 per 1,000 pregnant women. With an intervention program for the treatment of asymptomatic bacteriuria, acute pyelonephritis is reduced to 3 to 5 per 1,000 pregnant women.[91,92] Pregnant women should be screened for bacteriuria once at 12 to 16 weeks' gestation, and those with positive cultures should be treated. Repeated screening cultures later in the pregnancy are not recommended for asymptomatic women whose initial culture is negative. Pregnant women who have had bacteriuria or symptomatic urinary infections should be followed with urine cultures throughout pregnancy, usually every 4 weeks. Continuous low-dose prophylaxis until 6 weeks after the delivery is recommended for patients with recurrent infections.

The choice of a regimen for the treatment of asymptomatic bacteriuria or for the prophylaxis of symptomatic or asymptomatic infections in a pregnancy must address the potential adverse effects for the fetus.[92] Although TMP/SMX is generally considered safe during pregnancy, concerns about a potential teratogenic effect from the trimethoprim component limits its use, especially during the first trimester. Penicillins and cephalosporins are safe in pregnancy; nitrofurantoin is safe, but is avoided at term because of the potential hemolysis of fetal hemoglobin. Ampicillin or a cephalosporin may be used if the organism is known to be susceptible. Cephalexin may also be used for prophylaxis, at 250 mg per day, and nitrofurantoin may be used for either treatment or prophylaxis. Fluoroquinolones are contraindicated because of the potential harmful effects on fetal cartilage development.

Prevention of Catheter-Associated Upper Tract Infections

Catheter-associated infections are the most common hospital-acquired infection.[184,192,193] Prophylactic antimicrobial therapy is not recommended because of the predictable emergence of infections with organisms with increased resistance.[184] The most important interventions to prevent these infections are to limit use of an indwelling urethral catheter or, if a catheter is necessary, to minimize the duration of catheterization. In addition, catheter insertion using sterile techniques and appropriate catheter maintenance, such as

maintaining a closed drainage system, are necessary. Infection control programs must ensure that prevention practices are current, and should monitor adherence to appropriate practice and patient infection rates.

The Prevention of Infection in Patients with Neurogenic Bladders

Bacteriuria is an anticipated complication following neurologic injury or disease complicated by a neurogenic bladder. Prophylactic antimicrobials are not recommended in spinal cord–injured patients managed with intermittent catheterization.[194] Although TMP/SMX, trimethoprim, or nitrofurantoin prophylaxis may prevent infections in the acute or early injury phase, prophylaxis is not effective in preventing symptomatic infections in the long term, and the induction of resistant bacteria in subsequent infections outweighs any short-term benefits. Maintenance of a low-pressure voiding system is essential to prevent complications of urinary infection.[104]

Continuous Suppression

Continuous long-term suppression may be considered for highly selected patients with recurrent symptomatic relapses despite optimal antimicrobial therapy. Such patients include those with renal calculi or obstructive lesions that cannot be corrected, infections in a nonfunctioning kidney, and men with chronic bacterial prostatitis.

There is relatively little evidence addressing optimal approaches to suppressive therapy. Sheehan and colleagues[195] compared 12 to 24 weeks of norfloxacin therapy for complicated recurrent urinary infections in a prospective, randomized, and blinded study. The longer antimicrobial course served as prophylaxis, suppressive, or curative therapy for different patients and, overall, led to fewer failures or reinfections from 12 to 24 weeks compared to placebo. Chinn and associates[196] demonstrated that antibacterial suppression is effective in patients with renal calculi in whom stones cannot be fully removed. No patients had further loss of renal function during a cumulative 77 years of continuous observation despite the presence of stones and partial obstruction. Renal calculi increased in size in only 4 of the 22 patients during the period of antibacterial suppression, and 4 of the 6 patients with impaired renal function had a decrease in serum creatinine levels while receiving suppressive therapy.

The antimicrobial dosage required for the maintenance of suppressive therapy is not well studied but generally, after an initial 2 to 4 weeks at full therapeutic doses, one-half the treatment dose is continued. In stable patients without a recurrence of bacteriuria, the dose may sometimes be reduced further. Patients on suppressive regimens should be reviewed periodically to ensure compliance and to monitor renal function. "Breakthrough" symptomatic infections occur occasionally. If the initial infecting organism has reappeared, then susceptibility testing will determine whether resistance has developed or if there is a suboptimal dose of medication. New infections may occur with reinfections with a new pathogen, and organisms isolated from a reinfection will usually be resistant to the antimicrobial used for the suppressive therapy. If this occurs, the patient should receive a 7- to 10-day course of an appropriate alternate antimicrobial to eradicate the new pathogen, without discontinuing the antimicrobial being taken for suppressive therapy. No guidelines have been developed to address the duration for which suppressive therapy is continued. If the initial indications for continuous suppressive therapy persist, the antimicrobial therapy may be continued indefinitely.

VIRAL INFECTIONS OF THE KIDNEY

Viruses causing systemic diseases are frequently isolated from the urine. Utz,[197] in 1974, described 16 viruses that were reported to have been isolated from the urine. The number is likely substantially greater now given the many new viruses described and the more sensitive diagnostic techniques. However, viruria commonly occurs during viremic infections as a manifestation of acute generalized diseases with limited, if any, involvement of the kidneys. Many viruses multiply in the tubular epithelium and are excreted in the urine as exfoliated infected cells. In only a few instances are there acute inflammatory changes in the renal tissue or in symptoms of clinical renal illness.

Clinical disease with parvovirus B19, cytomegalovirus, or adenovirus, usually following viral reactivation, occurs in some immunocompromised patients including renal transplant recipients, hematopoietic stem-cell transplant recipients, and occasionally, HIV patients. Hemorrhagic cystitis is the more common clinical presentation, but nephropathy is well described.[198,199] Viral infections of the kidney following a renal transplant are discussed more fully in another chapter.

The hemorrhagic fever viruses frequently cause renal impairment and are important health problems in many parts of the world.[200] Viral hemorrhagic fever with renal syndrome, which occurs in Korea, Scandinavia, the Soviet Far East, and the Balkans, is caused by members of the Bunyaviridae genera. The prototype agent is the Hantaan virus, originally isolated in Korea in 1978. Rodents are the reservoirs for these agents, and transmission to humans occurs by respiratory aerosols with no intermediary vector. Secondary cases have not been described in humans. High fever, myalgias, severe headaches, and a petechial rash characterize the illness. Thrombocytopenia is common. Hypotension and oliguria develop on about the fifth day of the fever. A pathologic examination discloses widespread capillary damage with extravascular leakage of plasma and red cells. Acute oliguric renal failure with massive proteinuria is a frequent complication. Renal biopsy specimens show extensive necrosis of the tubular epithelium with anatomically normal glomeruli except for the presence of extravasated red cells and protein-rich fluid. The overall mortality can be markedly reduced by supportive therapy, including dialysis, and in recent series has been 5% or less.

Rubella, varicella zoster, measles, and cytomegalovirus are commonly isolated during clinical illnesses with these viruses, but renal disease attributed to these agents has not been recognized.[197] Mumps frequently produce a transient renal impairment with a reduction in glomerular filtration together with significant proteinuria and hematuria. These abnormalities disappear within 1 month of resolution of the illness. Rare fatal cases with mumps interstitial nephritis have been reported.[201] Histologic studies suggest that viral multiplication in renal tubular cells induces these changes. Coxsackie B virus has also been associated with mild renal impairment. Infectious mononucleosis may have renal manifestations; hematuria and proteinuria occur in 11% and 18% of patients, respectively.[202] Acute renal failure owing to interstitial nephritis with few glomerular changes has also been described in isolated case reports.

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CHAPTER

24 Renal and Perirenal Abscesses

Neha D. Nanda • Louise M. Dembry

Bacterial infections of the kidney and perinephric space include a spectrum of pathologic conditions that can be divided into intrarenal and perirenal abscesses. Both conditions are suppurative infections localized either within the parenchyma of the kidney (intrarenal abscess, i.e., renal cortical abscess and corticomedullary abscess) or within the perirenal fascia external to the kidney capsule (perinephric abscess), and each can be identified by specific diagnostic techniques. The incidence of intrarenal and perirenal abscesses ranges from one to 10 cases per 10,000 hospital admissions. In the preantibiotic era, most cases were caused by hematogenous seeding from distant foci of infection and were predominantly in young males without an antecedent history of renal disease. Currently, most cases occur as a complication of urinary tract infection and affect males and females with equal frequency. The incidence increases with age and if an abnormality of the genitourinary tract exists. This chapter covers only the more common types of these renal and perirenal infections.

INTRARENAL ABSCESS

Renal Cortical Abscess (Renal Carbuncle)

Etiology

A renal carbuncle (from the Latin, carbunculus, or "little coal") is a circumscribed, multilocular abscess of the renal parenchyma, which forms from a coalescence of multiple cortical microabscesses (Fig. 24.1). It is most commonly caused by staphylococci (Staphylococcus aureus) and is the result of metastatic spread from a primary focus of infection elsewhere in the body, most commonly the skin. Renal carbuncles were first described by Israel in 1905 in a presentation before the Free Society of Berlin Surgeons.[1] Although numerous reports and reviews[2–8] have been published since Israel's initial description, the total number of reported cases of renal carbuncle remains relatively small.

Pathogenesis

A renal cortical abscess results from a primary focus of infection elsewhere in the body. Common primary foci are cutaneous carbuncle, furunculosis, cellulitis, paronychia, osteomyelitis, endovascular infection, and infection of the respiratory tract. Important predisposing conditions that increase the risk of bacteremia and hematogenous spread are injection drug use, hemodialysis, and diabetes mellitus. S. aureus is the most common causative agent (90%) and infects the cortex of the kidney by hematogenous dissemination from the primary focus, often resulting in several interconnecting furuncles or microabscesses. Coalescence may occur with progression of the infection to a lesion consisting of a fluid-filled mass with a relatively thick wall. Rarely, the process may extend to the periphery of the renal cortex and rupture through the capsule, leading to formation of a perinephric abscess. The majority of renal cortical abscesses are unilateral (97%) single lesions (77%) occurring in the right kidney (63%), and are not associated with perinephric abscesses (90%). The reason for unilateral localization is not clear, although diminished resistance of the kidney resulting from previous disease or injury, including trauma, has been cited as a predisposing factor.[9] Infrequently, ascending infection causes a renal cortical abscess.[10,11] Because the interval between the original staphylococcal infection and the onset of clinical symptoms of a renal cortical abscess may vary from a few days to many months (average time of approximately 7 weeks),[9] the primary focus of infection may have healed and is not apparent in one third of affected patients.[5,7]

Clinical Features

Renal cortical abscesses are three times more common in males than females. The disease occurs at all ages but is most common between the second and the fourth decades of life.[9] The clinical picture of a renal cortical abscess is nonspecific. Most patients have chills, fever, and abdominal or back pain.[5,7,9] Some may have a palpable flank mass. Others present with a clinical picture of fever of undetermined origin, with few or no localizing signs.[12] Most patients have no urinary symptoms[9] because the abscess occupies a circumscribed area within the parenchyma of the kidney, which may not communicate with the excretory passages.

Physical examination often reveals tenderness in or near the region of the kidney. Pain on fist percussion of the

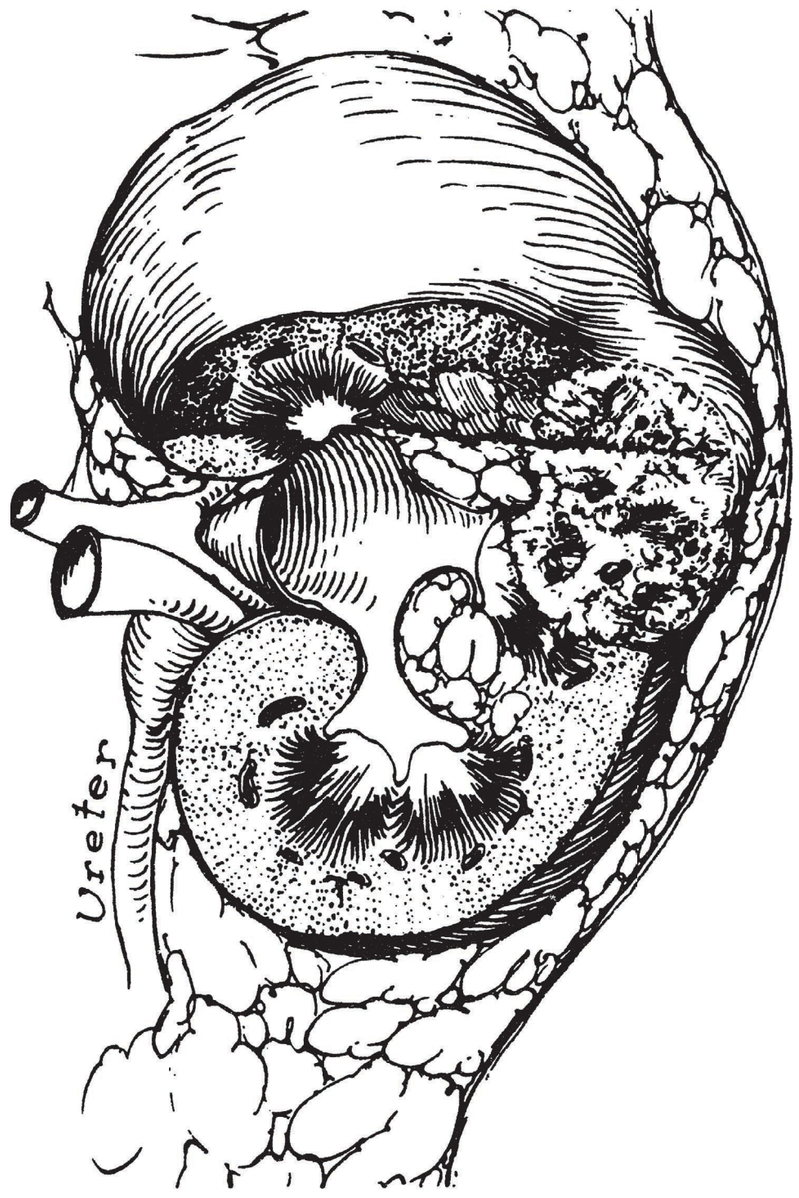


FIGURE 24.1 Diagram of the pathogenesis of a staphylococcal renal carbuncle. (From Andriole VT. Renal carbuncle. *Medical Grand Rounds*. 1983;2:259, with permission.)

costovertebral angle is the most constant physical finding, often accompanied by moderate muscle rigidity in the upper abdominal and lumbar muscles. A flank mass or a bulge in the lumbar region, with loss of the natural concave lumbar outline, may be present. Examination of the chest on the affected side may be abnormal, with decreased respiratory excursion, tenderness over the lower ribs, dullness, diminished breath sounds, increased fremitus, or rales.

Basic laboratory data are variable. Peripheral white blood cell counts are moderately elevated in 95% of patients.[9] The urinalysis usually presents no pathognomonic findings. Proteinuria, pyuria, or microscopic hematuria are usually present and a Gram stain of the urine will demonstrate the pathogen if the abscess communicates with the collecting system of the kidney. However, negative urinalyses are seen in most patients and blood cultures are usually negative.[9]

Diagnosis

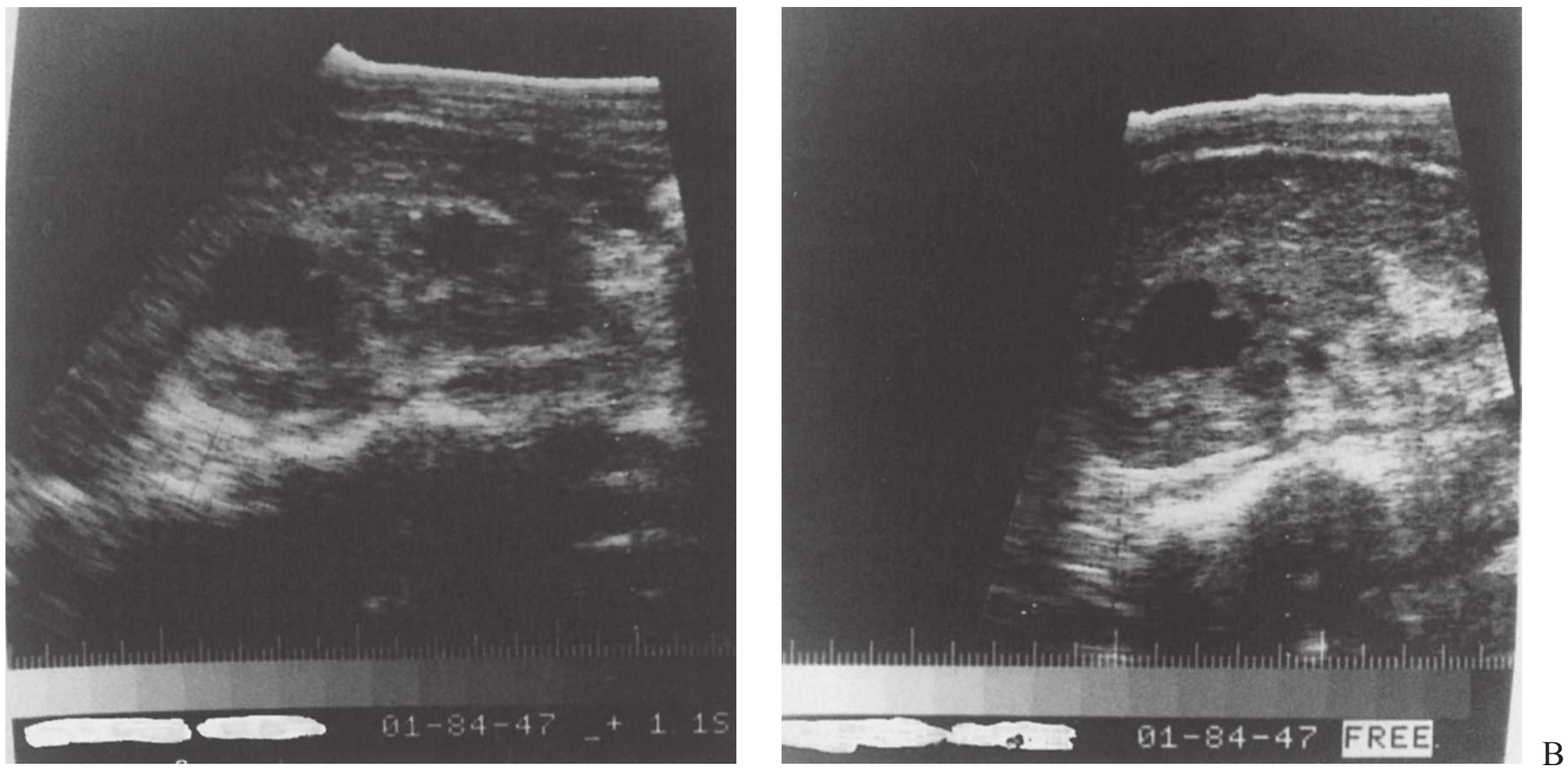
Renal cortical abscesses must be differentiated from other space-occupying lesions in the kidney. Renal tumors, cysts, intrarenal abscesses caused by aerobic gram-negative bacilli, and perinephric abscesses can mimic renal cortical abscesses. In the past, surgical exploration was performed to differentiate the renal mass from a carcinoma. The clinical presentation of a renal cortical abscess is nonspecific and not helpful in differentiating this disease from a renal tumor or perinephric abscess. Chills, fever, malaise, and back pain may be seen in each. A renal cortical abscess on the anterior surface of the kidney may produce abdominal symptoms and lead to an erroneous diagnosis of an intra-abdominal process. Renal cortical abscesses may also be confused with abscesses of the renal medulla, particularly in children.[7,9–11,13–15] Radiologic techniques can define the character of the renal mass and establish the correct diagnosis.[16–25]

In intravenous pyelograms, a renal cortical abscess appears as a mass of diminished density, frequently associated with distortion of the calyces, infundibulum, and renal outline. An abscess that extends to the periphery of the renal cortex may produce sufficient edema of the renal capsule to obliterate a segment of the perirenal fat shadow. However, there is no displacement of the kidney, as is frequently seen with a perinephric abscess. Thus, an abnormal intravenous pyelogram that demonstrates an intrinsic mass with calyceal distortion, but without displacement of the kidney in a patient with sterile urine, suggests a diagnosis of renal cortical abscess or tumor.

Ultrasonography has been extremely helpful in establishing the diagnosis of renal cortical abscess.[9] Renal ultrasonography is easily available, cost effective, and there is no exposure to radiation or contrast. Renal ultrasonography can provide morphologic detail of the kidneys; is capable of identifying cystic lesions, tumorlike masses, or abscess cavities; and can show the size and location of the lesion. Early in the development of a renal cortical abscess, however, internal echoes may be present, giving the appearance of a solid or semisolid mass. Because these findings are compatible with either a renal cortical abscess or tumor, computed tomography (CT) may be performed to define the lesion further and to establish the correct diagnosis.[26,27] Another disadvantage of renal ultrasonography is its dependence on the operator and the body habitus of the patient.[27] After coalescence, an abscess can be identified by ultrasound as a fluid-filled mass with a relatively thick wall (Fig. 24.2). Ultrasonography also can be used to guide aspiration of the lesion and to follow its resolution with antibiotic treatment[7,24,28] (Fig. 24.3).

CT is the most accurate noninvasive technique currently in widespread use and permits detection of abscesses smaller than 2 cm.[29–31] Contrast-enhanced CT is useful if ultrasonography is negative or equivocal and allows for the detection of pathologic lesions in the renal cortex and medulla in early stages. CT is also useful as a guide to percutaneous aspiration of an abscess and to follow a known lesion. An abscess appears as a sharply demarcated low-density lesion on CT. The abscess does not enhance with contrast because of its avascular nature; however, the wall of the abscess enhances because of the presence of dilated and inflamed vessels.[28,30,31] The finding of gas in a low density mass is pathognomonic for an abscess.[30]

Magnetic resonance imaging (MRI) is another noninvasive technique that is as accurate as CT to diagnose renal abscess and define the extent of involvement. With an MRI there is no exposure to radiation and ionizing contrast.[27] MRI with gadolinium and contrast-enhanced CT scans have comparable sensitivity to detect renal abscesses. Noncontrast CT and renal ultrasonography are not as good to detect renal parenchymal pathology.[32] To differentiate between a renal



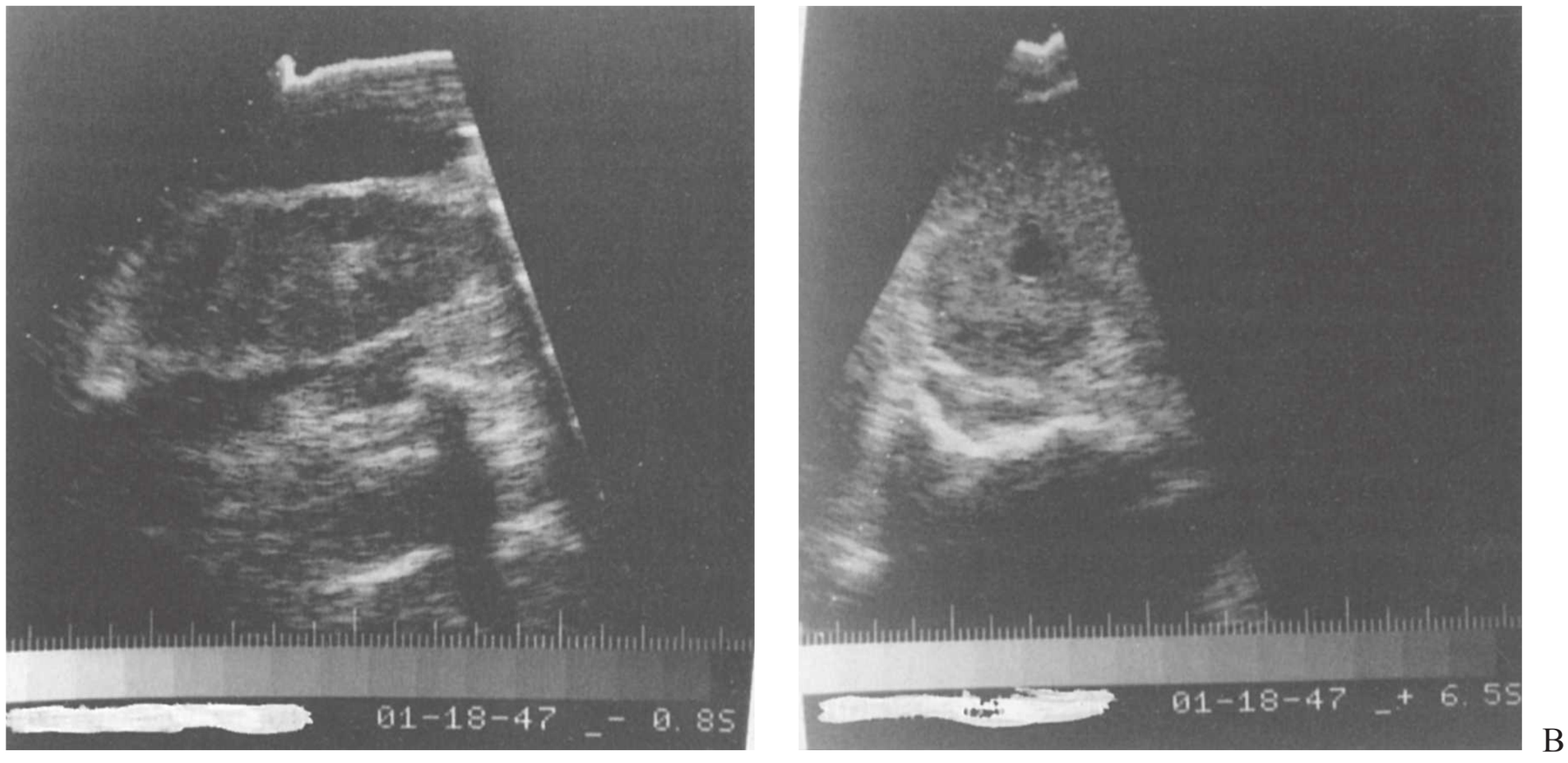
A B

FIGURE 24.2 Ultrasonogram of the right kidney on admission to the hospital. **A:** Longitudinal view, demonstrating two echolucent fluid-filled lesions. **B:** Transverse view, demonstrating fluid-filled masses with thickened margins. (From Andriole VT. Renal carbuncle. *Medical Grand Rounds*. 1983;2:259, with permission.)

malignancy and an isolated abscess in the kidney, MRI with diffusion-weighted images is helpful. The principle behind this is the diffusion of water molecules is reduced in the intracellular space compared to the extracellular space. Thus, highly cellular tumors may be more likely to have restricted diffusion than less cellular tumors/masses. This modality has been used extensively to characterize central nervous system lesions.[33] Several drawbacks to MRI are that it is expensive and time consuming to perform. Gadolinium use in patients with stage IV or V chronic kidney disease is also contraindicated.

Selective renal arteriography is an older modality used to differentiate renal cortical abscess from tumor. A renal cortical abscess can be identified angiographically as a mass that produces arcing, stretching, and attenuation of adjacent arteries, with the vessels located around the circumference rather than within the mass (Fig. 24.4). Early in the course, the rim around the abscess is poorly visualized, but arterial circulation to the periphery gradually increases with time so that a late study may identify a dense rim in the parenchymal phase. An untreated abscess may progress to a stage in which the rim is thick and poorly vascularized.

Renal and perirenal abscesses can be arteriographically distinguished from tumors because the major portion of an abscess is avascular whereas the wall of the abscess is hypervascular.



A B

FIGURE 24.3 Ultrasonogram of the right kidney after 4 weeks of antibiotic therapy (from the same patient as in Fig. 24.2). Longitudinal view **(A)** and transverse view **(B)** showing a decrease in the size of the fluid-filled echolucent lesions. (From Andriole VT. Renal carbuncle. *Medical Grand Rounds*. 1983;2:259, with permission.)

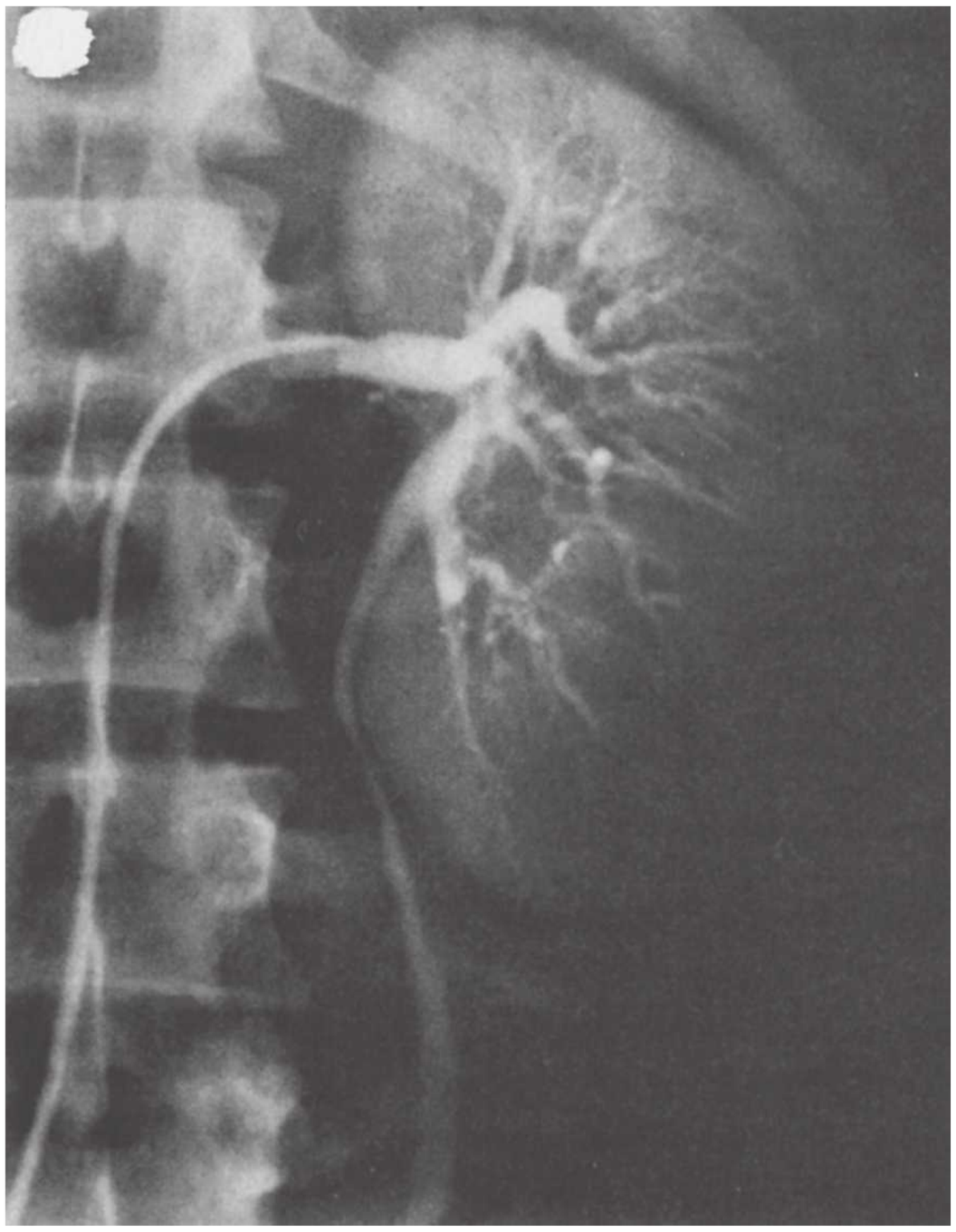


FIGURE 24.4 Arterial phase of left renal arteriogram. Peripheral vessels of the lower pole are attenuated and separated in comparison to normal vessels in the upper pole. No tumor vessels are present. (From Andriole VT. Renal carbuncle. *Medical Grand Rounds.* 1983;2:259, with permission.)

Renal carcinoma may be hypervascular or hypovascular (necrotic), but rarely both. In an abscess, the arteries retain their normal organization and branching pattern. Tumor neovascularity, in contrast, consists of abnormal vessels. Tumor vessels have no recognizable organization, may enlarge instead of taper as they course peripherally, and have an abnormal branching pattern.

Nuclear imaging was popular before CT and MRI became widely available. Renal scanning with gallium-67 (^{67}Ga) citrate (Fig. 24.5) also has been useful in localizing a renal abscess in adults.[17,28,34] A subtraction technique using ^{67}Ga citrate and technetium-99 (^{99}Tc) glucoheptonate can define the extent of perinephric involvement and eliminate any false-positive scans seen with gallium alone.[34] The latter may occur in patients with renal carcinoma, severe pyelonephritis without abscess formation, or ureteral obstruction. ^{111}In-labeled white cell scanning identifies a renal abscess but does not demonstrate renal carcinoma.

Noninvasive techniques such as ultrasound, CT, and MRI have reduced the need for intravenous pyelogram, selective angiography, and nuclear imaging to further define intrarenal masses.

Radiologic techniques can correctly establish a diagnosis of a renal cortical abscess only when this diagnosis is considered. Unfortunately, clinicians generally do not think of the diagnosis of renal cortical abscess early in its course. An average delay of 62 days before the correct diagnosis was established and proper treatment instituted has been reported.[9]

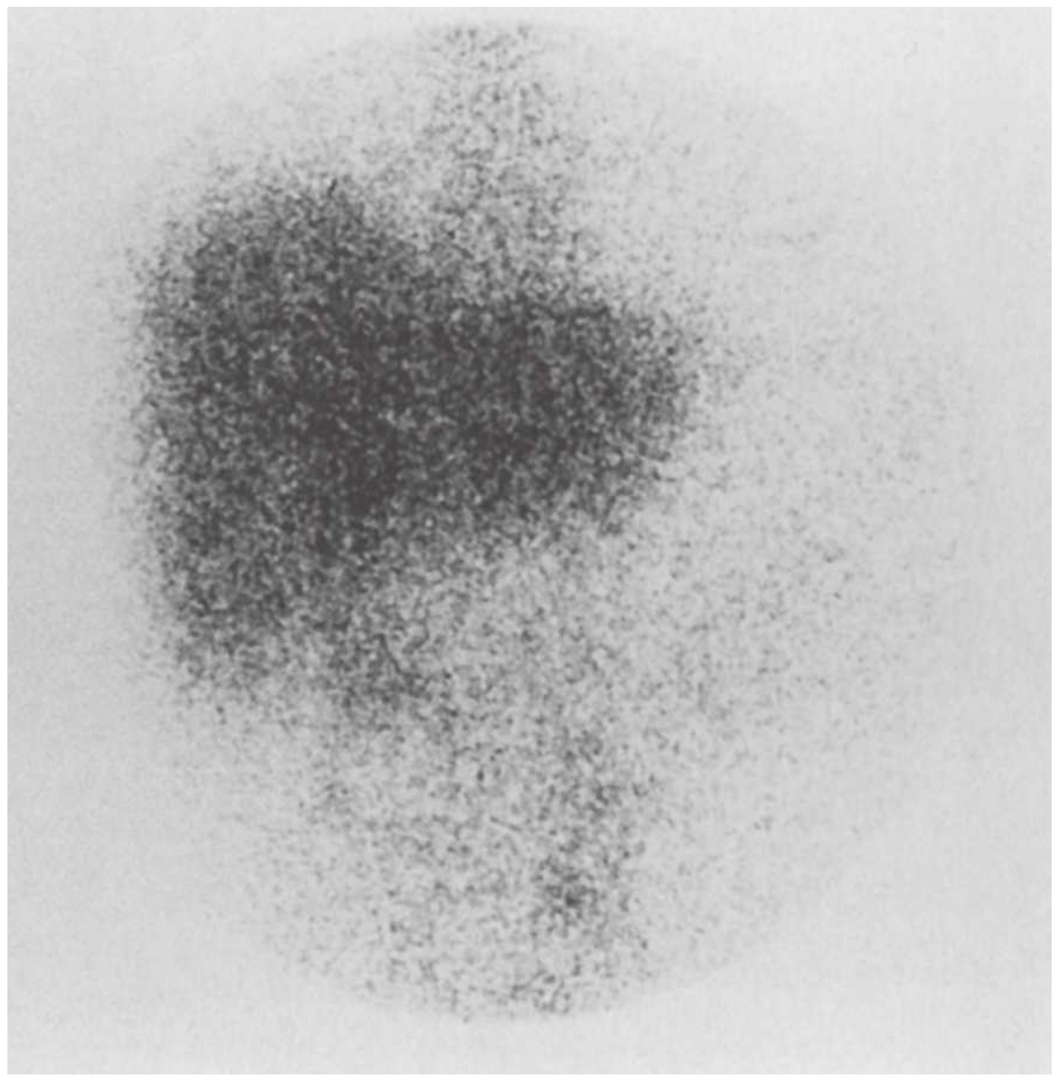


FIGURE 24.5 Radionuclide scan with ^{67}Ga citrate at 48 hours, showing abnormal uptake in the right upper quadrant, inferior to the liver and right in the area of the kidney. (From Andriole VT. Renal carbuncle. *Medical Grand Rounds.* 1983;2:259, with permission.)

Treatment

Historically, the treatment of a renal cortical abscess has been surgical and has varied with the condition of the patient.[8] However, because a renal cortical abscess is usually hematogenous in origin, and caused by S. aureus, it often responds to antistaphylococcal antimicrobial therapy alone, thus obviating the need for surgical intervention.[9]

If the diagnosis of renal cortical abscess is suspected from the history, physical findings, and renal ultrasonography (abscess localized to the renal parenchyma) or CT, and gram-positive cocci or no bacteria are seen on microscopic examination of the urine, antimicrobial therapy should be directed against S. aureus. Choice of empiric antistaphylococcal therapy depends on the susceptibility patterns of S. aureus in the community. If methicillin-sensitive S. aureus (MSSA) is prevalent in the community, oxacillin or nafcillin 1 to 2 grams intravenously (IV) every 4 to 6 hours is appropriate initial therapy. If a history of nonanaphylactic penicillin allergy (i.e., rash) is present, cefazolin (2 grams every 8 hours) is an alternative. Patients with severe immediate penicillin allergy may manifest cross-reacting allergy when a cephalosporin is administered and should receive vancomycin (1 gram every 12 hours) instead. If the prevalance of community-associated methicillin-resistant S. aureus (MRSA) is high, empiric therapy with vancomycin

is justified. Historically, MRSA infections have been associated with exposures to health care settings; however, since 2001, community-associated MRSA has become an increasingly recognized and prevalent pathogen.[35] With the indiscriminate use of glycopeptides, vancomycin-intermediate S.aureus (VISA) and vancomycin-resistant S. aureus (VRSA) are a growing concern. Daptomycin (6 mg per kg IV every 24 hours) is the agent of choice in this setting. The prevalence of VISA and VRSA is not high enough at this time to warrant empiric therapy with daptomycin. Renal cortical abscesses can be cured with parenteral antibiotic therapy administered for a minimum of 10 days to 2 weeks, followed by oral antistaphylococcal therapy for at least an additional 2 to 4 weeks. The decision to pursue percutaneous drainage for therapeutic and/or diagnostic purposes is guided by the size of abscess and response to antimicrobial therapy. Renal abscesses less than 5 cm in size can be treated with antibiotic therapy alone.[36,37] If there is optimal response, fever gradually subsides over a 5- to 6-day period without recurrence. Flank or back pain abates rather quickly, and patients display significant clinical improvement within 24 hours of initiating antibiotic therapy. A prompt response to treatment justifies continuing antibiotic therapy without surgical intervention, and serial ultrasound or CT examinations can be used to show progressive reduction and ultimate disappearance of the renal mass. A contrary clinical course should suggest misdiagnosis or uncontrolled infection, with the development of perinephritis, perinephric abscess, or infection with an organism resistant to the antibiotics being administered. In such cases, modification of therapy may be required, based on the results of cultures of blood and urine. Nevertheless, a trial of intensive antibiotic treatment is warranted in lesions measuring less than 5 cm that are localized to the renal parenchyma. If the patient does not respond within 48 hours, percutaneous, ultrasonically, or CT-guided needle aspiration of the intrarenal fluid-filled lesion can be attempted.[38–41] If the renal abscess measures more than 5 cm, therapeutic and diagnostic percutaneous drainage along with antibiotics should be attempted.[37] If this treatment is unsuccessful, operative intervention should be undertaken.

Renal Corticomedullary Abscess

Etiology

Enteric aerobic gram-negative bacilli, predominantly Escherichia coli, Proteus spp., and, less commonly, Klebsiella spp., Enterobacter spp., and Pseudomonas spp. are usually responsible for intrarenal corticomedullary infections in association with vesicoureteral reflux or other urinary tract abnormalities.

Pathogenesis

Renal corticomedullary bacterial infections include a variety of acute and chronic parenchymal inflammatory processes. The more severe forms of these infections include acute focal bacterial nephritis, acute multifocal bacterial nephritis, and xanthogranulomatous pyelonephritis, which almost always involve only one kidney.

Acute focal bacterial nephritis is an uncommon, severe form of acute infectious interstitial nephritis presenting with a renal mass caused by acute focal infection without liquefaction.[42] This entity is also referred to as focal pyelonephritis or acute lobar nephronia, because the pathology consists of a heavy leukocytic infiltrate confined to a single renal lobe with focal areas of tissue necrosis.

Acute multifocal bacterial nephritis is also a severe form of acute renal infection in which a heavy leukocytic infiltrate occurs throughout the kidney with frank intrarenal abscess formation. Acute focal bacterial nephritis may represent an early phase of acute multifocal bacterial nephritis.[43]

Xanthogranulomatous pyelonephritis is a very rare and atypical form of severe chronic renal infection. Schlagenhaufer initially described the pathologic features of xanthogranulomatous pyelonephritis[44] in 1916. Grossly, the entire kidney or its involved segment is enlarged and may be fixed by perirenal fibrosis or retroperitoneal extension of the granulomatous process, which often resembles an inoperable tumor. Xanthogranulomatous pyelonephritis is classified into three stages based on the extent of involvement of renal and adjacent tissue by the xanthogranulomatous process.[45] In stage I (nephric), the xanthogranulomatous inflammatory process is confined to the kidney. Stage II lesions (perinephric) involve the renal parenchyma and Gerota's fat, whereas stage III lesions (paranephric) involve the renal parenchyma and its surrounding fat with widespread retroperitoneal involvement. Each stage is further divided into focal or diffuse, depending on the amount of parenchymal involvement. Microscopically, the disease is characterized by massive tissue necrosis and phagocytosis of liberated cholesterol and other lipids by xanthoma cells (macrophages). These foamy xanthomatous histiocytes appear to simulate clear-cell renal carcinoma.[46,47]

Acute focal bacterial nephritis, acute multifocal bacterial nephritis, and xanthogranulomatous pyelonephritis most commonly occur as a complication of bacteriuria and ascending infection, associated with tubular obstruction or scarring from prior infections, renal calculi, vesicoureteral reflux, urinary tract obstruction, or other abnormalities of the genitourinary system or in association with the endocrinopathies of diabetes mellitus or primary hyperparathyroidism.[9,11,14,15,42,43,47–51] These predisposing factors, particularly vesicoureteral reflux in children and renal calculi or other forms of urinary obstruction in adults, lead to intrarenal reflux and provide an avenue for bacteria to inoculate the renal parenchyma. Parenchymal infection develops with abscess formation because the kidney is unable to clear the infection in the presence of reflux, urinary obstruction, medullary scarring, or other causes of tubular obstruction. In adults, two thirds of intrarenal abscesses caused by aerobic gram-negative bacilli are associated with renal calculi or damaged kidneys, whereas in children this process is often associated only with vesicoureteral reflux. The incidence of renal abscesses in patients with diabetes mellitus is twice that in nondiabetic persons. In contrast to the staphylococcal renal cortical abscess of hematogenous origin, the gram-negative bacillary corticomedullary abscess of the kidney frequently produces severe renal infection. Although renal corticomedullary infections are confined within the substance of the kidney, they may perforate the renal capsule and form a perinephric abscess, extend toward

the renal pelvis and drain into the collecting system, or develop into a chronic abscess.[50] The etiology of xanthogranulomatous pyelonephritis is undefined; however, it appears to be related to a combination of chronic urinary tract infection and renal obstruction. The majority of the cases have renal calculi with staghorn renal calculi being the most common type.[52] Additional predisposing factors include chronic segmental or diffuse renal ischemia resulting in alterations in renal or lipid metabolism or both, lymphatic obstruction, abnormal immune response, diabetes mellitus, and primary hyperparathyroidism.[47,53,54]

Clinical Features

Renal corticomedullary abscesses affect males and females with equal frequency except for xanthogranulomatous pyelonephritis in adults, where females are more frequently affected than males.[53,54] Although these infections occur in all age groups, the incidence increases with advancing age. Peak incidence for xanthogranulomatous pyelonephritis occurs in the fifth to seventh decade and has been reported to occur in transplanted kidneys as well as native kidneys.[55] Most patients with acute focal bacterial nephritis, multifocal bacterial nephritis, or xanthogranulomatous pyelonephritis experience fever, chills, and flank or abdominal pain. Two thirds of patients have nausea and vomiting but dysuria is not necessarily present thus mimicking an abdominal process. Some patients may have a palpable flank or abdominal mass. Clinical signs of severe urinary tract infection with urosepsis may be seen in patients with acute multifocal bacterial nephritis, half of whom have diabetes mellitus. Nonspecific constitutional complaints of malaise, fatigue, and lethargy are particularly common (74%) in patients with xanthogranulomatous pyelonephritis, who may also complain of weight loss (24%). Significant physical findings include a renal mass (60%), hepatomegaly (30%), and, rarely, a draining flank sinus in patients with a past medical history of recurrent urinary tract infection (65%), renal stones (30%), or prior urinary tract instrumentation (26%). Peripheral white blood cell counts are elevated in most patients. The urinalysis is often abnormal, with pyuria, proteinuria, bacteriuria, and occasionally hematuria. However, the urinalysis may be normal in as many as 30% of patients. E. coli, Proteus mirabilis, and Klebsiella spp. are the most common organisms recovered from urine cultures. Blood cultures are also frequently positive in patients with acute focal bacterial nephritis or acute multifocal bacterial nephritis. Anemia is present in 75%, abnormal liver function tests (bilirubin, asparate transaminase [AST], alkaline phosphatase, and prothrombin time) in 38% to 63%, hypoalbuminemia in 60%, hypergammaglobulinemia (α_1- and α_2-globulin) in 79%, and hyperuricemia in 50% of patients with xanthogranulomatous pyelonephritis.[47,56] In general, the clinical and laboratory findings may or may not point to the urinary tract as the focus of infection and may not distinguish renal abscess from other abnormalities of the urinary tract.

Diagnosis

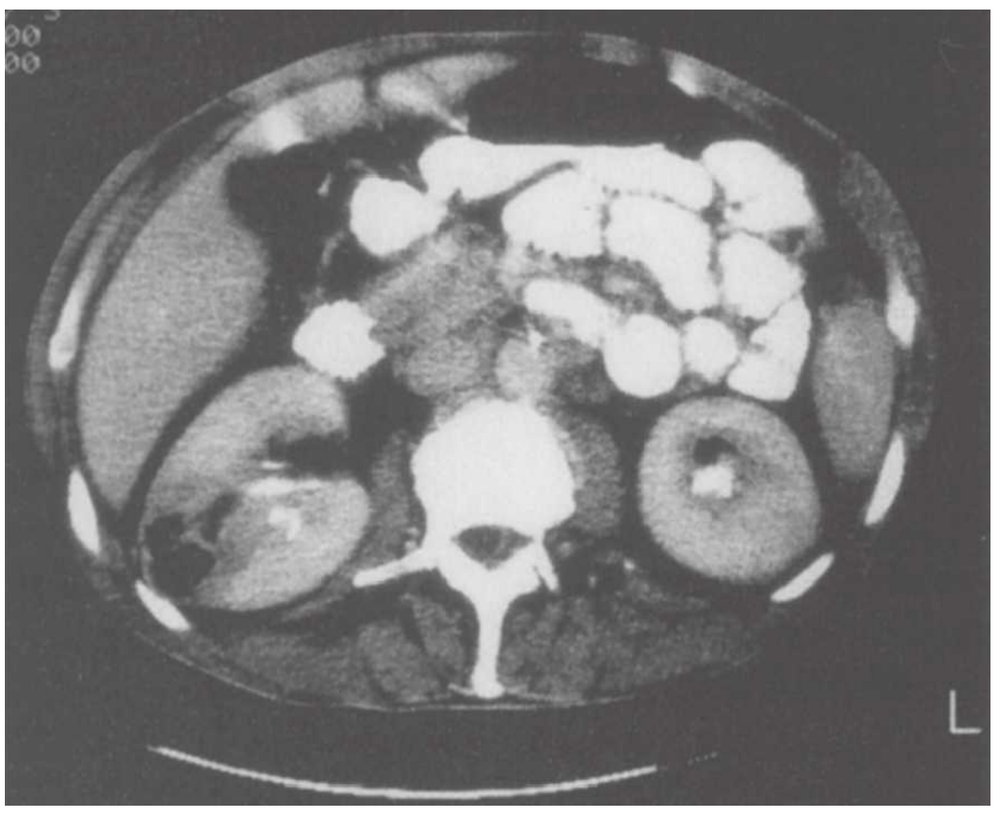
Renal corticomedullary abscesses must be differentiated from other space-occupying lesions in the kidney. Renal tumors, intrarenal cysts, renal cortical abscesses, and perinephric abscesses may mimic renal corticomedullary abscesses because the clinical presentation of each of these conditions is similar. Fever, chills, malaise, and back pain may be seen in each. Clinical signs of urosepsis may be present in patients with renal corticomedullary abscesses and, to a lesser extent, in patients with perinephric abscesses. In contrast, these signs are usually absent in patients with renal tumors, cysts, and renal cortical abscesses. Patients with renal corticomedullary abscesses often have an abnormal urinalysis (70%) with pyuria, proteinuria, and bacteriuria and blood cultures frequently are positive.[43]

Radiographic techniques are essential to identify renal corticomedullary abscesses. The urographic findings in patients with acute focal bacterial nephritis are: (1) a poorly marginated and relatively sonolucent ovoid mass disrupting the corticomedullary definition and producing some low-level echoes on ultrasound examination; (2) a solid-appearing mass on excretory urography, CT, or angiography; and (3) abnormal uptake of gallium at the location of the mass, which may be associated with increased activity elsewhere in the same or opposite kidney.[42] A fluid-filled mass or a fluid debris level typical of a frank renal abscess is not found in acute focal bacterial nephritis on ultrasonography.[42,43] In the pediatric population if there is evidence of nephromegaly (more than 3 standard deviations greater than the mean for age) on ultrasound or a focal mass is present, the probability of acute focal bacterial nephritis is high in the appropriate clinical setting.[57] On a non–contrast-enhanced CT, the lesion of acute focal bacterial nephritis is typically imperceptible. There are three characteristics seen with contrast-enhanced CT: lobar distribution of inflammatory areas, poorly defined wedge-shaped areas of diminished contrast enhancement without frank liquefaction, and masslike hypodense lesions in severe cases.[43,58] Renal abscesses, however, are usually round, have liquid centers, and are visible with and without contrast enhancement. The lack of a defined wall by ultrasound or CT in acute focal bacterial nephritis is an important factor distinguishing this entity from an abscess.[28] On angiography, narrowing and obstruction of veins within the mass, along with only minor arteriographic abnormalities, are characteristic of acute focal bacterial nephritis.[42] Focal abnormalities in the kidney on gallium images may be seen in some neoplasms and renal abscesses as well as acute focal bacterial nephritis. However, the diagnosis of acute focal bacterial nephritis is strongly suggested whenever the abnormalities are larger on the gallium image than on the urogram or sonogram or whenever bilateral abnormalities are seen on gallium images that correlate with a focal mass on the urogram or sonogram.[42] The distinction between acute focal bacterial nephritis, renal abscess, and tumor can be made also by needle aspiration. However, in most patients, a combination of imaging techniques is sufficient to diagnose acute focal bacterial nephritis and permit conservative medical therapy without confirmation by needle biopsy or surgery.[42,59] In this context, serial uroradiologic studies should be performed in 4 to 6 weeks, to follow the process to resolution.[26,51,60,61]

The urographic findings in patients with acute multifocal bacterial nephritis typically show severe impairment of

excretion of contrast material on the affected side, with renal enlargement, a diminished nephrogram, and a delayed pyelogram.[43,62,63] Ultrasonography may demonstrate areas of decreased echogenicity throughout the affected kidney. Poorly defined wedge-shaped areas of decreased contrast enhancement similar to those described in patients with acute focal bacterial nephritis can be seen on contrast-enhanced CT, except that multiple renal lobes are involved[43,64] (Fig. 24.6). If angiography is performed, the number and caliber of interlobar arterial branches are diminished, and fine linear stripes of alternating density and lucency in the angiographic nephrogram are present throughout the kidney.[43] CT is more sensitive than ultrasonography for the detection of intrarenal bacterial infections and defining the extent of disease.[26,28,65]

The radiographic findings in patients with xanthogranulomatous pyelonephritis are varied and uncharacteristic. The xanthogranulomatous process may occur in a localized (unifocal) or diffuse (multifocal) form in either a previously normal kidney or one that is obstructed, contains a stone, or has an anomalous collecting system or calyceal diverticulum.



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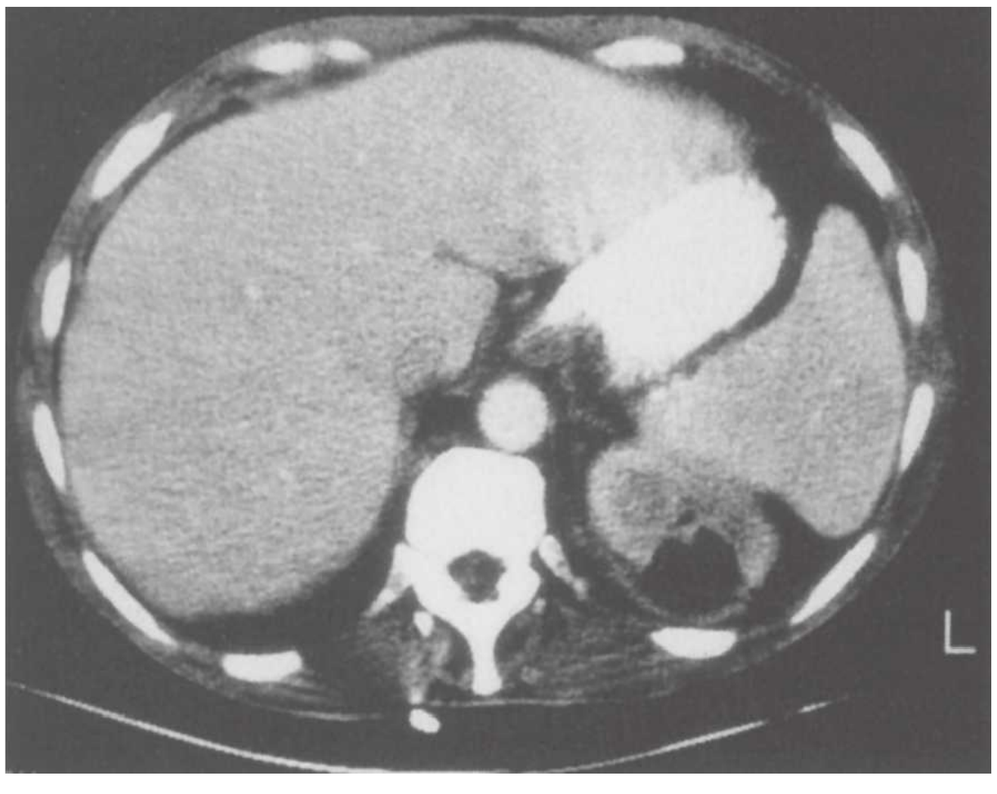

B

FIGURE 24.6 Computed tomography scans of a patient with bilateral intrarenal abscesses.

Perinephric extension of the xanthogranulomatous process produces obscure renal margins and ablation of the perinephric and paranephric fat.[47] Radiographically, xanthogranulomatous pyelonephritis appears as either localized or diffuse enlargement of one kidney with an indistinct renal outline. Urographically, the most frequent finding has been a stone-bearing (70%) and functionless (80%) kidney.[66] Calyceal deformity and irregularity (46%) may be present.[47,56] Neovascularity may be present on angiography, but most xanthogranulomatous renal masses are hypovascular or avascular, and the majority cannot be definitively distinguished from renal cell carcinoma.[47,67,68] There are no specific ultrasonographic findings that reliably distinguish renal tumors and abscesses from xanthogranulomatous pyelonephritis.[69] The sonographic features of xanthogranulomatous pyelonephritis include a diffusely enlarged kidney with multiple areas of increased anechoicity and a central echogenic focus with acoustic shadowing.[70] Although these findings are also seen in a hydronephrotic kidney, the presence of a central strongly echogenic focus suggests xanthogranulomatous pyelonephritis.[70,71] On CT, focal xanthogranulomatous pyelonephritis appears as low-density mass lesions, with wall enhancement surrounding dilated calyces, which may contain stones, or as a focal mass in one pole of a duplicated kidney.[72] In diffuse xanthogranulomatous pyelonephritis, CT demonstrates an enlarged kidney, often with central calcification in the renal pelvis without dilatation, and with multiple, rounded, low-density areas representing dilated calyces and abscess cavities. On enhanced scans, the walls of these cavities demonstrate a prominent blush because of increased vascularity within the granulation tissue and the compressed normal parenchyma.[66,72] Extension of the xanthogranulomatous process through the renal capsule, with involvement of the perirenal and pararenal spaces and the psoas muscle, when present, also can be observed on CT.[66,72] The diagnosis is suggested by CT findings in 44% of cases. This imaging study plays an important role in determining the extent of extrarenal disease and planning of operative treatment. Ultrasound examination is less specific than CT, and MRI generally offers no additional information over CT scans but may be useful in patients with renal insufficiency or allergy to iodinated contrast material.[28,73]

Treatment

In the past, surgical drainage, débridement, or nephrectomy was the accepted treatment for renal corticomedullary abscesses. Recent experience indicates that successful therapy of acute focal and multifocal bacterial nephritis with antimicrobial agents alone will produce a symptomatic response within 1 week in most patients and result in no sequelae.[29,34,41,43,49,50,74] Radiologic techniques should be used to document resolution of the infection[26]; nevertheless, the time at which the intrarenal infection is discovered and its degree of suppuration should guide its management. A well-established large abscess cavity may be more difficult to eradicate with antibiotics alone than an earlier lesion in the preabscess state; however, a trial of intensive antibiotic treatment is appropriate for lesions localized to the renal parenchyma before determining the need

for operative drainage, particularly in a promptly diagnosed and otherwise healthy person. Intravenous fluids and parenteral antibiotics should be started as soon as the diagnosis is entertained. In patients with acute focal or multifocal bacterial nephritis, initial antibiotic selection (empiric therapy), before the results of urine cultures and sensitivities are available, should be aimed at the most common uropathic, enteric gram-negative aerobic bacilli (e.g., E. coli, Klebsiella, and Proteus spp.). Monotherapy with a third generation cephalosporin (e.g., cefotaxime, ceftriaxone, or ceftazidime), an extended-spectrum penicillin (e.g., piperacillin), or ciprofloxacin is acceptable empiric therapy.

Combined therapy with a β-lactam, such as ampicillin or cefazolin, with an aminoglycoside is no more successful than single-agent therapy in the treatment of acute focal or multifocal bacterial nephritis.[41] Empiric therapy should be modified to the most effective single agent based on the results of the antibiotic sensitivities of the organisms recovered from cultures of urine, blood, or both. Although the duration of treatment has not been defined, current recommendations are to continue parenteral antibiotics for at least 24 to 48 hours after the resolution of fever and clinical improvement are attained. Oral antibiotic therapy, based on the results of antimicrobial susceptibility tests, can then be continued for an additional 2 weeks. Patients with acute focal bacterial nephritis typically respond to medical therapy alone (at least 14 days of an appropriate antimicrobial agent), and follow-up studies have shown resolution of the intrarenal lesion without the need for surgical drainage.[43,51,59] Although many patients with acute multifocal bacterial nephritis slowly improve with antibiotic therapy alone, some may require surgical intervention.

Patients who are likely to fail appropriate antibiotic therapy alone are those who have radiologic evidence of a large intrarenal abscess, significant obstructive uropathy, severe vesicoureteral reflux (primarily in children with gram-negative bacillary multifocal bacterial nephritis) with extensive parenchymal involvement, patients with diabetes mellitus with gas-forming infections, and patients of advanced age or with urosepsis.[14,43,48–50,75] In general, surgical intervention is indicated in the patient who has radiologic evidence of a large intrarenal abscess and persistent fever, with an absence of clinical response after 5 to 7 days of adequate antibiotic therapy. In patients requiring drainage, percutaneous aspiration of the abscess combined with systemic antibiotic therapy has been successful.[50,76,77] If a significant obstructive uropathy is present, prompt drainage, usually by percutaneous nephrostomy, is necessary with correction of the lesion, if possible, when the patient is afebrile and stable.[43] If surgical intervention is necessary, the abscess should be incised and drained, and nephrectomy should be reserved for diffusely damaged kidneys or for patients of advanced age who are septic and require urgent surgical intervention for survival.[48] Also, all children with renal parenchymal infection caused by gram-negative bacilli should undergo voiding cystourethrography to look for lower urinary tract abnormalities.[14] Thus, current clinical experience indicates that many patients with acute multifocal bacterial nephritis may not require surgery as they did in the past but may be treated successfully with antibiotics alone. The decision to drain the abscess mechanically should be based on the radiologic findings and response of the patient to initial drug therapy.

In contrast, patients with xanthogranulomatous pyelonephritis generally are not cured by antibiotic therapy alone. These patients often require surgical removal of the xanthogranulomatous process to cure this disease; however, there have been several case reports of successful treatment with antibiotics without surgical intervention.[53] The diagnosis of xanthogranulomatous pyelonephritis is not commonly made preoperatively; however, once the involved tissue is resected, the xanthogranulomatous process ceases and does not seem to recur. The prognosis in patients who have an otherwise normal urinary tract is excellent. Total nephrectomy is the usual procedure, but Malek and Elder[47] suggested that partial nephrectomy for selected localized disease, such as cases confined to the kidney (stage I) or involving the perinephric fat (stage II), may be sufficient. Partial nephrectomy is especially suitable in children, who usually present with localized disease.[47] In adults, the disease is frequently diffuse throughout most, or all, of the kidney and in advanced stages extend to the perinephric fat (stage II) and beyond (stage III). Although removal of the kidney and perirenal fat is preferred, it may be technically difficult and complicated by fistulization of adjacent bowel. Open surgical nephrectomy is preferred over laparoscopic nephrectomy as it is quicker, leads to less complications, and results in a similar postoperative course.[78] Even though xanthogranulomatous pyelonephritis does not recur following successful surgery, bacteriuria may continue in some patients and will require appropriate treatment.[47,56]

Infected Renal Cyst

Spontaneous infection of preexisting solitary renal cysts has been described.[43,79,80] In contrast, patients with autosomal dominant polycystic renal disease may have one or more cysts that become infected.[81] The most common etiologic agents are gram-negative uropathogens (especially E. coli) that are thought to infect the cysts as a consequence of bacteriuria and ascending infection.[82,83] Infection may also occur as a result of iatrogenic cyst instrumentation.[43] The clinical features of infected cysts are similar to those of an acute renal abscess and include nausea, chills, fever, flank or back pain, and dysuria. The diagnosis is made radiographically. Ultrasonography or CT may demonstrate a solitary renal mass that is compatible with an uncomplicated simple renal cyst or multiple lesions characteristic of polycystic renal disease. Gallium or indium imaging, gadolinium-enhanced MRI, or positron emission tomography may help to identify the infected cyst (or cysts) in patients with polycystic kidneys.[83,84] A definitive diagnosis can be made by ultrasound or CT-guided percutaneous cyst puncture with culture.

Effective treatment for infected solitary cysts includes percutaneous drainage combined with 2 weeks of appropriate antimicrobial therapy.[41] Fluoroquinolones and third generation cephaloporins have better penetration into the intrarenal cyst

compared to β-lactams.[83] Surgical drainage is rarely required. In contrast, the therapy of infected cysts in patients with polycystic renal disease is more difficult and not well defined. A conservative approach with long-term (6 to 8 weeks) oral antibiotic treatment directed against the most likely pathogens or those isolated from urine or blood cultures is successful in some cases.[82] In general, surgical drainage is generally avoided because of the difficulty in identifying which cyst is infected.

PERINEPHRIC ABSCESS

Etiology

S. aureus, E. coli, and Proteus spp. are the most common causes of perinephric abscesses. Other, less common causes include Klebsiella spp., Enterobacter spp., Pseudomonas spp., Serratia spp., and Citrobacter spp. Occasionally enterococci and streptococci are implicated, including two cases of S. pneumoniae[85,86] and one case of group B streptococcus in a diabetic patient.[87] Perinephric abscesses also may be caused by various anaerobic bacteria, including gram-negative bacilli and anaerobic cocci, Clostridium spp., and Actinomyces spp.[88] These anaerobic bacteria may be the pathogens in patients with abscess cultures reported to be sterile.[29,50] Mycobacterium tuberculosis is also an important cause of perinephric abscess, as are certain fungi, particularly Candida spp. Nocardia has been reported as a cause of perinephric abscess in immunocompromised patients.[89] More than one microorganism has been simultaneously recovered from perinephric abscesses in as many as 25% of patients.[48,50,90,91] Although bacteria isolated from the urine frequently correlate with those isolated from the abscess, in some patients, urine cultures are positive for microorganisms different from those subsequently isolated from the abscess material.[50,90,92] Blood cultures may be positive (20% to 40%) in some patients.[90]

Pathogenesis

A perinephric abscess is a collection of purulent material in the space between the capsule of the kidney and Gerota's fascia (Fig. 24.7). The abscess usually is confined to this space but may extend beyond Gerota's fascia into the pararenal space or even into the flank muscles or psoas muscle.[93] It may present as a draining flank abscess through Petit's triangle or as an abscess in the groin or paravesical area by extending caudally between the diverging layers of Gerota's fascia. It rarely perforates into the peritoneal cavity or ruptures into the colon. Cephalad extension may result in a subphrenic abscess, penetration of the diaphragm and empyema, lung abscess, or formation of a nephrobronchial fistula.[2,50,76,91] The most frequent initiating event is the direct extension or rupture of an abscess within the renal parenchyma into the perinephric space.[10,13] This is the most common mechanism responsible for staphylococcal perinephric abscesses that occur when a renal cortical abscess ruptures into the perinephric space.[9] Other causes include hematogenous or regional lymphatic seeding of the perinephric space, usually from sites of skin infection.[50,90] Rarely, spread of

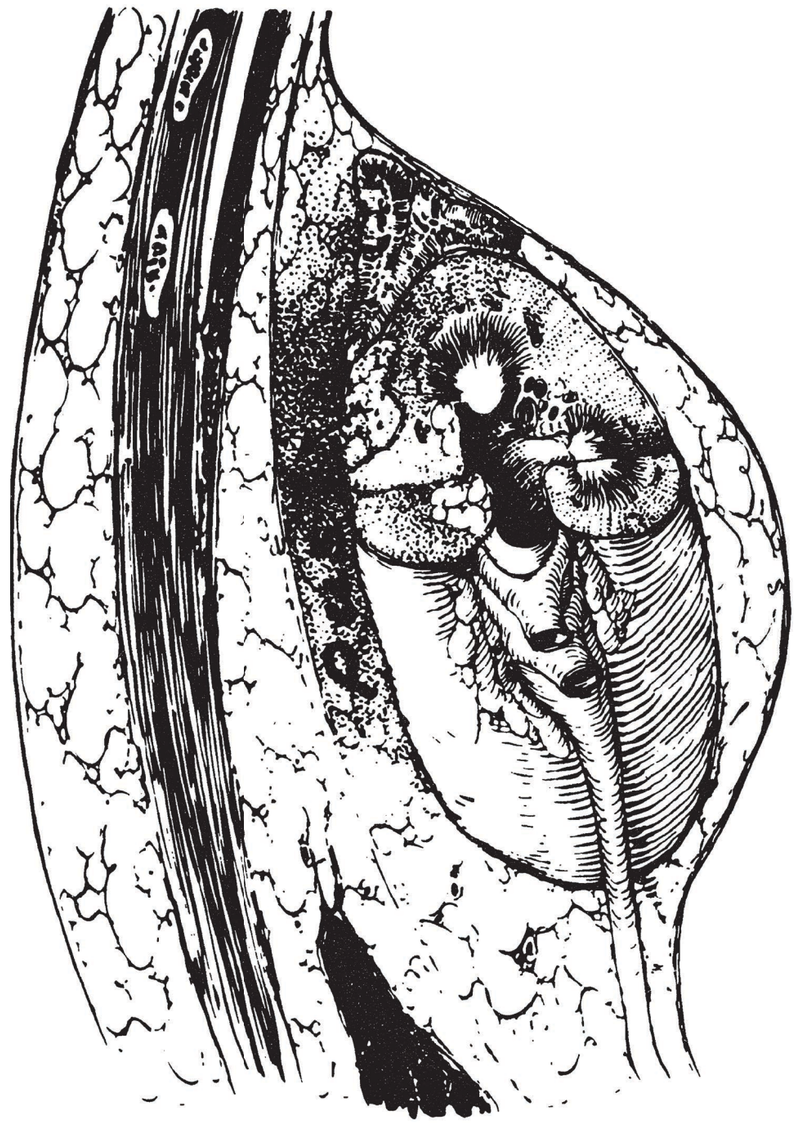

FIGURE 24.7 Diagram of the pathogenesis of a perinephric abscess.

infection from inflammatory lesions of the liver, gallbladder, pancreas, pleura, prostate, or female reproductive organs as well as diverticulitis, appendicitis, perforated carcinoma of the colon, and osteomyelitis of adjacent ribs or vertebrae has been implicated in the pathogenesis of perinephric abscess.[29,50,94–96] The majority of patients with perinephric abscesses have some form of obstruction to urinary outflow. Specific predisposing factors include renal or ureteral calculi, ureteral stricture, neurogenic bladder, vesicoureteral reflux, mechanical bladder outlet obstruction, neoplasm, renal papillary necrosis, polycystic kidney disease, genitourinary tuberculosis, immunosuppresion including renal transplantation, trauma (including urinary tract instrumentation, renal biopsy, or aspiration), and the associated conditions of diabetes mellitus (a major contributing factor), glucocorticoid therapy, and injection drug use.[43,50,81,85,97]

Clinical Features

The onset of perinephric abscess is characteristically insidious. Patients are often ill for 1 to 3 weeks before they seek medical care, and early recognition of this condition is difficult. The most common symptoms are fever, which occurs in almost all patients; unilateral flank pain, in 70% to 80%; and chills and dysuria, in 40% of patients.[50,90,98,99] Weight loss, nausea, and vomiting are less common. Diarrhea is very rarely a symptom associated with perinephric abscess.[100] Interestingly, in renal transplant recipients, the aforementioned symptoms are not masked despite immunosuppression.[101] On physical examination, flank and costovertebral angle tenderness are the most common findings, but abdominal tenderness may be present in about 60% of cases. In some patients, the pain may be

referred to the corresponding hip, thigh, or knee. Scoliosis, with splinting on the affected side, pain on bending toward the contralateral side and during either active flexion of the ipsilateral thigh against pressure, or extension of the thigh while walking may be present in some patients. A flank or abdominal mass is present in less than half the patients.[29,43,50,90] Routine laboratory studies are nonspecific. The peripheral white blood cell count is usually modestly elevated with associated neutrophilia. Anemia and azotemia may be present in 40% and 25% of patients, respectively.[98] Pyuria and proteinuria are common; however, hematuria is present in only 10% of patients and the urinalysis may be entirely normal in 25% to 30% of cases. Two thirds of patients have positive urine cultures, with more than 10^5 bacteria per mL of urine. Approximately 40% of patients are bacteremic.[29,43,50,90]

Diagnosis

A perinephric abscess must be differentiated from other infections of the urinary tract and from other occult abscesses. Patients with this disease may present with fever of undetermined origin or with unexplained peritonitis, empyema, or pelvic abscess resulting from extension of the perinephric abscess. The diagnosis should also be considered in patients with urinary tract infection who do not respond promptly to antibiotics and have an abnormality of the urinary tract or diabetes. Prompt diagnosis of this disease is made in less than one third of patients at the time of admission.[50,90] Up to 25% to 30% of patients are diagnosed only at autopsy.[50,90] This disease should be considered in the differential diagnosis of patients who present with the signs and symptoms described previously. Radiologic examinations with ultrasonography and CT are essential diagnostic aids in most cases. Roentgenogram of the chest may be normal or may show a pleural effusion, elevated hemidiaphragm either with or without decreased diaphragmatic excursion, or a lower lobe infiltrate.[50,90] Supine abdominal roentgenogram may demonstrate an upper quadrant mass, obliteration of the renal outline, vertebral scoliosis, or absent psoas shadows. However, obliteration of the psoas margin is not a reliable diagnostic sign for perinephric abscess.[9,13] Although uncommon, the presence of extraluminal, retroperitoneal gas bubbles in the area of the kidney suggests a perinephric abscess produced by gas-forming organisms. This condition, termed emphysematous pyelonephritis, occurs primarily in patients with diabetes mellitus, with or without urinary obstruction, and more rarely in nondiabetic patients who have urinary obstruction.[75,102,103] On excretory urography, important radiographic findings of perirenal abscesses include decreased renal mobility with respiration or position, absent or diminished renal function, caliectasis and other calyceal abnormalities, and displacement (usually medially and upward) of the kidney or ureter. Extrarenal extravasation of contrast material, although uncommon, is virtually diagnostic of perirenal abscess.[50,93] Also, fistula formation occurs occasionally between the perirenal space and other structures, such as the colon. Retrograde pyelography is usually not necessary, but it is occasionally helpful in identifying obstructive lesions distal to the renal pelvis. Ultrasonography may demonstrate an intrinsic mass in addition to the more characteristic findings of a perinephric fluid collection, along with displacement of the kidney, loss of a distinct renal outline, and renal fixation. These findings indicate extension of the inflammatory process to the perinephric space.[9,13] The sonographic appearance may vary from a nearly anechoic mass, displacing the kidney, to an echogenic collection that merges with normally echogenic fat within Gerota's fascia.[43] Ultrasound also may be useful to determine the extent of the abscess (Fig. 24.8)

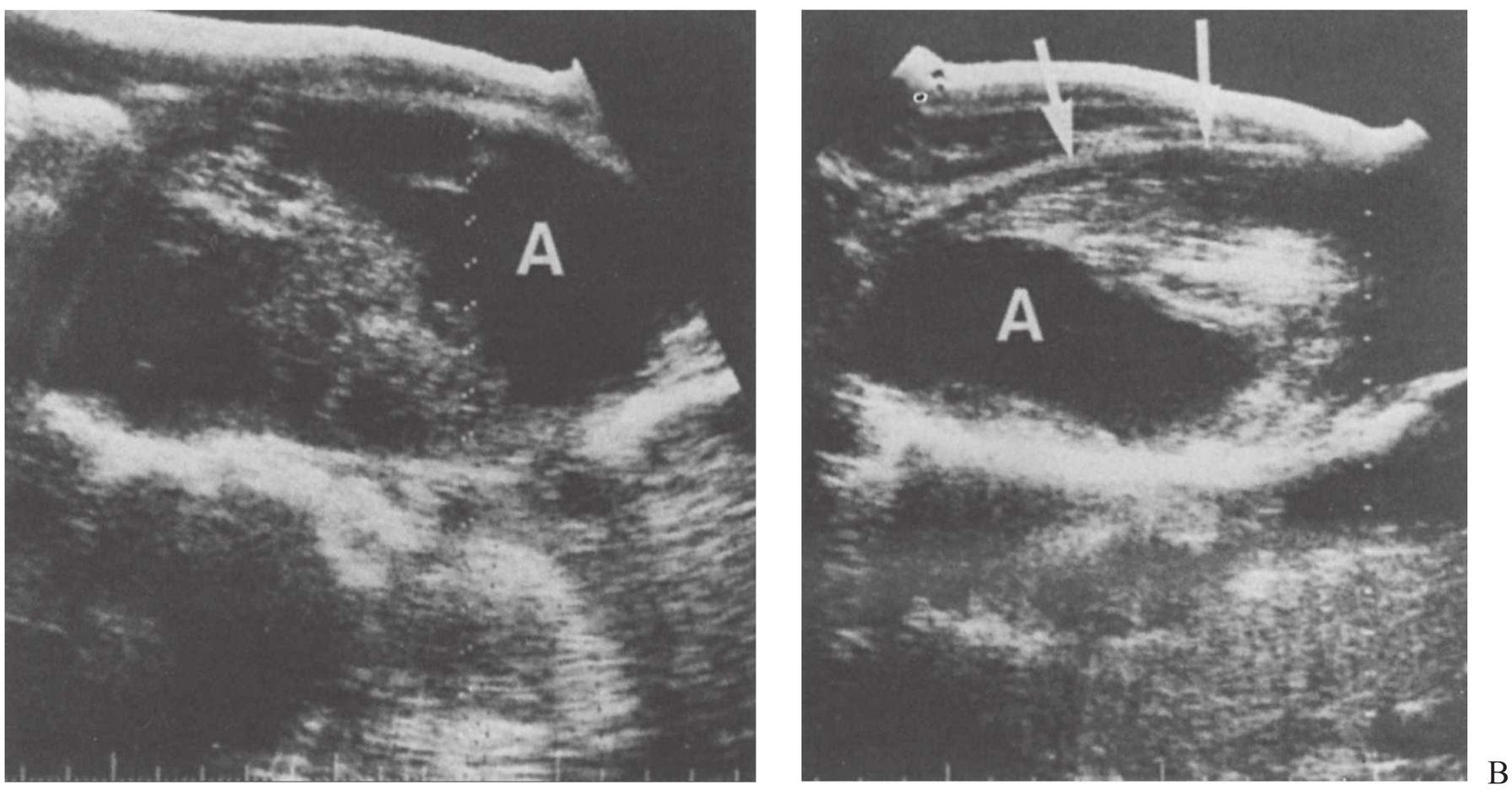

FIGURE 24.8 **A:** Longitudinal ultrasonogram down the right paravertebral region, demonstrating a huge complex mass with a large irregular fluid component, labeled *A*. **B:** Marked enlargement of the psoas major muscle *(arrows)* with a large contained fluid collection, labeled *A*. (From Andriole VT. The clinician's viewpoint. *Clin Diagn Ultrasound.* 1982;11:1, with permission.)

and detect associated obstruction of the collecting system. However, ultrasound may be falsely negative in 36% of cases as compared to CT.[98] Findings on CT include thickening of the renal fascia and perirenal fluid collection. In most cases, pus can be differentiated from other causes of perirenal fluid collections such as urine, blood, lymph, exudates, and transudates.[36] CT also provides the most precise anatomic information and can demonstrate the extent and route of the abscess beyond the renal capsule (e.g., extension to the flank or the psoas muscle) (Fig. 24.9). This detail is important in planning surgical drainage of the abscess. MRI has been used in conjunction with ultrasonography to detect perinephric abscess during pregnancy as CT is contraindicated.[104] Other modalities not used as frequently are ^{67}Ga or indium-111 (^{111}In) imaging and arteriography. Radionuclide imaging with ^{67}Ga or ^{111}In may be used occasionally to confirm the presence of renal or perirenal inflammation or evaluate renal function. Gallium or indium imaging may provide the first evidence of a perirenal abscess in patients with suspected infections but without localizing signs or symptoms. However, ^{67}Ga is not sufficiently definitive to exclude renal carcinoma, pyelonephritis, intrarenal abscess, or ureteral obstruction.[9,30,34] Thus a subtraction technique using ^{67}Ga citrate and ^{99m}Tc glucoheptonate has been used to define the extent of perinephric involvement as well as to eliminate any false-positive scans seen with gallium alone.[9,34] On angiography, characteristic findings of perinephric abscesses include an increase in number and size of the perforating arteries extending from the kidney into the abscess, stretching, and prominent tortuous capsular arteries around the abscess and a contrast blush.[7,9,21,22] Perirenal and renal abscesses can be arteriographically distinguished from tumors, as described previously; however, angiography is not necessary in most patients with perinephric abscess because of the availability of the newer, noninvasive imaging modalities.

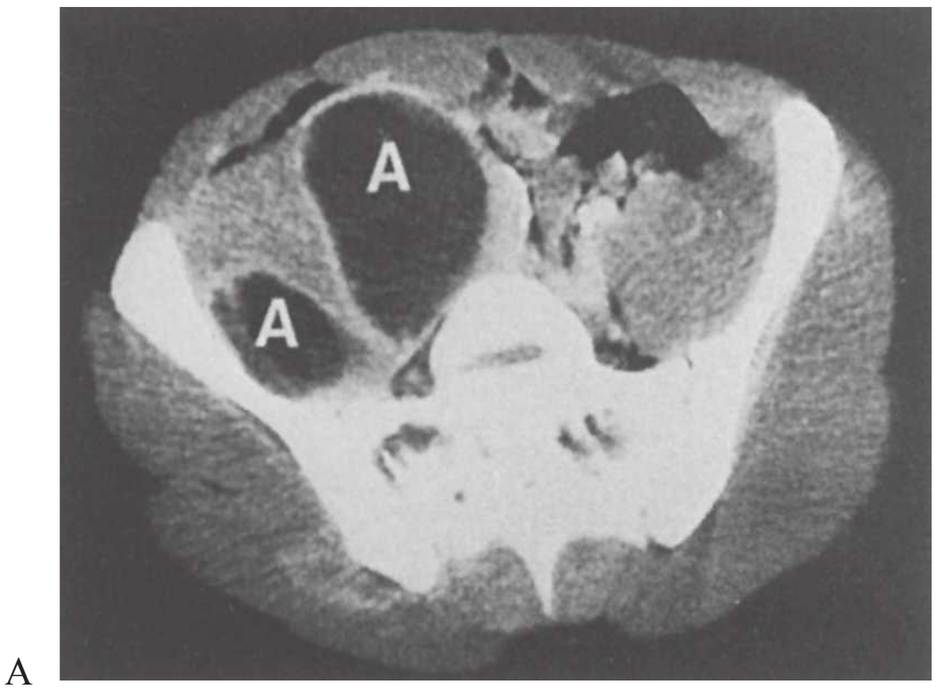

FIGURE 24.9 **A:** Computed tomography (CT) scan through midabdomen, demonstrating marked enlargement of the right psoas major muscle with a bilobed fluid-filled cavity, labeled *A* (from the same patient as in Fig. 24.8). **B:** Transverse CT scan through the level of the femoral head, showing the inferior extent of the abscess *(arrow)* pointing below the inguinal ligament. (From Andriole VT. The clinician's viewpoint. *Clin Diagn Ultrasound.* 1982;11:1, with permission.)

Treatment

Early surgical or percutaneous (under imaging guidance) drainage of the perinephric abscess is imperative.[43,50,90,105] Antibiotic therapy alone is inadequate and should be used as adjunctive treatment. In some patients, the perinephric abscess has been drained by percutaneous tube placement, aspiration of pus, and antibiotic irrigation prior to definitive surgery (nephrectomy), which is frequently necessary.[46,50] In others, acute nephrectomy is performed at the time of initial surgical drainage. There are rare case reports of immunocompetent patients with small (≤3.5 cm) abscesses who were successfully treated with prolonged courses of intravenous antibiotics without drainage[36,77]; however, immunocompromised patients or those with abscesses larger than 2 cm did poorly and had high mortality rates when treated with antibiotics alone. CT should be used to follow the response to treatment.

Initial antimicrobial therapy should be aimed at the most common uropathic gram-negative bacilli as well as against staphylococci, because some perinephric abscesses are a consequence of staphylococcal renal carbuncles. An aminoglycoside (gentamicin or tobramycin) in a dose of 1 to 1.5 mg per kg of body weight every 8 to 12 hours in patients with normal renal function should be combined with an antistaphylococcal β-lactam, oxacillin, nafcillin, or cefazolin, intravenously. The dose of gentamicin and tobramycin must be adjusted for those patients with compromised renal function. If suspicion of MRSA is high, vancomycin can be given empirically instead of a β-lactam. If an extended spectrum β-lactamase producing gram-negative organism is suspected, a carbapenem (e.g., doripenem, meropenem, and ertapenem) should be used in place of a β-lactam. Therapy should be modified based on the results of the antibiotic sensitivities of the organisms recovered from cultures of the abscess material. An antipseudomonal β-lactam (e.g., ticarcillin, piperacillin, cefoperazone, or ceftazidime) or a quinolone (ciprofloxacin) should be added to the aminoglycoside if Pseudomonas aeruginosa is the cause of the infection, and clindamycin or metronidazole should

be added if anaerobic bacteria are involved. A combination of penicillin or ampicillin plus gentamicin is the treatment of choice in enterococcal infections. Isoniazid plus rifampin and ethambutol or streptomycin is necessary for abscesses caused by M. tuberculosis and azoles (fluconazole or voriconazole) for those caused by fungi.

The prognosis in patients with perinephric abscess is poor. Even though there have been major improvements in diagnostic technology, surgical therapy, and antimicrobial treatment, the mortality associated with perinephric abscess remains high and is in a range from 20% to more than 50%.[29,48,50,77] Prompt diagnosis of perinephric abscess, immediate surgical or percutaneous drainage, appropriate antimicrobial therapy, followed by definitive surgical therapy for cases with poor response may be effective in reducing this high mortality rate.

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CHAPTER

25 Complicated Urinary Tract Infections

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Complicated infections of the genitourinary (GU) tract refer to those infections that occur in the presence of anatomic or functional abnormalities in the kidney, bladder, or collecting systems (including vesicoureteral reflux and neurogenic bladders in patients with spinal cord injury); obstruction to normal urine flow (including renal, ureteric and bladder calculi, and prostatic hypertrophy); urinary tract catheterization or instrumentation; cystic renal disease; specific diseases such as diabetes mellitus; and abnormalities in host defense mechanisms and those infections that follow surgery or renal transplantation (Table 25.1). In addition, infections that are caused by organisms that resist antimicrobial therapy (such as multidrug-resistant gram-negative rods and enterococci) or that might otherwise be difficult to eradicate may be considered in this category. Aerobic or anaerobic bacteria, mycobacteria, fungi, parasites, and even viruses may cause complicated infections.

Other conditions associated with complicated urinary infections include prostatic, kidney, or perinephric abscesses; pyonephrosis; emphysematous pyelonephritis and cystitis; malakoplakia and xanthogranulomatous pyelonephritis; intramural vesicle abscesses; pyelonephritis with bacteremia and sepsis; and tuberculosis. Some of these conditions are discussed in other chapters. Complicated urinary infections occur either in the upper or lower urinary tract and may be acute or chronic. Not included in this category are asymptomatic bacteriuria, urethritis, acute cystitis, acute pyelonephritis, or recurrent upper or lower urinary tract infections (UTIs) occurring in the presence of a normal urinary system.

ANATOMIC OR STRUCTURAL RISK FACTORS

Obstructive Uropathy

Obstructive uropathy includes: calculi at any level of the urinary tract; prostatic hypertrophy; cancer of the prostate, bladder, or uterus; external compression by uterine or other tumors; neurogenic bladder; and congenital abnormalities. Any of these conditions may be associated with a complicated UTI. An obstruction above the bladder can lead to renal pelvic dilatation and hydronephrosis with subsequent pressure atrophy of renal cortical tissue. When an infection does occur in the setting of partial or complete obstruction, the clearance of an infection is more difficult because drainage may be limited, antibiotic penetration might decrease, and host responses may be impaired.

Obstruction of the urinary tract may be acute or chronic, unilateral or bilateral, and complete or incomplete. Acute obstruction of the upper urinary tract may be associated with retroperitoneal or flank pain, especially if calculi are present. Obstruction at the bladder level increases the risk of infection by decreasing the effect of micturition on reducing bacterial inocula as well as allowing for the multiplication of bacteria to the degree that mucosal antibacterial and other host factors are overwhelmed or inactivated. An obstruction at higher levels of the urinary tract may predispose the patient to infection because dilatation and pressure necrosis may decrease defense mechanisms in the kidney and may allow disseminating hematogenous bacteria to alight and form a nidus of infection in the renal cortex or medulla. Also, once the normal architecture of the urinary tract has been damaged, whether as a result of reflux or obstruction, bacteria that lack the virulence factors necessary to cause renal infection in the absence of structural lesions may be responsible for serious upper tract infection once introduced into the kidney.

The treatment of urinary infections in the face of obstructive uropathy or stone disease usually requires antibiotic therapy for a longer duration than that for uncomplicated infections; treatment may be required for up to 6 weeks. Obviously, correction of the obstruction and removal of calculi are important adjunctive measures. In general, a 6-week course of a bactericidal antibiotic that achieves adequate concentration in renal tissue and bladder urine is recommended. Depending on the organism and susceptibility testing results, intravenous or oral therapy can be used; for example, a fluoroquinolone or a β-lactam antibiotic. Susceptibility testing is particularly important given the increased frequency of antibiotic-resistant bacteria in complicated urinary tract infections.[1] Special consideration is needed in the

25.1 Complicated Infections of the Urinary Tract

Anatomic or Structural Risk Factors

- Obstructive uropathy—stones, strictures, tumors
  - Prostate associated (see Chapter 23)
- Instrumentation—catheter associated and nosocomial
- Renal cystic disease
- Ureteral stents and surgical urinary diversions
  - Ileal loop constructions
- Other anatomic risk factors
  - Vesicoureteral reflux
  - Urachal remnants

Functional Risk Factors

- Diabetes mellitus
- Renal transplantation
- Spinal cord injury and neurologic dysfunction
- Neutropenia
- HIV

Miscellaneous Complicated Infections

- Pyonephrosis
- Emphysematous pyelonephritis and cystitis
- Malakoplakia and xanthogranulomatous pyelonephritis
- Intramural vesicle abscess
- Urosepsis
- Tuberculosis (see Chapter 27)
- Infections caused by atypical or resistant organisms
  (e.g., vancomycin-resistant enterococci, ESBL-producing gram-negative rods, anaerobes)

ESBL, extended spectrum beta-lactamase.

face of staghorn calculi, which often form in the presence of urease-producing organisms such as Proteus spp. Once the organism has been eradicated with a long course of antibiotics, "prophylactic" or "suppressive" therapy can be given with low-dose trimethoprim-sulfamethoxazole, daily or every other day, or with a combination of trimethoprim and a methenamine compound. Nephrolithiasis is considered in more detail in Chapter 20.

Catheterization and Instrumentation

The distal third of the urethra is normally colonized with perineal and skin flora. Instrumentation for any reason may introduce these organisms into the bladder. In the presence of an indwelling catheter, bacteria can ascend from the periurethral area along the mucous sheath that develops between the urethral mucosa and the latex rubber catheter.[2] Although several techniques have been introduced to prevent urinary infection in chronically catheterized patients, such as closed sterile drainage, continuous irrigation through a three-way catheter with antibiotic or acetic acid, and systemic antibiotic prophylaxis, all patients with prolonged urinary catheter drainage ultimately become colonized with high counts of bacteria (from 2 to about 21 days).

Catheter-associated bacteriuria and catheter-associated urinary tract infections (CA-UTIs) are the most common infection acquired in hospitals and long-term care facilities (LTCF);[3] elderly patients are at greatest risk. Over 40% of nosocomial infections originate in the urinary tract and Escherichia coli is responsible for most of these infections, followed by Enterococcus spp., Pseudomonas aeruginosa, and Candida spp.[3–5] Bacteremia, an important sequela of complicated UTIs, develops in about 4% of patients with indwelling bladder catheter-associated urinary infections, and case-fatality rates of 13% to 30% have been reported for these bacteremic nosocomial urinary infections.[3,5–6]

Catheter-associated nosocomial UTIs (CA-UTI) have been described in a study of 1,497 patients. These infections were more frequent in women (23.2%) than men (8.9%). They were unimicrobial in 94% and polymicrobial (primarily with enterococci and gram-negative bacilli) in 6%. The distribution of single isolates in these patients included gram-negative bacilli in 34%, enterococci or staphylococci in 27%, and Candida spp. in 27%. Patients with CA-UTIs only rarely have symptoms (<10%) in the face of infections and pyuria, and they may not have peripheral leukocytosis.[7,8] Catheters left in place without bona fide medical necessity often contribute to nosocomial infections and are accompanied by an increase in associated antibiotic costs.[9]

Environmental factors may relate to the nosocomial acquisition of bacteriuria in catheterized patients. Prevalent bacteria in the hospital colonize patients, or the patient's endogenous flora may enter the urinary collecting system or drainage bag. Within 24 to 48 hours they may be found in the bladder, and they increase to high colony counts over the subsequent 48 hours.[10,11] Bacteria may attach to the luminal surface of the catheter in association with the production of a mucoid biofilm, and this may predispose the patient to urinary infection or catheter blockage and obstruction.[7,10]

Urea splitting bacteria may lead to mucosal encrustations and encrusted cystitis and pyelitis. Corynebacterium group D_2 also have been implicated.[12] There is often a history of a prior urologic procedure or chronic illness, including immune compromise or renal transplantation. Patients may describe symptoms of cystitis, dysuria, gross hematuria, passage of encrusted debris, often with complaints of an ammonia odor to the urine. Failure to diagnose this condition can lead to renal impairment or ureteric obstruction and loss of renal graft as a result of infection, renal abscess, or obstructive uropathy. Treatment consists of antibiotics. The glycopeptides vancomycin and teicoplanin have in vitro activity against Corynebacterium group D_2, which are frequently resistant to fluoroquinolone antibiotics (>50%).

Additional treatment includes acidification of urine as well as chemolysis and the removal of infected calcified plaques that contain the organisms.[12]

Some caution is warranted in interpreting the results of cultures of material obtained from urinary collection devices. Several populations of bacteria may grow within the catheter and include planktonic bacteria in the urine and surface bacteria associated with the bacterial biofilm.[13] Bacteria may be cultured from catheter lumen encrustations when bladder urine might be sterile.[14] Because bladder urine is normally sterile, Garibaldi and associates[15] suggested that the presence of 100 or more organisms per milliliter should be considered as evidence of a positive urine culture in a catheterized patient. These authors demonstrated that breaks in the catheter-collecting system junction were associated with an early acquisition of bacteriuria. Current guidelines from the Infectious Diseases Society of America (IDSA) suggest that infection is likely in the presence of compatible signs and symptoms and an indwelling catheter if the bacterial counts of one or more organisms are equal to or greater than 10^3 CFU per milliliter.[3]

Condom catheters are the usual alternatives to indwelling bladder catheters in incontinent male patients without obstructive uropathy. Although associated with fewer infectious complications, at least one outbreak implicated these devices in 64 geriatric patients, 40 (63%) of whom had asymptomatic infections, frequently with mechanical obstruction of urine flow including kinking of the outlet, or blockage of flow by adhesive devices with associated penile cyanosis and ulceration. These problems may lead to urinary stasis, bacteriuria, and bladder wall distention, all of which may predispose a patient to complicated urinary infections.[16]

Treatment of catheter-associated urinary infections depends on the clinical setting. In general, asymptomatic bacteriuria in catheterized patients is not treated. In patients with catheters in place for the long term, there is some risk of dissemination of bladder bacteriuria to the blood during manipulation of the urinary tract as during catheter changes (generally done to minimize concretions and obstruction). Antimicrobial agents have not been shown to prevent catheter associated UTIs in persons with long-term indwelling urethral catheters.[3] Preventive strategies that avoid antibiotics are needed for these patients. We occasionally advocate treating the colonizing bacteria 8 to 24 hours prior to the catheter change, with a single dose of a bactericidal antibiotic based on susceptibility testing of the organisms (e.g., a quinolone or an aminoglycoside). This topic is extensively reviewed in the new IDSA guidelines,[3] and they do not support antibiotics at catheter replacement.

Bacteriuria

Interventional attempts to decrease the incidence of nosocomial UTIs using a silver-alloy, hydrogel-coated latex urinary catheter have been compared with standard silicone-coated latex catheters.[17] In older studies, silver-coated catheters significantly reduced the rates of bacteriuria in male surgical patients not receiving antibiotics.[18] In the more recent study,[17] silver-coated catheters were associated with a 32% decrease in the infection risk in male patients. Infection rates in females were similar in both catheter groups. In addition to a decrease in nosocomial infection rates, significant savings on hospitalization and other infection-related costs were described. Another study estimated that economic consequences of nosocomial symptomatic UTIs can reach over US$650 and catheter-related bacteriuria over US$2,800 per incident.[19] A study by Rupp et al.[20] demonstrated additional cost savings with the silver-alloy hydrogen-coated urinary catheters and also showed a decline in nosocomial UTIs. No evidence of silver-resistant urinary pathogens was found.

Symptomatic urinary infections or urosepsis in the presence of an indwelling catheter is best treated with rapidly bactericidal antibiotics such as an aminoglycoside, a fluoroquinolone, or a β-lactam-aminoglycoside combination based on antimicrobial susceptibility testing.[1] Bacteremia is usually easily cleared, but eradication of the urinary infecting organism may be difficult in the continued presence of the catheter. Guidelines for the prevention and management of catheter-related urinary infections have been updated recently.[3]

Renal Cysts (Including Polycystic Renal Disease)

Complicated infections within or associated with isolated renal cysts, autosomal dominant polycystic renal disease (ADPKD), or acquired renal cystic disease (three or more renal cysts or cystic involvement of >25% of renal mass in the absence of autosomal dominant polycystic kidney disease) remain important diagnostic and therapeutic challenges.[21]

Patients with polycystic kidney disease may develop typical infections of the urinary bladder and ascending pyelonephritis with renal parenchymal involvement as well as infection within the renal cysts themselves.[22] The presence of polycystic kidney disease is associated with a 50% to 70% lifetime risk of some form of UTI.[23] In an autopsy study of 23 patients with polycystic kidney disease, 13 (56%) had findings consistent with pyelonephritis.[24] It may be difficult to implicate infection as a cause of hematuria or flank pain in patients with cystic abnormalities of the kidney because these symptoms may be present in the absence of infection.[25] Also, pyuria (≥10 leukocytes/high-power field) may be present in more than 40% of patients with polycystic kidney disease, with or without other symptoms suggestive of urinary tract infection; however, infection is documented in only about 10% of these patients.[26] Findings suggestive of a UTI in the presence of cystic renal disease include positive blood cultures, leukocytosis, fever, and lower GU tract symptoms such as dysuria. Negative urine cultures do not exclude infection of a renal cyst.

In a classic review of renal infections in patients with polycystic kidney disease, Sklar and associates[25] described

four types of infections according to anatomic involvement: (1) localized infected cyst (pyocyst), (2) pyonephrosis (intrarenal abscess associated with ureteral obstruction), (3) acute bacterial interstitial nephritis, and (4) perinephric abscess. Clinical findings may vary with the anatomic location of bacterial infections in these patients.

The diagnosis of complicated UTIs in patients with renal cystic disease is usually based on the results of clinical examination, laboratory testing, and diagnostic imaging. Radiologic evaluation with plain radiography, ultrasound, computed tomography (CT), and gallium imaging has been used to determine the presence and location of infection.[25] Gallium imaging may show uptake within the kidney, but it does not provide specific information to determine whether an abscess or an infected cyst is present. CT may be necessary to define pyocysts but CT scans are not optimal in distinguishing infected from noninfected cysts. Plain radiography may be useful if calculi are contributing to the clinical presentation. Renal ultrasonography also may identify calculi and can differentiate hydronephrosis from pyonephrosis and perinephric abscess. Recent studies suggest that positron emission tomography (PET) scanning and diffusion-weighted magnetic resonance imaging (MRI) might be useful in differentiating infected from noninfected cysts.[21,27] The percutaneous drainage of infected cysts in adult polycystic kidney disease has been described, as has laparoscopic cyst decortication using transperitoneal or retroperitoneal access.[28,29]

When cysts are infected, the Enterobacteriaceae (especially E. coli, Klebsiella spp., and Proteus spp.) and P. aeruginosa are most frequently implicated, with Staphylococcus aureus, Salmonella spp., Streptococcus spp., Corynebacterium spp., and others isolated less frequently.[21,30] A gas producing Clostridium perfringens infection in a renal cyst has been reported in a patient with ADPKD.[31]

Attempts should be made to isolate the infecting organism from the blood, urine, or cyst drainage. Appropriate treatment of infections in patients with polycystic kidney disease depends on the use of antibiotics that are able to concentrate within the infected cysts in addition to providing bactericidal activity against the infecting organism. Aminoglycosides, penicillins, and cephalosporins have relatively poor penetration into renal cysts, although pH, cyst physiology, and histology may affect the diffusion.[32] These antibiotics are relatively lipophobic and do not diffuse across cyst epithelial layers. Lipophilic agents such as clindamycin, chloramphenicol, macrolides, metronidazole, and trimethoprim are able to penetrate and accumulate within cysts, but they may or may not be active against the infecting organisms.[33] Fluoroquinolone antibiotics such as ciprofloxacin accumulate in cystic fluid, and they have been used successfully to treat infected renal cysts.[34,35] Because most of the causative organisms in infected cysts are facultative gram-negative bacilli with presumed fluoroquinolone susceptibility, these agents may still be quite useful clinically. Oral therapy is acceptable unless patients are septic. Infection in multiple cysts has been associated with sepsis and may require surgical intervention (nephrectomy) in rare cases.[36] Intravenous therapy should be used in bacteremic patients and in patients with decreased gastrointestinal (GI) quinolone absorption (e.g., antacid use). Antibiotic choice should be based on pharmacology and antibiotic susceptibility testing given the increased incidence of antibiotic resistance among gram-negative bacilli.[1]

Urinary Diversion

Ureteral Stents

Ureteral stents are placed in the treatment of hydronephrosis and obstruction caused by nephrolithiasis or malignancies, and as adjuncts to lithotripsy and open surgical procedures on the urinary tract. These stents are made of synthetic polymers and are either indwelling (self-retained in place between the renal pelvis and the bladder) or external.

Complications of indwelling ureteral stents include fever, infection, gross or microscopic hematuria, biofilm development and stent rupture, catheter migration, encrustation and bladder stone formation, and vesicoureteral reflux.[37] Infection in the presence of stent obstruction is problematic and difficult to eradicate. Also, it is often difficult to differentiate symptoms caused by an infection from those associated with the presence of a stent, which include hematuria, dysuria, increased urinary frequency, nocturia, and loin pain. Bacterial colonization of stents is common and Enterobacteriaceae, staphylococci, streptococci, and Pseudomonas aeruginosa are most frequent.[38,39]

Positive urine cultures with supporting clinical evidence for urinary infection should stimulate prompt antibiotic treatment to eradicate the infecting pathogens. Ureteral stents that develop biofilm formation and encrustations treated in vivo with oral ciprofloxacin or ofloxacin absorb these antibiotics.[40] Attempts to determine the presence of bacterial stent colonization are not reliable. Despite negative urine cultures, stents may be colonized with bacteria or fungi. Double-J ureteral stents can become colonized with gram-negative bacteria within 2 weeks of placement. Risk factors for stent colonization and urinary infection include diabetes mellitus, chronic renal failure, and pregnancy.[39] Silicone ureteral stents compared with low surface energy stents and hydrogel-coated stents demonstrated less encrustation in the presence of urease-producing bacteria (Proteus mirabilis). These results may indicate a reduced risk of encrustation and P. mirabilis infection with this stent,[41] but biofilm formation and subsequent colonization and encrustation remain important challenges.[37] Triclosan-eluting stents might result in a reduction in symptomatic infections and antibiotic use, but their ultimate role needs more study.[42] The presence of bacteremia and other signs of systemic infection suggest infection in the face of possible occlusion or obstruction of the stent, and stent removal is essential.

Surgical Urinary Diversion

Urinary diversions are performed to reroute urine in patients with obstructive uropathy from many causes—urinary bladder carcinoma or prostatic or gynecologic malignancies—and in

patients with congenital abnormalities, neurologic disorders, and pelvic trauma. Although intermittent catheterization may be preferable in some patients with neurogenic bladder dysfunction (e.g., multiple sclerosis, paraplegia), the creation of a ureteroileal conduit is a popular alternative to achieve control of urine excretion. This procedure does not carry the associated metabolic and electrolyte complications seen with jejunal bypass procedures. The surgical construction of an ileal loop conduit is associated with few serious complications and a low mortality rate.

Infectious complications have been well described in pediatric and adult populations, and an increased incidence of infections has been noted when the ureteric component becomes obstructed; pyelonephritis may result. Renal calculi are encountered frequently after urinary diversion and are often caused by urea-splitting organisms such as P. mirabilis and Proteus morganii. The urease produced by these organisms splits urea to form an alkaline pH, and the solubility product constant for calcium and phosphate is exceeded with the resultant precipitation of crystals, which form the nidus for renal stones.[43,44] Newer diversion procedures have been introduced (such as orthotopic urinary diversion or the neobladder) and also carry a risk of urinary infection.[45]

Recommendations for the management of these patients include aggressive control of the infection using bactericidal drugs active against urea-splitting organisms and acidification of the urine or avoidance of alkaline urinary pH, which encourages stone formation. The detection of urinary infection in these patients is difficult because the ileal loops are almost always colonized. Asymptomatic bacteriuria in the presence of a ureteroileal conduit should not be treated and prophylactic antibiotics are not recommended; this is less clear with orthotopic diversions.[45] However, positive urine cultures associated with physical findings of fever, chills, and flank pain should prompt the initiation of appropriate bactericidal antibiotics directed against gram-negative enteric rods including Proteus spp. Aminoglycosides, fluoroquinolones, third- or fourth-generation cephalosporins, carbapenems, and penicillin-β-lactamase inhibitor combinations (e.g., ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanate) may be useful in this setting. When susceptibilities are known, specific therapy can be instituted. Septic complications often lead to failure of ileal diversions.[46]

Other Anatomic Risk Factors

Vesicoureteral Reflux

Conditions that allow for the reflux of urine from the bladder to the ureters and subsequently the kidneys are associated with increased frequencies of acute and chronic pyelonephritis. Various degrees of vesicoureteral reflux may occur congenitally, and once the bladder urine is infected, an upper tract infection is facilitated by the retrograde flow. In this setting, organisms that cause infection may be relatively free of virulence factors such as hemolysins, pili, and other adhesions (Chapter 55). Vesicoureteral reflux is associated with progressive renal scarring in children, even in the absence of infected urine, and these patients have an increased risk of renal damage and even renal failure when infection does occur.[47]

In a study of the etiologies of renal scarring, Huland and Busch[48] evaluated 213 patients with recurrent UTIs. Forty-two patients (26%) were found to have pyelonephritic scarring and renal insufficiency. Twenty-eight (67%) of these patients had urinary infections in the presence of vesicoureteral reflux. Young children under 4 years of age with intrarenal reflux have a well demonstrated risk of renal scarring.[47]

The approach to the treatment of patients with documented vesicoureteral reflux and recurrent UTIs includes antibiotic management plus surgical reconstruction (antireflux operation). Hendren[49] reported that more than 70% of very young children no longer had urinary infections after the surgical procedure. Long-term antibiotic chemoprophylaxis to prevent recurring UTIs has been studied in addition to surgical reconstruction. However, a recent review has questioned the use of this approach. A Cochrane Database review of several published randomized placebo-controlled trials showed a benefit of long-term antibiotics (10 to 52 weeks) to reduce the frequency of symptomatic UTI recurrences. However, this benefit was small and is associated with a risk of antimicrobial resistance.[50]

Two recent publications highlight ongoing controversies in the management of vesicoureteral reflux in children given newly developed endoscopic procedures in the face of the traditional approach of continuous antibiotic prophylaxis.[51,52] The optimal approach to the prevention of renal scarring remains under active investigation.[53]

Infected Urachal Remnants/Cysts

Acute and recurrent UTIs in the presence of abnormalities of urachal development with patent urachal remnants may be difficult to diagnose. Four primary developmental defects exist and present with varying signs and symptoms: patent urachus, umbilical urachal sinus, vesicourachal diverticulum, and urachal cyst.[54] Umbilical urachal sinuses and urachal cysts are only rarely infected and many urachal cysts are found incidentally during surgery or radiographic studies. Although most patent urachal remnants in adults are undetected and asymptomatic, umbilical urachal sinuses occasionally may be infected and present with purulent umbilical drainage or periumbilical erythema. These infections may present as infraumbilical abdominal wall abscesses or with contiguous involvement of the peritoneal cavity with an enteric fistula. Adult and pediatric cases have been reviewed.[54–56]

A vesicourachal diverticula may present with recurrent urinary infections, and urethral discharge, pneumaturia, and a suprapubic mass also may be found. Imaging studies may help make the diagnosis, but often cystoscopy may be necessary, especially if the diverticulum is associated with malignant transformation of the remnant. The organisms that infect the vesicourachal diverticula include E. coli and other Enterobacteriaceae, S. aureus, Enterococcus spp., and Actinomyces spp.[54,57]

FUNCTIONAL RISK FACTORS

Diabetes Mellitus

Many factors that predispose the diabetic patient to infections in the urinary tract have been described. Diabetes mellitus is putatively associated with an increased risk of these infections as a result of poorly controlled plasma glucose concentrations, which in turn may impair granulocyte function and cell-mediated immunity. Also, the neurologic dysfunction associated with diabetic neuropathy may result in a neurogenic bladder with incomplete bladder emptying, urinary stasis, and retention. The increased likelihood of urethral instrumentation may predispose these patients to infection, as may diabetic microangiopathy, which can contribute to local ischemia and impaired host defenses.[58,59]

Table 25.2 lists the manifestations of UTIs in patients with diabetes mellitus. Asymptomatic bacteriuria has been described as occurring two to four times more frequently in diabetic women[59,60] and as being more prevalent in diabetic women than men.[61] Although the treatment of asymptomatic bacteriuria in diabetic women reduced the duration of long-term bacteriuria, recolonization occurred after most treatment regimens.[62] A recent study in type 1 diabetic women suggested that sexual activity is more likely to be a risk factor for cystitis and pyelonephritis than diabetes control or complications.[63] In one recent small series, the distribution of responsible pathogens was similar among diabetic and nondiabetic patients, as was the frequency of infection with antibiotic-resistant organisms.[64] Bacteriuria in diabetic patients may be associated with a disproportionate risk of infection in the upper urinary tract and kidneys, and one study reported that upper tract infection could be documented in 79% of diabetic women with asymptomatic bacteriuria.[60] Other renal parenchymal complications of UTIs in diabetic patients include pyelonephritis, emphysematous pyelonephritis, papillary necrosis, and perinephric abscesses; these conditions should be considered in the evaluation of nonresponse to appropriate antibiotic therapy for urinary infections in diabetic patients.

Diabetic patients with urinary infections are more likely to be bacteremic or uroseptic than are nondiabetic patients.[65] These patients are also more likely to develop acute pyelonephritis at a fivefold greater risk than nondiabetic patients.[66] Diabetic patients with serious systemic signs of urinary infections should be studied with abdominal radiography to detect renal emphysematous pyelonephritis. Ultrasound or CT scans should be performed if an obstruction or an abscess is suspected. The urinary tract is implicated as the source of bacteremia more frequently in diabetic than in nondiabetic patients.[65] Postmenopausal women with diabetes are at a higher risk of a UTI and this is related to the duration of diabetes and insulin dependence.[67] Type 2 diabetic women with histories of UTIs (especially upper UTIs) are at increased risk for renal scarring and damage, as demonstrated by renal cortex scans.[68] Most of the bacteria responsible for urosepsis in diabetics are gram-negative rods, with E. coli and Klebsiella spp. accounting for about 70%. Notably, Klebsiella spp. are isolated frequently in diabetic patients with bacteremic urinary infections, and a large proportion of these patients have indwelling urinary bladder catheters.

In diabetic patients taking oral hypoglycemic agents, trimethoprim-sulfamethoxazole may lead to further hypoglycemia. No such potentiation is seen with most fluoroquinolone antibiotics, but hypoglycemia has been reported with gatifloxacin.[69] Invasive staphylococci also can cause complicated infections that might or might not result in abscess formation. Given the current high frequency of methicillin-resistant Staphylococcus aureus (MRSA), the treatment for staphylococcal urinary infections should begin with vancomycin, and can be modified based on susceptibility testing. Intravenous antibiotics should be administered for the first 2 to 4 days in complicated infections while monitoring for decreasing symptoms and fever. Oral step-down treatment should be continued for 14 days or longer in the presence of complicated infections.

Opal and associates[70] described 29 adult patients with Streptococcus agalactiae (group B streptococcus) bacteremia reviewed over 10 years at the Walter Reed Medical Center. Nine (31%) of these bacteremic patients had diabetes, and six of these had involvement of the urinary tract.

Renal papillary necrosis is a well-known complication of UTIs in diabetic patients. More than half of patients with renal papillary necrosis are diabetic, possibly reflecting microvascular insufficiency leading to ischemia and necrosis of the renal papilla.[71] These patients may present with flank pain, chills, and fever, and 15% may have renal insufficiency.[59]

25.2 Manifestations of Urinary Infections in Patients with Diabetes Mellitus

Clinical Manifestation	References
Asymptomatic bacteriuria	59,60,61,172
Acute papillary necrosis	59,71
Bacteremia	62, 65
Emphysematous cystitis	72,173,174
Emphysematous pyelonephritis	73–76,174,175
Fungal infections	176,177
Perinephric abscess	178,179
Xanthogranulomatous pyelonephritis	128,180

Emphysematous infectious processes may involve the bladder (emphysematous cystitis) or the kidney (emphysematous pyelonephritis) and are more frequent in diabetic patients. Emphysematous cystitis is usually caused by common facultative bacteria such as E. coli, although a few cases caused by Clostridium perfringens have been reported.[72] This condition is identified on plain films, urographic roentgenograms, or CT scans by finding gas in the bladder wall. Tissue ischemia or trauma is usually involved in the pathogenesis.

Emphysematous pyelonephritis usually results from an invasion of the renal parenchyma by gas-producing organisms. In patients with diabetes mellitus, high levels of blood glucose offer an enhanced environment for bacteria, and gas formation may result from the accompanying mixed acid fermentation of glucose by enteric organisms. In patients with diabetes, emphysematous pyelonephritis presents with fever, chills, flank pain and tenderness, and often the finding of a flank mass or a gas-containing renal mass on imaging studies.[73,74] E. coli is the most frequently isolated bacteria, followed by Proteus, Pseudomonas, and Klebsiella spp., with Clostridium spp. and Candida spp. reported occasionally. The diagnosis is established by the radiologic finding of gas in the renal parenchyma and bacteremia is usually present. Urine cultures are positive, and renal failure and hematuria may occur.[75,76]

Treatment includes intravenous fluid support, appropriate intravenous antibiotics, and percutaneous or renoureteral catheter drainage.[74–77] Hyperbaric oxygen might be useful as an adjunct to antibiotics.[78] Laparoscopic or open nephrectomy may be required if the response to medical therapy is delayed, especially in patients with extensive renal involvement and/or multiorgan system dysfunction. Emergency nephrectomy in these patients carries a high mortality and should be delayed for antibiotic treatment if possible.[74–77]

Renal Transplantation

UTIs may occur in patients following renal transplantation, with an incidence rate as high as 80% in some series.[79,80] Most of these infections occur within the first 6 months following transplantation and are usually caused by common Enterobacteriaceae, including E. coli, Klebsiella pneumoniae, or by P. aeruginosa, or fungi, especially Candida spp. and Aspergillus spp. Infections that occur 6 months or later after transplantation are less common and are more likely to involve the lower urinary tract, including the bladder. UTIs due to anaerobes, Ureaplasma urealyticum, and Gardnerella vaginalis have been reported posttransplant and the latter was implicated as a cause of a perinephric abscess in one patient.[81] Transmission of multidrug resistant (MDR) E. coli during kidney transplantation has been reported recently. A donor with MDR E. coli caused infection in two different kidney recipients. One developed sepsis and a complicated UTI, the other developed a perinephric abscess. This resulted in renal graft failure in both patients, but both survived.[82]

Risk factors for posttransplant UTIs include advanced age, pretransplant UTIs, female gender, diabetes mellitus, postoperative instrumentation and catheterization, cadaveric donors, intraoperative ureteral stents, immunosuppression, and acute graft rejection.[80,83]

In the early posttransplantation period, symptoms of a urinary infection may be mild or absent and fever may be absent; however, pyelonephritis and associated bacteremia are not uncommon.[79] Uremia and corticosteroid or other immunosuppressive therapy may contribute to this situation. Posttransplantation renal infection (especially with Enterococcus faecalis and occurring 6 months or more after a transplant) may lead to elevated serum creatinine levels and may result in a cascade of immunologic responses that ultimately precipitate allograft dysfunction or rejection.[80,83] In addition to common cytomegalovirus infections in transplant patients, infections with hepatitis C virus and BK polyomavirus have been described after renal transplantation, and viral-associated interstitial nephritis may occur. In one center, BK polyomavirus with an associated transplant dysfunction and a graft loss was reported in 2.5% of transplanted patients. Clinical features include ureteral obstruction, lymphocele, bacterial urinary infection, and hematuria. The diagnosis may be established with renal biopsy and electron microscopy of urine. Interruption of the progression may be achieved with immunosuppressive treatment.[84,85] Relapse rates may be high if posttransplantation urinary infections are not treated aggressively with antimicrobial agents.

Chronic urinary infections may occur in these patients and can be particularly problematic if associated with anatomic or structural defects in the ureter, bladder, or urethra as a result of the surgical procedure itself or secondary to a fistula formation. Vesicoureteral reflux may develop at the ureteral anastomotic site in up to 25% of patients and may lead to hydronephrosis and infection. Graft failure may also occur as a result of mesangiocapillary glomerulopathy.[86,87]

Prophylactic use of trimethoprim-sulfamethoxazole for 6 months following a renal transplantation may prevent complicated UTIs, including gram-negative rod bacteremia and sepsis[79,80,88,89] and may decrease the incidence of posttransplantation urinary infection to less than 10%. This drug also might reduce the development of opportunistic infections caused by Listeria monocytogenes, Nocardia asteroides, and Pneumocystis jiroveci.[79,80] However, reports of trimethoprim-sulfamethoxazole–induced alteration of renal function or synergistic exacerbation of cyclosporine nephrotoxicity have stimulated the search for other prophylactic compounds.

The treatment of pyelonephritis in the posttransplantation patient should include 6 weeks of an appropriate antibiotic based on susceptibility testing plus chronic "suppressive" antibiotic therapy thereafter.[79] A late UTI after renal transplantation may be a risk factor for serious complications including graft loss and death as described in a retrospective review of 728,000 renal transplant patients.[91]

Pharmacokinetic or pharmacodynamic interactions between ciprofloxacin and cyclosporine have not been reported, which suggests that they can be used together without additional monitoring.[90]

Patients are at increased risk of catheter-associated urinary infection because they often receive Foley catheters in the post-transplantation period. Catheter-tip cultures have been used to screen for these infections. Tolkoff-Rubin and colleagues[89,92] used prophylactic trimethoprim-sulfamethoxazole (160 mg of trimethoprim, 800 mg of sulfamethoxazole) for 4 months after urinary catheter removal and reported a decrease in the catheter-associated infection rate from 38% to 8%.

Spinal Cord Injury and Neurologic Dysfunction

Frequent bladder catheterization is necessary as a result of bladder neuropathy in paraplegic or quadriplegic patients following traumatic or surgical injury to the spinal cord. This leads to colonization of the lower urinary tract with pathogenic bacteria and results in bacteriuria in about 80% or more of these patients.[93] Bacteremia may follow and urinary infection may be associated with high mortality rates in patients with spinal cord injury and neuropathic bladders.

Urinary infections and the related sepsis as well as the high frequency of renal disease in these patients are probably associated with vesicoureteral reflux, hydronephrosis, accompanying renal calculi, and pyelonephritis, which are often responsible for death.

These patients may not present with typical symptoms of fever, chills, dysuria, or flank pain, and the presence or absence of symptoms usually is not helpful in predicting the results of urine cultures. Perkash and Giroux[94] found typical symptoms in only 3% of 110 patients with spinal cord injury and bacteriuria ($>10^5$ bacterial colonies per milliliter).

The prevention of infection in patients with spinal cord injury is a major priority. Unfortunately, intermittent catheterization or self-catheterization as an alternative to indwelling bladder catheters may be associated with complications, including urethral fistulization, stricture, periurethral abscess formation, and epididymitis.[3] Although several older publications reported modest success with methenamine compunds[95] or with some antibiotics including trimethoprim-sulfamethoxazole,[96] the current IDSA guidelines do not support these modalities in long-term catheterized patients.[3] In almost every case, modest reduction in bacteriuria or symptomatic infection was associated with an increased risk of antimicrobial resistance. Bladder irrigants are also not recommended and neither is routine screening for bacteriuria in these patients.[3]

The IDSA guidelines do recommend that a urine culture be obtained prior to treatment in catheterized patients who do develop symptomatic UTIs. Replacement of the catheter prior to the onset of treatment can hasten the response and can minimize the occurrence of polymicrobic bacteriuria. Optimal culture results are obtained through the replacement catheter. These guidelines recommend treatment for several days if the clinical response is prompt, or 10 to 14 days if not. The specific antibiotic choice should be made according to susceptibility test results.[1]

Recent attention to the development of adherent biofilms in the urinary tract may offer explanations for recurring and difficult-to-treat infections in this population. Uropathogens can develop dense urethral biofilms with glycocalyx material on the bladder wall or catheters. Recently, biofilms have been described with intracellular bacteria that cause bulges appearing like pods on the bladder surface of infected mice. The pods have been shown to have polysaccharide matrices with a protective shell of uroplakin. It is becoming increasingly more evident how bacteria may evade antimicrobial killing within an environment that protects microorganisms in a uroplakin shell. These mechanisms support the ability of biofilms to allow bacterial microcolonies to survive and cause chronic or recurrent infections.[97,98] This may contribute to the pathogenesis of recurrent urinary infections. Bacterial biofilms also can be detected on bladder epithelial cells and may respond better to fluoroquinolones than to trimethoprim-sulfamethoxazole. In addition to the clinical cure of urinary infections, ofloxacin eradicated bladder biofilms in patients with spinal cord injury.[99] Although silver alloy-hydrogel coated catheters might reduce biofilm formation and delay bacteriuria, they have not yet been shown to reduce bacteriuria or UTIs in patients with neurogenic bladders who require long-term catheterization.[100] Increasing antibiotic resistant gram-negative bacteria and MDR staphylococci are being reported in outpatients with spinal cord injury and UTIs.[101]

An experimental approach to prophylaxis has been reported that uses a nonpathogenic E. coli strain 83972 to colonize urinary bladders in spinal cord injury patients. Intravesical inoculation and colonization with this bacteria was not associated with urinary infection symptoms but was associated with an improved quality of life.[102] Other approaches, including cranberry products, may lead to a reduction of uroepithelial cell biofilms,[103] but these are not recommended in the IDSA guidelines because supporting data were not definitive.

As mentioned already, frequent monitoring and the treatment of symptomatic infection with appropriate bactericidal antibiotics may reduce the morbidity of urinary infections in spinal cord–injured patients with long-term indwelling catheters.

Neutropenia

Patients whose neutrophil count has fallen below 1,000 granulocytes per microliter are at an increased risk of bacterial infections. Most of these patients have received anticancer chemotherapy for leukemia, lymphoma, or solid tumors. The gastrointestinal (GI) flora is the usual source of bacteremia in these patients, and unless instrumentation in the urinary tract has been performed, urinary infections are not particularly frequent. In a recent series, about 6% of the infections in neutropenic patients arose from the urinary tract.[104] In patients with profound and prolonged granulocytopenia

(<100 cells per microliter), bacteremia is not uncommon; however, the urinary tract is infrequently the source of these infections. In fact, bacteremia in this population is more frequently caused by gram-positive cocci than gram-negative rods, probably in part because of the presence of chemotherapy-induced oral mucositis and the dissemination of oral gram-positive cocci to the bloodstream.

The usual symptoms and signs of bacterial urinary infection may not be manifest because granulocytopenic patients may not respond locally to the presence of infection. Dysuria and burning may or may not be present, and pyuria is often minimal because of the absence of granulocytes[105]; therefore, it is important to culture the urine in febrile granulocytopenic patients even though the yield may be low. Fungal urinary infections may occur, especially following long courses of antibiotics. Patients with hematologic malignancies and neutropenia have been reported to have Achromobacter and Alcaligenes spp. bacteremic infections, with urinary tract involvement or origin. Resistance patterns for these species show susceptibility to antipseudomonal penicillins, carbapenems, and trimethoprim-sulfamethoxazole, but resistance to fluoroquinolones and aminoglycosides.[106]

The empirical use of antimicrobial agents early in the course of a fever after appropriate cultures of blood, urine, and material from other presumed infected sites are obtained has reduced the mortality owing to infections in neutropenic patients. A Gram stain should be performed on urine specimens from these patients and antibiotics directed against the common bacterial pathogens in this population, such as E. coli, S. aureus, P. aeruginosa, and streptococci (especially viridans Streptococcus). Fluconazole or amphotericin B should be considered for candidal UTIs, but the new echinocandins do not achieve adequate concentrations in the urine.

AIDS and HIV Infection

AIDS and infection with HIV have been associated with infectious and noninfectious complications in the GU tract.[107–109] Noninfectious complications include HIV-associated nephropathy, which has become the third leading cause of end-stage renal disease in African Americans between 20 and 64 years old, but the progression of HIV nephropathy has been slowed by the use of highly active antiretroviral therapy (HAART).[110] Acute renal failure also develops in HIV-infected patients at rates that are enhanced at lower CD4 counts and are decreased with antiretroviral therapy.[110–112]

Other noninfectious complications are associated with antiretroviral drugs, especially protease inhibitors, and are usually used in combination with two other highly active agents to prevent HIV replication. Indinavir sulfate has been implicated with crystallization and stone formation in the urinary tract, which is not usually visible on plain radiographs. Patients complain of ipsilateral flank pain with nausea, vomiting, dysuria, and hematuria. CT scans are not diagnostic, but calcifications may be seen ultrasonically in approximately 35% of cases. Up to 20% of patients treated with indinavir may have urologic side effects. Conservative treatment with hydration, analgesia, and a brief discontinuation of therapy is effective, but permanent withdrawal may be necessary in 5% of patients.[113] Tenofovir and lopinovir-ritonavir combinations have been associated with an increased risk of acute renal failure (ARF), and renal function should be monitored during therapy.[114] However, more recent studies have suggested that these drugs were not as predictive of ARF as were levels of immunodeficiency.[110–112, 115]

Infections caused by commonly encountered bacteria are frequent and may present as cystitis, pyelonephritis, or renal abscesses. Also, Mycobacterium tuberculosis as well as atypical or nontuberculous mycobacteria may be found in upper and lower UTIs in these patients. Of the usual infecting bacteria, E. coli accounts for the most, and Pseudomonas, Proteus, Klebsiella, Enterobacter spp., and Enterococcus spp. are found frequently. Acinetobacter, Salmonella, and Candida spp., Aspergillus spp., Cryptococcus spp., and Mycobacterium spp. also may be encountered and should be looked for in patients whose CD4 counts are <500 or who do not respond quickly to antibiotics.[107,108] Upper and lower UTIs are probably more common in HIV-infected than in non-HIV-infected patients.[107,108]

Urethritis, prostatitis, and prostatic abscesses may occur due to infections with S. aureus, Enterococcus spp., Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, and Histoplasma capsulatum.[107,116] In the case of a histoplasma abscess, urine cultures are positive only rarely. Granulomatous interstitial nephritis resulting from disseminated histoplasmosis has been reported.[117] Microsporidial infection of the urinary tract caused by Vittaforma corneae has been described.[118]

Urinary infection with Aspergillus presenting as a renal aspergilloma has been reported in an AIDS patient who was treated successfully with amphotericin B instillation via a nephrostomy tube, as well as with systemic antifungals.[119] Nosocomial infections with Stenotrophomonas maltophilia of presumed urinary tract origin have been reported in HIV-infected patients and were associated with high-level resistance to β-lactam antibiotics, monobactams, carbapenems, and aminoglycosides.[120]

Cytomegalovirus can cause cystitis with hematuria in HIV-infected persons,[92] and toxoplasmic cystitis also has been described.[121] Trichomonas vaginalis urethritis, documented both by wet-mount and culture and polymerase chain reaction, has been reported in men with and without symptoms.[122] Latent urinary BK virus, a human polyomavirus, can be activated in patients with AIDS, and nephritis may result.[123] Adenovirus hemorrhagic cystitis with gross hematuria and dysuria has been described in an HIV-infected patient with Burkitt lymphoma.[124] Noninfectious vesicle involvement with Kaposi sarcoma and urethral lymphoma in HIV-infected patients may have unusual presentations that initially could be confused with urinary infection.[108]

Patients with a neurogenic bladder that complicates HIV infection or AIDS may present with urinary retention, urinary frequency, and altered urinary flow as a result of bladder hyperreflexia or hyporeflexia. Most of the patients

with bladder hyperreflexia had concomitant cerebral toxoplasmosis.[125] Other patients with AIDS may have urinary retention secondary to central nervous system lymphoma, myelopathy, or prostatic hypertrophy. The incidence of many of these conditions has declined with the use of HAART.

MISCELLANEOUS CAUSES

Pyonephrosis is an acute suppurative infectious process with gross pus within the renal parenchyma that usually results from ureteral obstruction.[126] The clinical differentiation of pyonephrosis from infected hydronephrosis is difficult even with the use of ultrasound evaluation, but the finding of fluid-debris levels on MR urography is a strong indicator of pyonephrosis.[127] This infection may be associated with an obstruction secondary to congenital anomalies, calculi, malignancy, ureteral strictures, nephrolithiasis, diabetes, and functional disorders of the renal collecting system.

In a review of 23 patients with pyonephrosis, 15 patients presented with virgin stone formation that produced an obstruction at various sites including the calyces, renal pelvis, and middle and distal aspects of the ureter.[126] Clinical presentation may vary from asymptomatic bacteriuria to resembling that of pyelonephritis with fever, flank or abdominal pain, leukocytosis, pyuria, and septic shock. The responsible organisms include Enterobacteriaceae and anaerobic bacteria such as Bacteroides spp., and Candida spp.

Radiographic determination of pyonephrosis may be limited even with the use of ultrasonography and CT; the correct diagnosis requires a high degree of suspicion, and MR urography may be helpful.[127] Ultrasound or CT-guided renal urine aspiration or retrograde ureteral catheter placement may be required. Nephrectomy may be necessary if drainage and appropriate antibiotic therapy directed at organisms isolated from aspirated and drained infected material does not prove successful.

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is a unique pathologic presentation of chronic bacterial pyelonephritis. Schlagenhaufer initially described it in 1916, and more than 500 cases have been reported in the literature. However, xanthogranulomatous pyelonephritis remains relatively uncommon and accounts for less than 1% of surgically or pathologically proved cases of chronic pyelonephritis. Most cases are diagnosed in elderly patients, with almost 70% occurring in women. A recent review highlighted the risk factors for xanthogranulomatous pyelonephritis, which included female gender, obesity, elevated creatinine, and the presence of renal stones or staghorn calculi.[128]

The pathogenesis of xanthogranulomatous pyelonephritis is uncertain. Although P. mirabilis infection is present in most patients, it is not essential for the pathologic process to occur. Urinary obstruction usually has a role. Macrophages filled with periodic acid-Schiff (PAS)-positive granules have been produced in a rat infection model with P. mirabilis, E. coli, and S. aureus. On electron microscopy, macrophages appear to have ingested bacteria and developed phagolysosomes filled with amorphous material. It is hypothesized that xanthogranulomatous pyelonephritis may be caused by a lysosomal defect of macrophages that prevents the complete digestion of ingested bacteria. Familial disease has not been described.

Presenting symptoms are recurrent flank pain, fever, and constitutional fatigue. Persistent anemia and leukocytosis occur in about 75% of patients. The urinalysis shows pyuria and, often, hematuria. Urine cultures are positive for P. mirabilis in about two-thirds of patients, and E. coli, Klebsiella spp., and S. aureus are each reported from a small proportion of patients. Multiple pathogens occur in about 25% of patients. In a small proportion of patients, urine cultures are negative despite ongoing disease activity, and positive cultures may be obtained from resected renal tissue.[129] S. aureus may produce localized disease that might not be associated with nephrolithiasis, and MRSA infections have been reported.[130] Occasionally, pathogens isolated from resected renal tissue are different than those from voided urine.[129]

Most patients have a history of recurrent urinary infection, often complicated by renal calculi, obstructive uropathy, and previous urologic procedures. Often, patients have had a chronic undiagnosed illness for several months before the diagnosis of xanthogranulomatous pyelonephritis has been considered. On physical examination, a renal mass is palpable in more than 50% and hypertension is present in about 25% of patients.

Intravenous pyelography (IVP) discloses a nonfunctioning kidney in 85% of patients. Struvite renal calculi occur in 80% and are virtually universal with P. mirabilis infection. Radiologic investigation may also demonstrate cavitary masses and calyceal deformities. Angiography usually discloses hypovascular renal masses with no neovascularization. CT scan is the diagnostic procedure of choice. It demonstrates the extent of involvement of perirenal structures and may permit a specific diagnosis by the recognition of abnormal fatty tissue in the renal mass.

The disease is almost always unilateral and appears to be caused by chronic renal infection with an unusual inflammatory response. On a gross examination, the kidney is enlarged with either local or generalized involvement of renal tissue. Calyces are usually dilated and the renal parenchyma is replaced by yellow-orange soft tissue, which is usually surrounded by abscesses. The localization of this peculiar tissue in the renal pelvis is characteristic. Perirenal fat is usually inflamed, edematous, and adherent to the kidney. The inflammation may spread beyond Gerota's fascia and involve the perirenal fat of the retroperitoneal space. On a microscopic examination, the lipidlike tissue is composed of a mixture of large foamy lipid-laden macrophages (xanthoma cells) together with neutrophils, plasma cells, fibroblasts, and necrotic debris. The cytoplasm of the xanthoma cells stains strongly with PAS. Although these cells form the basis of the microscopic identification of the lesion, they are not specific

and may only reflect phagocytosis of tissue within the lipid. Foreign-body giant cells and microscopic calcification are also frequently present.[129]

Xanthogranulomatous pyelonephritis is frequently mistaken for renal carcinoma or renal tuberculosis. Prior to the availability of CT scanning, the diagnosis was seldom considered preoperatively. Kidneys were often removed surgically because of an incorrect preoperative diagnosis. Current organ imaging technologies enable a preoperative diagnosis. If the disease is localized in the kidney, total nephrectomy may be avoided, and local resection with the removal of renal calculi and intensive treatment of the urinary infection may salvage residual functioning renal tissue.[129] The disease rarely involves both kidneys and does not recur after treatment. The disease has not been observed to progress serially from one kidney to the other, so the radical removal of involved tissue is not necessary. Laparoscopic approaches have been successful,[131] and a recent case report documents successful treatment with antibiotics alone in a patient with leukemia and a splenic abscess.[132]

Malakoplakia

Renal malakoplakia is a rare granulomatous disease of uncertain etiology that occurs in similar clinical settings to xanthogranulomatous pyelonephritis. The term malakoplakia is derived from the Greek word for soft plaque. Over 200 cases have been reported to date, predominantly in women and the elderly. Many of the clinical and laboratory features of this disease resemble those of xanthogranulomatous pyelonephritis, but most patients have an E. coli rather than a P. mirabilis urinary infection.[133]

The gross lesion is a soft yellow-brown plaque of variable size. Renal tissue is involved in one-fifth of patients and is bilateral in about 50%. The renal pelvis and ureters are involved in an additional one-fifth of patients, and a ureteral stricture may develop. Renal involvement appears frequently to be an ascending progression of bladder malakoplakia. Histologically, the plaques show large histiocytes with a foamy eosinophilic cytoplasm, called von Hansemann macrophages. The cytoplasm contains PAS-positive granules and large renal concentric crystals, named Michaelis-Gutmann bodies, which differentiate malakoplakia from xanthogranulomatous pyelonephritis.[134] These bodies show a typical crystalline structure on electron microscopy and are primarily calcium and iron on chemical analysis. These lesions may be confined to the urinary tract but occasionally are seen on the skin, in the prostate, testes, and the gastrointestinal tract.

The disease is caused by a defect in macrophage function, with impairment of bactericidal activity of monocytes for E. coli, and this organism may play a particular role in its pathogenesis.[134,135] The movement of lysosomes to phagocytic vacuoles is delayed owing to low levels of cyclic guanosine monophosphate. A cholinergic agonist, such as bethanechol, can correct this defect.[136,137] The Michaelis-Gutmann bodies are presumed to result from the abnormal deposition of calcium phosphate and iron in the overloaded phagosomes.[134]

Renal parenchymal malakoplakia usually occurs as an upper tract infection with fever and flank pain or tenderness.[134–136] A palpable flank mass may be present. Imaging reveals enlarged kidneys with multiple filling defects. Renal malakoplakia may progress to renal impairment and failure. The disease occurs more frequently in immunosuppressed patients and has been observed in several patients following renal transplantation.[134,137]

Several patients have been treated successfully with the cholinergic agonist bethanechol chloride and long courses of trimethoprim-sulfamethoxazole or a fluoroquinolone.[134–137] Immunosuppression may have to be modified when the disease occurs in renal transplant recipients.

Urosepsis

The urinary tract is the most common site of origin for gram-negative rod bacteremia, and the development of bacteremia from a urinary focus is termed urosepsis. Urosepsis is one of the most common presentations for bacteremic illness in nursing homes and hospitals. As discussed already, E. coli accounts for almost half of these uroseptic infections, with other enteric gram-negative bacilli and enterococci following in frequency. Interleukin 8 (IL-8), a small chemotactic protein, plays an important role in neutrophil migration during a UTI and increased urinary interleukins occur in urinary tract infections and urosepsis.[138,139] A recent report even suggests that urinary IL-8 can be used for the rapid diagnosis of urosepsis in children.[140] Patients with acute pyelonephritis have higher IL-8 levels in the urine than in the plasma. During urosepsis, this phenomenon stimulates the delivery of neutrophils to the GU system and results in pyuria. Serum IL-1 receptor antagonist, IL-10, and soluble tumor necrosis factors are elevated in urosepsis, suggesting a systemic anti-inflammatory response.[141] Although endotoxin (the bacterial cell wall lipopolysaccharide) may enter the circulation from a well localized focus of gram-negative bacterial infection, the presence of sepsis originating from the urinary tract usually implies bacteremia, which in turn usually results from an infection in the kidney or the renal pelvis.

Clinically, most uroseptic patients present with fever, shaking chills, flank pain, hypotension, cloudy urine, and leukocytosis. However, obtunded patients may not have urinary complaints or clinical signs referable to the urinary tract, and patients with obstructive uropathy might not present with the usual laboratory clues of pyuria and bacteriuria. Bacteremia in a patient with a urinary infection usually implies that the infection originates from the kidney, but in the presence of an indwelling bladder catheter, erosive urethritis or cystitis might be the responsible focus. Also, sepsis may follow the instrumentation or manipulation of the lower urinary tract such as occurs during a percutaneous nephrolithotomy or a ureteroscopy. Staghorn calculi (struvite or apatite) can become embedded with gram-negative bacteria. A recent study identified endotoxins associated with sepsis in stone fragments in a child who died from sepsis syndrome after percutaneous staghorn stone manipulation.[142]

The diagnosis of urosepsis is usually confirmed by blood cultures positive for the same organism cultured from the urine. Despite initial hopes that rapid diagnostic tests for the presence of a circulating endotoxin might speed the diagnosis of bacterial sepsis, no such test is available currently. A careful examination for the presence of bacteria on a drop of unspun urine (under a cover glass, or a gram-stained smear) plus the findings of pyuria in a septic patient point to the urinary tract as the source of the infection in most cases. However, strong clinical suspicion is necessary for less typical patients and at least two and preferably three blood cultures should be obtained to identify the responsible organism.

Treatment requires intravenous fluid support, the maintenance of blood pressure with pressors if necessary, and antibiotics. If the organism is known (as in the case of recently cultured urine in a septic patient), then a single active antibiotic can suffice. If no clues exist as to the identity of the organism, intravenous therapy with an extended-spectrum penicillin (e.g., piperacillin), a penicillin-penicillinase inhibitor combination (e.g., ticarcillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam), or a carbapenem (e.g., imipenem, ertapenem, meropenem, doripenem) can be used. Third-generation cephalosporins also can be used, but these agents are not effective against enterococci. Aminoglycosides (gentamicin, tobramycin, or amikacin) may be used in combination with a β-lactam if enterococci or P. aeruginosa are suspected. Combination therapy with a third-generation antipseudomonal cephalosporin such as ceftazidime, or a carbapenem or an extended-spectrum penicillin plus aminoglycoside is suggested if gram-negative rod bacteremia occurs in a patient with granulocytopenia. Intravenous fluoroquinolones (e.g., ciprofloxacin, levofloxacin) also are useful, but resistance is increasing in many centers. Carbapenems have extended activity against gram-negative bacilli, although ertapenem has limited activity against Pseudomonas aeruginosa.[143]

β-Lactamase–mediated carbapenem resistance has been well described in Klebsiella pneumoniae and other Enterobacteriaceae, and these organisms as well as Acinetobacter spp. are widely spread in hospitals.[144,145] Multiple antibiotic resistance is common in these organisms, including resistance to aminoglycosides, cephalosporins, and fluoroquinolones. Some might retain susceptibility to carbapenems, but polymyxin or colistin might be needed to eradicate MDR bacteria.[146] Nephrotoxicity and neurotoxicity warrant caution with these antibiotics.

A new glycylglycine, tigecycline, has antibacterial activity against some MDR bacteria, as well as ESBL-producing Acinetobacter, MRSA, and vancomycin-resistant enterococci. However, tigecycline is unstable in urine and is not used as a first-line agent if gram-negative rod bacteremia originates in the urinary tract.[147,148]

Adjunctive therapy with corticosteroids is not recommended. Considerable research activity has been directed to blocking cytokine activity in sepsis (not limited to urosepsis), but recent trials of antiendotoxin monoclonal antibodies, antitumor necrosis factor antibodies, and IL-1 receptor antagonists have been disappointing.

Others

Tuberculosis (Chapter 27) and renal and perinephric abscesses (Chapter 24) also are discussed elsewhere. Abscesses may rarely develop in the bladder wall. These intramural vesicle abscesses are only rarely reported and are usually caused by coliform bacteria, often in the presence of inflammatory bowel disease, diverticular disease, or a foreign body.

Infections Caused by Unusual or Resistant Organisms

In the face of increasing bacterial resistance to many antibiotics, the treatment of complicated UTIs is less simple given the resistance to fluoroquinolones, TMP-SMX, and β-lactams.[1,149] At least 4 weeks of therapy may be needed and the treatment choice must be based on susceptibility testing. Enterococci are not uncommon causes of UTIs, and GU sites account for the majority of clinical bacteriology laboratory isolates. These organisms are more commonly isolated from nosocomial infections than from the community. Enterococci may be the causative agents in lower UTIs (e.g., cystitis), in catheter-related infections, in infections following urinary tract instrumentation, and in patients with GU anatomic abnormalities.[150,151]

The incidence of enterococcal infection, in general, is increasing at an alarming rate according to the National Nosocomial Infection Surveillance (NNIS) Study; for example, from 1980 to 1989, the incidence of bloodstream infections with enterococci has increased 120%,[149,151,152] and the urinary tract is frequently identified as the source. These infections may originate from the patient's own GU or GI flora or may be nosocomially spread from other patients.

Unfortunately, antimicrobial resistance in enterococci is also increasing dramatically, not unlike the rise in methicillin-resistant S. aureus seen over the past 30 years. A large number of enterococcal strains, especially Enterococcus faecium, show vancomycin resistance. This has been attributed to the almost 160-fold increase in vancomycin usage per 1,000 patient-days in hospitals between 1978 and 1992.[153] Vancomycin resistance is often classified by phenotypic expression of the presence of certain resistance genes such as VanA, which mediates resistance to vancomycin and teicoplanin; VanB with strains susceptible to teicoplanin but resistant to vancomycin; and VanC, which imparts low-level resistance to vancomycin in strains of Enterococcus gallinarum and Enterococcus casseliflavus.[154]

Also, enterococcal strains that were previously susceptible to penicillin and gentamicin have been identified. Even penicillin plus gentamicin therapy may fail to eradicate some of these infecting organisms.[154] Although vancomycin had been used as a mainstay in the therapy of complicated nosocomial enterococcal urinary infections, vancomycin-resistant

E. faecium and E. faecalis threaten the use of this drug. Alternative treatments with drugs such as linezolid or quinupristin-dalfopristin are effective against vancomycin- and penicillin-resistant E. faecium; nitrofurantoin or fosfomycin trometamol may be effective in simple UTIs.[155,156] Vancomycin-resistant E. faecalis may be treated with a penicillin, nitrofurantoin, or fosfomycin. MDR enterococcal infections may be treatable with linezolid, daptomycin, or tigecycline, but the emergence of resistance is possible.[155–158] Daptomycin is also active against MRSA.[159] Recent publications do not recommend the routine treatment of asymptomatic enterococcal bacteriuria.[155] Increasing MDR strains of E. coli causing community-acquired pyelonephritis and other complicated UTIs are being reported, with identified clonal groups and widespread circulation of ESBL-producers responsible for the antimicrobial resistance.[160–161] Some of these organisms might be treatable with carbapenems (imipenem, meropenem, doripenem, ertapenem) or they might require colistin treatment.[146,162]

Hemolytic-uremic syndrome (HUS) associated with enterohemorrhagic E. coli urinary infections has been reported. Hemolytic-uremic syndrome includes a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal impairment. Thirteen cases of HUS associated with E. coli UTIs have been described. Most of these cases were not associated with diarrhea or a prodrome, which usually occur when HUS occurs as a result of the consumption of fecally contaminated products from cattle or sheep.[163] A few other cases have been reported,[164] but routine screening for shiga toxin–producing E. coli is not recommended.[165]

Atraumatic ruptures of the urinary bladder associated with MRSA have been reported.[166] MRSA UTIs usually require therapy with intravenous vancomycin. Involvement of the upper urinary tract, pyelonephritis, renal abscess, and bacterial endocarditis should be excluded in these patients. Bladder ruptures have been reported as a result of a Candida tropicalis bezoar[167] and secondary to a multiorganism infected urachal cyst.[168]

Anaerobic bacteria are unusual causes of urinary infection, but they are implicated in some patients with genitourinary abscesses, scrotal gangrene, and in patients with urinary diversions.[169,170] Although they are major components of the normal urethral, periurethral, and vaginal flora, their presence in these areas may prevent other, more invasive aerobic or facultative organisms from colonizing these sites and subsequently causing infection. Anaerobic bacteria may be identified in the urine in chronically catheterized patients but this is often short lived and without clinical significance; however, anaerobic bacteriuria is more frequent in patients who have undergone a renal transplantation.[171] Anaerobes that have been implicated in complicated UTIs include Bacteroides fragilis, B. melaninogenicus, Fusobacterium nucleatum, Peptococcus spp., Peptostreptococcus spp., and Clostridium perfringens, among others.[169]

The mechanism by which these organisms cause disease in the bladder, kidney, or prostate depends on their ability to ascend from the periurethral area to the bladder or kidney. This process is accelerated in the presence of obstruction, urinary stasis, calculi, trauma, and catheters or other foreign bodies. Anaerobic bacteria have been documented to cause prostatitis and prostatic abscesses as well as bacteriuria, pyelonephritis, and urosepsis. Some of these organisms (e.g., Bacteroides or Sphaerophorus spp.) have been implicated in urinary or prostate infections following colonic surgery.[169] Clindamycin and metronidazole are usually active against most anaerobic organisms (although resistance is increasing) and cefoxitin or cefotetan, carbapenems, and β-lactams with β-lactamase inhibitor combinations also may be effective. The surgical correction of underlying causes is often required.

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CHAPTER

26 Fungal Infections of the Urinary Tract

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Urinary tract infections due to fungi are much less common than those due to bacteria. Among the fungi, relatively few target the urinary tract (Table 26.1). Candida species are responsible for most urinary tract infections.[1] Other opportunistic yeasts, such as Cryptococcus neoformans, involve the urinary tract usually only when widespread disseminated infection has occurred. Mold infections, such as aspergillosis and mucormycosis, rarely spread to the urinary tract, but have disastrous consequences when they do. Finally, the group of geographically restricted endemic mycoses, histoplasmosis, coccidioidomycosis, and blastomycosis, can cause localized lower urinary tract infections, but rarely cause symptomatic upper tract infection.

Candiduria is not a disease, but is usually the initial event triggering the question as to whether a fungal urinary tract infection is present.[2] Most patients who have Candida in their urine do not have a urinary tract infection, but are merely colonized with these yeasts. Diagnostic tests to define whether candiduria reflects colonization or infection are often not helpful, and localization of the site of infection either to the bladder or the kidneys can be difficult. For this reason, much of the literature on urinary tract involvement with Candida species is actually based on candiduria and much less often on specific infections due to these organisms.

CANDIDA

Epidemiology

Candida species are common members of the microbiota of the perineum but are found in urine in less than 1% of healthy persons.[3,4] In hospitalized patients, especially those in the intensive care unit, candiduria is very common presumably because of the multitude of risk factors that allow ingress of organisms into the bladder and subsequent growth of Candida species in the urine.[5–11] A recent point prevalence survey of positive urine cultures obtained from hospitalized patients in hospitals throughout Europe found that Candida species were the third most common microorganism isolated from urine.[5] Although it has been thought that candiduria could serve as a prelude to candidemia, this appears to be uncommon. Investigation of candiduric and candidemic isolates by molecular genotyping failed to show a relationship between the two sites in over half of the patients in one study.[12] In a large prospective surveillance study only 7 of 530 (1.3%) candiduric patients followed for 10 weeks developed candidemia.[13]

The risk factors for candiduria have been better defined than those for either bladder or kidney infection with Candida. This is due to the fact that firm diagnostic criteria for infection have not been defined, but candiduria is easily and simply defined as the growth of Candida species from a urine culture. Prospective surveillance studies and case-controlled studies have shown that increased age, female sex, antibiotic use, urinary drainage devices, prior surgical procedures, and diabetes mellitus are important risk factors for candiduria[6,8,11,13] (Table 26.2).

In the largest multicenter surveillance study, which assessed 861 hospitalized patients, urinary drainage devices, consisting mostly of indwelling urethral catheters, were present in 83%, diabetes in 39%, and urinary tract abnormalities in 37% of patients who had candiduria. Only 11% of patients with candiduria had no obvious risk factor identified.[13] In a multicenter study from Spain assessing candiduria in patients in an intensive care unit (ICU) setting, the independent risk factors associated with candiduria were age over 65 years, female sex, diabetes mellitus, prior antibiotics, mechanical ventilation, parenteral nutrition, and length of hospital stay before admission to the ICU.[6]

Among children, low-birth-weight neonates who are in an ICU are at the highest risk for candiduria and Candida urinary tract infections.[14–16] Fewer data are available for patients in the community than for hospitalized patients. Risk factors appear to be similar to those in hospitalized patients and include diabetes, indwelling catheters, and the use of antibiotics.[11]

Several studies, especially those focused on the ICU population, have noted increased mortality rates in patients who have candiduria when compared to similar patients without candiduria.[6,9,13,17,18] In all of these studies it appeared that Candida urinary tract involvement was not responsible for death but was most likely a marker for seriously ill patients

26.1 Fungi That Cause Urinary Tract Infection

Yeastlike Fungi
- Candida species[a]
- Cryptococcus neoformans
- Saccharomyces cerevisiae
- Trichosporon asahii

Molds
- Aspergillus species
- Mucorales

Endemic Fungi
- Histoplasma capsulatum
- Blastomyces dermatitidis
- Coccidioides species

[a]The vast majority of fungal urinary tract infections are due to Candida species. All of the other fungi listed only rarely cause urinary tract infections.

who died of their underlying illnesses. Treatment of candiduria did not impact mortality rates.[18]

Pathogenesis

Candida species can cause renal infection by either the hematogenous or ascending routes. In contrast, most bacterial upper tract infections are related to ascending infection from the bladder. It is likely that most kidney involvement with Candida occurs by hematogenous seeding from a distant focus but almost all of these infections cause no urinary tract symptoms. Rather, the patient is ill from candidemia or other foci of infection due to Candida. The pathogenesis of hematogenous seeding of Candida to the kidney has been studied extensively in experimental rodents and rabbits given an intravenous bolus of C. albicans.[19] Multiple microabscesses develop throughout the cortex. As the infection progresses, the yeasts penetrate through the glomeruli into the proximal tubules and are shed into the urine. Healthy animals eventually clear the organisms from the kidney, usually within 2 weeks; however, animals given immunosuppressive drugs cannot clear the infection. In agreement with experimental studies, renal microabscesses have been identified at autopsy in most patients who die of invasive candidiasis.

The pathogenesis of ascending infection with Candida has not been studied as extensively as that of hematogenous spread. Not surprisingly, it has been shown that those Candida strains found in the vagina are genetically related to the strains that cause candiduria in women who have indwelling bladder catheters while in the ICU.[20] There is no animal model that replicates the mode of spread that occurs in humans, which is presumably from the perineum into the bladder and then retrograde to the collecting system of the kidney.[21] Creating the milieu in which Candida persist in the bladder has been difficult in experimental animals. Studies from the 1970s showed that rats made diabetic were unable to clear C. albicans inoculated into the bladder, and also that the presence of a concomitant Escherichia coli urinary tract infection allowed retrograde spread of Candida to the kidney.[19] Unfortunately, these experiments have not been repeated nor has use of this model continued. Another model using bladder tissue explants from rabbits confirmed the essential role of adherence to the epithelial cells in colonization of the explants, but could not further explore the pathogenesis of retrograde spread.[22]

Microbiology

C. albicans is the yeast most commonly isolated from urine, accounting for 50% to 70% of isolates. C. glabrata is the second most common yeast found in urine, accounting for about 20% of isolates.[2,13] However, the proportion of urine isolates that are C. glabrata varies with different risk groups. Older adults frequently have C. glabrata isolated from urine, but neonates rarely are colonized or infected with C. glabrata. In patients who have hematologic malignancies, and in kidney transplant recipients, C. glabrata is more commonly isolated, possibly because of increased use of fluconazole in units that care for these patients. In one series of kidney transplant recipients, over half of all urine isolates were C. glabrata and only one third were C. albicans.[23] A study among hospitalized patients who had indwelling bladder catheters found that independent risk factors for C. glabrata candiduria were diabetes, ICU admission, and prior treatment with antibiotics and with fluconazole.[24]

C. parapsilosis, C. tropicalis, and C. krusei are less commonly found in urine although some centers have reported C. tropicalis more often than C. glabrata.[8] In general, there are no distinguishing characteristics of urinary tract infections due to the different Candida species.

Many laboratories do not identify yeast isolates to species level. This is reasonable because most yeasts that are

26.2 Risk Factors for Candiduria[a]

- Older age
- Female sex
- Diabetes mellitus
- Antibiotic use
- Urinary drainage device
- Urinary tract surgery or instrumentation
- Urinary tract obstruction

[a]Most patients have more than one predisposing factor present.

isolated are merely colonizing the urinary tract. However, knowledge of the species is needed if treatment of infection is required. Almost all isolates of C. albicans, C. tropicalis, and C. parapsilosis are susceptible to fluconazole, the antifungal agent of choice for treating Candida urinary tract infections. However, many isolates of C. glabrata and all isolates of C. krusei are resistant to fluconazole. Additional benefit is obtained when the laboratory performs susceptibility studies for fluconazole by helping the clinician to tailor therapy to the specific infecting organism.

Clinical Manifestations

Most patients with candiduria are asymptomatic, reflecting the fact that most do not have infection. In one large prospective surveillance study of patients with candiduria, fewer than 5% had symptoms suggestive of urinary tract infection.[13] Patients who have had hematogenous spread to the renal parenchyma in the course of candidemia may have fever, hypotension, and other manifestations of sepsis associated with invasive candidiasis. They do not have symptoms suggesting urinary tract infection. In these patients candiduria is a clue to the presence of invasive candidiasis, but the urinary tract is not the primary site of infection or the source of candidemia.

In those patients who do have symptomatic urinary tract infection, symptoms are indistinguishable from those noted with bacterial infections. Cystitis is manifested by dysuria, frequency, urgency, and suprapubic discomfort. Rarely pneumaturia and the passage of particulate matter may be present. Fever is uncommon. Patients who have an indwelling bladder catheter rarely complain of symptoms other than suprapubic discomfort, and if they are in the ICU they often are unable to communicate about symptoms that they might have.

Patients who have pyelonephritis usually have chills, fever, and flank pain. Some patients are afebrile whereas others have predominantly lower tract symptoms, but upper tract infection is noted on imaging studies.[25] Pyelonephritis is more common in diabetics, women, and older adults. Complications of pyelonephritis are uncommon but include emphysematous pyelonephritis, perinephric abscess, and papillary necrosis—all of which are associated with increased morbidity and usually require surgical intervention.[26] Formation of a fungus ball composed of a mass of hyphae and yeast cells in the collecting system is frequently found with pyelonephritis and causes obstruction.[25,27–30] Neonates and infants are especially prone to develop fungus balls.[27,30] If obstruction is present oliguria may occur and candidemia is common. Fungus balls can also form in the bladder and obstruct one or both ureters, causing hydronephrosis.[28]

Diagnosis

The initial task when approaching a patient who has candiduria is to decide if the presence of candiduria represents infection or merely reflects contamination of a urine sample or colonization of the bladder or urinary catheter. Repeating the urine culture to determine if the candiduria disappears tells one that the previous specimen was contaminated and no further diagnostic workup is indicated. If the patient is unable to perform a clean-catch collection of urine, bladder catheterization may be required. In those patients who have an indwelling bladder catheter, the catheter should be replaced and the second urine specimen collected from the newly inserted catheter.

Distinguishing colonization from infection is not simple as there are no standardized criteria that enable one to distinguish the two situations, especially in the setting of an indwelling bladder catheter.[2] Specifically, pyuria and quantitative cultures have not been shown to be definitive markers for the diagnosis of Candida urinary tract infection.[31] In patients who have an indwelling bladder catheter, pyuria is routinely noted and thus is not helpful to differentiate infection from colonization. On the other hand, in patients who do not have an indwelling bladder catheter, the presence of pyuria is helpful. One must be sure that bacteriuria is not present as a cause for pyuria.

Early studies by Wise and colleagues in the 1970s showed broad ranges of colony counts for both colonization and infection.[31,32] For patients who did not have indwelling catheters kidney infection was documented with colony counts in urine as low as 10^4 yeasts per mL. For patients who had indwelling catheters colony counts varied between 2×10^4 to $\geq 10^5$ colony-forming units (CFU) per mL, and the correlation of urine colony counts with biopsy-proved renal infection was poor. In a murine model of renal candidiasis initiated by intravenous inoculation of organisms, urine colony counts varied widely and no specific amount in the urine correlated with the burden of organisms in the kidney.[33]

Identification of casts containing yeasts in the urine is specific for kidney infection.[34] However, the techniques required to evaluate the presence of casts are complicated and time consuming, and this assay is not useful clinically. Finding pseudohyphae in urine may not be indicative of infection, especially because some Candida species, specifically C. glabrata, cannot form pseudohyphae.

Occasionally a patient has symptoms suggesting a urinary tract infection and yeasts are seen on microscopic examination of a urine sample, but the urine culture shows no growth. In this circumstance it is likely the patient has infection with C. glabrata, and the culture plates have not been held long enough for detection of this slowly growing species. Although the standard urine culture techniques used in clinical laboratories detect most Candida species, they can miss C. glabrata strains which may not appear for 48 hours. Asking the lab to culture urine specifically for fungi ensures that plates are kept for at least 5 days, and C. glabrata will then be found.

Imaging procedures including abdominal ultrasound and computed tomography (CT) scan are essential to document obstruction in the bladder, ureters, or renal pelvis.[31] It is important to discover the presence of fungus balls in the bladder or kidneys as surgical intervention is often required

for effective treatment. Perinephric abscess and emphysematous pyelonephritis, although unusual, are serious consequences of upper urinary tract Candida infection and are best detected by CT scan. Cystoscopy is helpful to ascertain the presence and extent of mucosal invasion by Candida.

Treatment

As a general rule asymptomatic patients should not be treated with antifungal agents.[35–38] However, there are two circumstances in which asymptomatic patients should be treated (Table 26.3). One such circumstance is when candiduria likely represents a marker for invasive candidiasis in high-risk patients, especially neutropenics and very low-birth-weight neonates.[35] The other circumstance is when the patient has candiduria and is about to undergo a urologic procedure that is likely to lead to candidemia.[35,39] Asymptomatic candiduria in a kidney transplant recipient does not warrant systemic antifungal treatment unless obstruction is present or the patient develops symptoms suggesting infection.[23]

For many patients simply removing the indwelling bladder catheter will allow the host to clear the candiduria.[2] If catheterization cannot be discontinued, the existing catheter should be removed and a new one inserted. Many times this will eradicate candiduria transiently, but it is highly likely that the organisms will return within a short time period. Relieving obstruction to urine flow in the upper or

26.3 Treatment of *Candida* Fungal Urinary Tract infections

Infection	Preferred Treatment	Comments
Asymptomatic candiduria with no high risk factors	Remove risk factors (bladder catheter, antibiotics)	Antifungal treatment not recommended
Asymptomatic candiduria in low birth weight neonates or neutropenics	Fluconazole, 400 mg (6 mg/kg) daily × 2 weeks	Treat for disseminated candidiasis; for fluconazole-resistant Candida, AmB, 0.5–1.0 mg/kg daily × 2 weeks
Asymptomatic candiduria in patient about to undergo urologic procedure	Fluconazole, 200–400 mg (3–6 mg/kg) daily for a few days periprocedure	For fluconazole-resistant Candida, AmB 0.3–0.6 mg/kg daily for a few days periprocedure
Cystitis	Fluconazole, 200 mg (3 mg/kg) daily × 2 wk	For fluconazole-resistant Candida, AmB, 0.3–0.6 mg/kg daily × 1–7 days OR 5-FC, 25 mg/kg qid × 7–10 days
Pyelonephritis	Fluconazole, 200–400 mg (3–6 mg/kg) daily × 2 wk	For fluconazole-resistant Candida, AmB, 0.5–0.7 mg/kg daily ± 5-FC, 25 mg/kg qid × 2 weeks OR 5-FC alone× 2 weeks
Renal infection—hematogenous spread	Fluconazole, 400 mg (6 mg/kg) daily × 2 wk	Treat for disseminated candidiasis; for fluconazole-resistant Candida, AmB, 0.5–1.0 mg/kg daily × 2 weeks
Prostatitis	Fluconazole, 400 mg (6 mg/kg) daily until resolved	Surgical drainage usually needed in addition to antifungal therapy
Epididymoorchitis	Fluconazole, 400 mg (6 mg/kg) daily until resolved	Surgical drainage usually needed in addition to antifungal therapy
Fungus balls (bladder, ureter, or kidney)	Fluconazole, 200–400 mg (3–6 mg/kg) daily until resolved	Surgical or radiologic intervention almost always required; local instillation of AmB an effective adjunct

AmB, amphotericin B deoxycholate; 5-FC, flucytosine; qid, four times a day.
Modified from Pappa PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:503.

lower urinary tract is essential for the long-term eradication of Candida from the urinary tract. Stopping broad-spectrum antibiotics will help the host clear candiduria without having to resort to antifungal agents.

Patients who have symptoms suggesting cystitis or pyelonephritis, and in whom bacteria as well as Candida are found in the urine culture, should be treated with an antibacterial agent alone to see if this leads to resolution of symptoms. However, many times there is a dual infection and both infections, bacterial and fungal, will require treatment.

Antifungal Agents

When therapy is indicated oral fluconazole is the drug of choice (Table 26.3). A loading dose of 400 mg should be given and then the daily dosage is 200 mg for a total of 14 days.[35,40] Fluconazole achieves high urine levels and effectively treats all Candida species with the exception of those due to C. glabrata, which are relatively or completely fluconazole-resistant, and C. krusei, which are uniformly fluconazole resistant. The dosage of fluconazole is reduced when the creatinine clearance falls to 50 mL per minute (Table 26.4).

26.4 Dosages of Antifungal Agents in Patients with Renal Insufficiency

Antifungal Agent	Creatinine Clearance (mL/min)	Recommended Dosage
Fluconazole	>50	400 mg every 24 hours
	21–50	200 mg every 24 hours
	11–20	200 mg every 48 hours
	Hemodialysis	400 mg after each dialysis
Flucytosine[a]	>50	25 mg/kg every 6 hours
	21–50	25 mg/kg every 12 hours
	11–20	25 mg/kg every 24 hours
	Hemodialysis	25 mg/kg after each dialysis

[a]Flucytosine peak levels should be measured 1–2 hours after dosing and should be <75 ug/mL.

The other available azoles (itraconazole, voriconazole, and posaconazole) and all of the echinocandins (micafungin, anidulafungin, and caspofungin) are not excreted into the urine as active drug, greatly limiting their use for Candida urinary tract infections.[37] It is possible that the tissue concentrations achieved with these agents might be adequate to treat invasive Candida infections of the kidney. However, clinical data are limited and both failures and success have been reported in individual patients.[41–43] It is highly likely that the echinocandins and voriconazole are able to eradicate those Candida organisms that have seeded into the kidney because all of these agents are effective therapy for candidemia and residual infection in the kidney after treatment of candidemia does not seem to be a common problem. However, currently no echinocandins or other azoles are recommended for the treatment of Candida urinary tract infections.

Intravenous amphotericin B deoxycholate has been used for decades as an effective treatment for Candida urinary tract infections.[35] However, because of its well-known toxicity, it is generally reserved for patients who have upper tract infection or documented bladder infection, and not merely colonization with C. glabrata or C. krusei (Table 26.3). The usual dosage is 0.3 to 0.6 mg/kg/d for 5 to 7 days, but even single-dose treatment with 0.3 to 1.0 mg per kg has been shown to be effective.[36,44] Infusion-related side effects that are seen in some patients, even when treated with low dosage amphotericin B deoxycholate, are rigors, fever, nausea, vomiting, and headache. Even a few days of therapy can cause transient renal insufficiency in some patients.

Unfortunately, the lipid formulations of amphotericin B, liposomal amphotericin B, amphotericin B lipid complex, and amphotericin B colloidal dispersion are not effective in treating fungal urinary tract infections.[45] It is postulated that the addition of the lipid component, which decreases nephrotoxicity with these agents, also precludes these drugs from achieving adequate levels in the urinary tract. This has been documented in one patient undergoing nephrectomy.[46]

Oral flucytosine can be used to treat fluconazole-resistant C. glabrata urinary tract infections either in concert with amphotericin B or as the sole agent.[35] Flucytosine is excreted into the urine as active drug in high concentrations. Unfortunately, it is fairly toxic with reversible bone marrow toxicity and hepatotoxicity being common. These adverse effects correlate directly with high serum levels and can be avoided in most patients who have normal renal function by using no more than 25 mg per kg orally every 6 hours for 7 to 10 days. Treatment longer than this is likely to lead to the development of resistance to flucytosine. The risk of toxicity increases greatly with renal dysfunction and the dose must be reduced (Table 26.4).

Local Antifungal Administration

Instillation of amphotericin B into the bladder to treat candiduria is used much less frequently now than previously.[47] Although local instillation will eliminate candiduria the effect is brief and Candida colonization returns in 1 to 2 weeks

in most patients.[48–50] Bladder instillation obviously is not efficacious for patients who have upper tract infection.

The usual daily dosage has been 50 mg amphotericin B deoxycholate per liter of sterile water. An indwelling catheter must be placed for instillation and most often a triple-lumen catheter is used for this purpose. This allows continuous slow infusion of amphotericin B. However, it has been questioned whether this effectively allows the infusate to "wash" the whole bladder or just the local area where the catheter is located. The alternative method for infusing amphotericin B is to administer a bolus of drug several times daily through a standard indwelling catheter, clamp the tube after the administration of drug, and then after about 30 minutes allow the infusate and urine to drain.

Most physicians and patients prefer oral azole therapy rather than bladder instillation, and recent guidelines do not recommend bladder instillation as a therapeutic option.[35] However, there are some specific circumstances in which local administration of amphotericin B can be useful. For example, bladder instillation might be helpful for treating patients who have documented cystitis with C. krusei, or C. glabrata resistant to fluconazole. In some of these patients local infusion of amphotericin B has proved useful.[51] Some have advocated the use of fluconazole bladder infusion (200 mg in 1 liter sterile saline daily) for patients who have renal insufficiency and have failed oral fluconazole therapy and are not candidates for systemic amphotericin B therapy.[52] The other circumstance in which local administration of either fluconazole or amphotericin B is indicated is when a patient has a fungus ball as discussed next.

Treatment of Complications

Patients who have a fungus ball should be treated with systemic antifungal agents, either fluconazole or amphotericin B deoxycholate with or without flucytosine, and surgical or radiologic interventions to relieve obstruction caused by the fungus ball.[35] Nephrostomy tubes placed into the collecting system are usually irrigated with amphotericin B, but fluconazole has also been used in this context.[53] This causes no damage to the kidney but achieves very high local levels of antifungal drug in the fungus ball. Other methods to break up fungus balls include irrigation with saline or streptokinase[54] and debulking the hyphal mass by percutaneous endoscopic disruption.[55,56] Treatment of emphysematous pyelonephritis almost always requires nephrectomy, as is the case with some patients in whom papillary necrosis has occurred following severe Candida pyelonephritis. Perinephric abscess can sometimes be drained without loss of the kidney. For all of these complications of upper tract candidiasis antifungal treatment with either fluconazole or amphotericin B is indicated in concert with surgical management.

Prostatitis

Candida prostatitis presents with symptoms similar to those noted with bacterial prostatitis, but generally patients are not as acutely ill as they can be with bacterial infection. Perineal discomfort, pressure behind the pubis, dysuria, difficulty voiding, and sexual dysfunction are some of the symptoms that may be present.[57–60] The patient may or may not be febrile. Diffuse infection or abscess formation can occur and rarely, emphysematous prostatitis.[61] The prostate is tender on examination and urine obtained after prostatic examination may reveal yeasts. Patients may present with urinary retention, thought to be due to benign prostatic hyperplasia or cancer, only to have Candida infection found on biopsy.

A combination of surgery and antifungal treatment is usually required for effective treatment of Candida prostatitis or prostatic abscess. Fluconazole is recommended as the preferred antifungal agent provided the organism is susceptible.[35] Fluconazole concentrations in prostatic tissue are approximately 30% of serum concentrations.[62] For infections due to C. glabrata or C. krusei that are resistant to fluconazole, amphotericin B is preferred and has been shown to be efficacious.[57] No data are available regarding the penetration of voriconazole, posaconazole, or the echinocandins into prostatic tissue, and their efficacy is unknown.

Epididymoorchitis

Candida epididymoorchitis is uncommon and appears to occur more often in patients who have diabetes and an indwelling catheter or urinary tract instrumentation.[63] A tender scrotal mass usually brings the patient to the physician's attention.[63–66] The presence of candiduria is a clue to the etiology of the epididymoorchitis.[63] Ultrasound or CT scan to assess for abscess formation should be performed. Drainage or orchiectomy is usually required for resolution. Fluconazole, 400 mg daily, is the preferred antifungal agent,[35] but there is experience using amphotericin B with flucytosine.[64,65] Treatment should continue for several weeks and until all signs of infection have resolved.

OTHER YEASTS

Yeasts other than Candida species are an uncommon cause of urinary tract infections. The most common among the non-Candida yeasts is Cryptococcus neoformans, a heavily encapsulated environmental yeast. This fungal infection occurs predominantly in persons who are immunosuppressed but normal hosts also can be infected by C. neoformans. The pathogenesis of infection begins with inhalation of the yeast, which is nonencapsulated in the environment and thus more easily dispersed. The initial infection involves the lungs and usually is asymptomatic. Because of the neurotropism of this organism, meningitis is the most common clinical manifestation.

Widespread disseminated infection, usually with accompanying meningitis, occurs commonly in those who are markedly immunosuppressed, especially patients with AIDS. In autopsy series kidney involvement has been noted in 25% to 50% of AIDS patients, but it is usually asymptomatic.[67] Symptomatic genitourinary tract involvement can occur as one aspect of widely disseminated infection or present as isolated prostatitis or epididymoorchitis.[67–69] Even focal

involvement implies disseminated infection, however, and other sites of infection should always be sought in such patients. The prostate can remain as a reservoir for viable Cryptococcus organisms after other sites of infection are successfully treated leading to subsequent relapse.[70]

The diagnosis of cryptococcosis is established by growth of C. neoformans in culture, by visualization of the encapsulated yeast in tissue sections with special stains, and/or by detection of capsular antigen in serum or body fluids. Treatment is based on whether meningitis is present and on the state of immune suppression in the host. In general, patients who have meningitis and/or disseminated infection should receive initial therapy with amphotericin B and flucytosine and subsequent consolidation and maintenance therapy with fluconazole.[71] In those rare cases that appear to have focal genitourinary tract cryptococcal infection, fluconazole 400 mg daily for at least 6 months is the preferred treatment.[71]

Saccharomyces cerevisiae has been described in a few cases as a cause of urinary tract infection.[72,73] The presentation is the same as that noted with Candida species. If the clinical microbiology laboratory does not identify yeasts isolated from urine to the species level, this organism will not be differentiated from Candida. S. cerevisiae is often resistant to fluconazole, and successful treatment may require amphotericin B.[74]

Trichosporon asahii is an opportunistic yeastlike organism that causes disseminated infection in immunosuppressed hosts, most often in those who have leukemia and are neutropenic. Genitourinary involvement can be related to disseminated infection or in certain settings can occur as an isolated infection. This appears to be the case in kidney transplant recipients who can be merely colonized, develop local fungus ball formation and obstruction, or have progressive invasion of the transplanted kidney without the occurrence of disseminated infection.[75] Treatment of disseminated infection is usually with voriconazole, and treatment of localized infection is voriconazole with surgical relief of obstruction, if present.

ASPERGILLUS AND OTHER MOLDS

Aspergillus and other molds uncommonly involve the urinary tract. In most instances involvement is noted for the first time at autopsy in patients who have widely disseminated infection. However, symptomatic localized urinary tract infections of the kidneys, the prostate, and the epididymis have been reported.[1,76–83] Most commonly these infections are due to Aspergillus species, but infections with the Mucorales, including Rhizopus and Mucor species, and other more rare molds have also been reported.[1] In most cases the patients who have urinary tract mold infections are immunosuppressed due to bone marrow or solid organ transplantation, HIV/AIDS, or neutropenia related to hematologic malignancies.[76,79–83] However, a few patients have been reported who had only diabetes or corticosteroid use as risk factors.[77,78]

The usual pathogenesis in most cases is hematogenous spread to the urinary tract from an initial pulmonary infection. However, primary renal aspergillosis that developed post-lithotripsy in a diabetic patient and localized Aspergillus prostatic abscess related to indwelling bladder catheterization have been reported with no other focus of infection.[77,78]

Kidney involvement is usually manifested by numerous microabscesses and infarcts, and, in some cases, obstruction to the collecting system occurs due to the development of fungus balls.[1] Patients who have obstructing renal lesions present with decreased urine output, flank pain, and fever. Prostatic and epididymal abscesses present with dysuria, frequency, or localized painful scrotal swelling.

Fungus balls or lower tract abscesses can be visualized by CT scan or ultrasound examination. Diagnosis of a mold infection is dependent on tissue biopsy showing invasion by hyphae and culture of the biopsy sample revealing the specific organism. Urine cultures cannot be relied on to yield the organism. Removal of an obstructing fungus ball, and many times nephrectomy, is required to treat mold infections of the kidneys. Surgical drainage of a prostatic abscess and epididymoorchiectomy are usually needed to effectively treat lower tract mold infection. Systemic antifungal therapy, either with amphotericin B or voriconazole for aspergillosis and with amphotericin B for mucormycosis, is necessary in all but exceptional cases in which truly localized infection has been documented. Mortality remains high in patients who have kidney involvement.

ENDEMIC FUNGI

The endemic fungi are those fungi that are restricted to certain geographical areas and that are dimorphic. They exist in the environment as molds, which are the infectious forms. In the body, and in the laboratory at temperatures from 35°C to 37°C, they transform to the yeast phase or in the case of Coccidioides, the spherule phase. The major endemic mycoses are Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis, and Coccidioides posadasii. Paracoccidioides brasiliensis is restricted to South America and Penicillium marneffei to Southeast Asia and will not be discussed further. All of the major endemic mycoses have been reported to infect the genitourinary tract. Infection occurs by the hematogenous route as a sequel to the initial pulmonary infection. There are two major manifestations involving the genitourinary tract. Kidney involvement occurs as one manifestation of widespread disseminated infection with these fungi and is generally asymptomatic, but can be associated with renal insufficiency. The other manifestation is infection of the prostate, epididymis, or testicles presenting either as an isolated finding or as one manifestation of active disseminated infection. In either case, the pathogenesis of infection is via the hematogenous route.

B. dermatitidis has the greatest propensity to cause symptomatic genitourinary tract infection. In patients who have disseminated blastomycosis, involvement of the

genitourinary tract occurs in as many as a third of cases.[1] Kidney involvement is generally asymptomatic and found only at autopsy.[84] In most cases symptomatic infection involves the prostate and less commonly the epididymis or testicles. In some patients, dysuria, hesitancy, and trouble initiating urination are the presenting symptoms of what later is shown to be disseminated blastomycosis.[85] In others, biopsy of a prostatic nodule or a nontender epididymal mass, thought to be cancer, shows granulomas, and B. dermatitidis is seen on histopathologic examination and/or grown from the tissue.[84,86] Every patient who has blastomycosis found to involve the genitourinary tract should have a workup to define the extent of involvement of other organs.

Symptomatic genitourinary tract involvement with histoplasmosis is rare, but autopsy findings document spread to the kidneys not uncommonly in patients who have disseminated histoplasmosis.[87,88] Individual case reports of testicular or prostatic abscesses, epididymitis, and ulcerations of the bladder have been published.[87,89,90] In most of these cases a prostatic nodule or an epididymal or testicular mass, thought to be cancer, is found on biopsy to show granulomas and the small budding yeasts typical of H. capsulatum. In a few cases immune complex glomerulonephritis with H. capsulatum antigen demonstrated in the mesangium has been described.[87,88]

Coccidioidomycosis rarely causes symptomatic urinary tract infection but autopsy series of disseminated coccidioidomycosis have noted kidney involvement in 30% to 40% of cases.[1] Rarely, in the course of severe disseminated coccidioidomycosis, renal insufficiency can be ascribed to kidney involvement. In some patients with disseminated coccidioidomycosis, coccidioiduria can be found with an absence of symptoms. Localized infection, presenting as abscesses or mass lesions of the epididymis, testicles, or prostate, also occurs in patients who have coccidioidomycosis. Sometimes this occurs in the absence of documented disease elsewhere, but in other patients urinary tract involvement is just one manifestation of disseminated infection.[91–93]

Treatment of genitourinary infection due to the endemic mycoses depends on the pathogenesis of the infection. In most cases urinary tract involvement is one manifestation of disseminated disease and systemic therapy with amphotericin B or an azole, as recommended for disseminated infection, is given.[94–96] For those patients who have a focal mass or abscess in the epididymis or testicle, surgical removal has usually been accomplished before the diagnosis is suspected. Although cure has been reported with surgical excision alone,[93] additional treatment with an azole agent is recommended because the pathogenesis of infection is always related to hematogenous dissemination. If biopsy of a prostatic nodule shows infection with one of the endemic mycoses further surgical drainage is generally not necessary, and the infection can be treated successfully with an azole agent.

The specific azole agent to use should be discussed with an infectious diseases consultant. Itraconazole, the azole of choice for histoplasmosis and blastomycosis, does not achieve very high levels in the prostate. However fluconazole, a second-line agent for these infections, achieves excellent prostatic tissue levels.[62] Fluconazole is a first-line agent for coccidioidomycosis and should be used for focal Coccidioides infection of the genitourinary tract. Little is known about the penetration into prostatic tissue by voriconazole or posaconazole, and neither is currently recommended for treatment of the endemic mycoses.

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CHAPTER

27 Urinary Tract Tuberculosis

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Over a century ago, Osler noted that tuberculosis is the net result of two pathologic processes: "In all tubercles two processes go on: the one—caseation—destructive and dangerous; and the other—sclerosis—conservative and healing. The ultimate result in a given case depends upon the capabilities of the body to restrict and limit the growth of the bacilli."[1]

Perhaps in no other form of tuberculosis are these processes so important in determining the impact of the tubercle bacillus on an organ system as in genitourinary tuberculosis. Progressive destruction and caseating necrosis of the kidney ultimately leading to "autonephrectomy" have long been recognized as possible catastrophic complications of renal tuberculosis. However, progressive ureteral and calyceal scarring seen during healing can result in severe obstructive uropathy and comparable loss of renal function. Therefore, medical and surgical management of genitourinary tuberculosis must focus on both aspects of the tuberculous process, with emphasis on the early diagnosis and prevention of both tissue damage and excessive scarring. Achieving these goals can be challenging because genitourinary tract tuberculosis can be a particularly occult process. This chapter outlines a practical approach to this problem, based on established epidemiologic, pathogenetic, and clinical principles.

ETIOLOGY

Robert Koch first identified the tubercle bacillus in 1882. His classic report defined staining procedures for the direct observation of bacilli in clinical specimens (including the use of aniline dyes for "acid-fastness"), culture techniques on solid medium for the in vitro passage of bacilli isolated from clinical or experimental lesions, and subsequent inoculation of guinea pigs with cultured material to confirm its etiologic role in tuberculosis.[2] Demonstrating an etiologic role for the tubercle bacillus in tuberculosis became the basis of "Koch's postulates," the standard criteria for etiologic research in infectious disease.

Mycobacterium tuberculosis, the human tubercle bacillus, is one of approximately 90 species of higher bacteria with unusual shared structural and tinctorial properties.[3,4] All mycobacteria, members of the genus Mycobacterium, have the ability to take up aniline dyes, such as those contained in carbolfuchsin, and to resist decolorization by washing in alcohol acidified with inorganic acid (e.g., 95% ethanol, 3% HCl). This unique property correlates with the extremely high lipid content of mycobacterial cell walls. Although all mycobacteria are obligate aerobes, they are found in nature in disparate settings: some species are soil and water saprophytes, whereas others are true pathogens of amphibians, reptiles, birds, and various mammals. M. bovis, the bovine tubercle bacillus, has virtually disappeared as a human pathogen in modern societies through tuberculin testing of cattle and pasteurization of dairy products. A variety of mycobacterial species can be pathogenic in humans (e.g., M. avium-intracellulare), whereas others have been characterized as human saprophytes (M. gastri, M. smegmatis). M. tuberculosis is distinguished from the many other "atypical" mycobacteria by its metabolic properties, rate of growth, pigment production, and virulence in experimental infection in guinea pigs, as well as by genomic features that facilitate direct speciation. M. tuberculosis characteristically appears as a small, slender, slightly curved rod 2 to 4 μm in length with a diameter of 0.3 to 0.6 μm. Bacilli can appear singly or in small clusters on clinical specimens. Unlike infected pulmonary secretions where the density of organisms commonly is high, the low density of bacilli in urine samples, as well as their possible confusion with saprophytic mycobacteria, makes urine acid-fast stains impractical for rapid diagnosis. Although M. tuberculosis can grow on simple synthetic media, typically in intertwining aggregates known as serpentine cords, its slow growth rate (15 to 20 hours doubling time) necessitates culture periods of up to 6 to 8 weeks for the appearance of visible colonies. Optimal growth requires high partial pressures of oxygen, as in air, although bacilli can remain viable but metabolically dormant under greatly reduced PO_2. This is particularly relevant for the progression of renal tuberculosis (see later).

The mycobacterial cell wall accounts not only for acid-fast staining, but for some of the important host–parasite

interactions as well. In addition to a peptidoglycan cell wall layer common to conventional bacteria, a second glycan layer encases the organism.[5,6] This arabinogalactan layer is covalently linked to the peptidoglycan layer and also contains esters of mycolic acids, which are very large fatty acids that are unique to mycobacteria. A number of complex glycolipids reside in the outermost layer—"cord factor" (trehalose dimycolate), phosphatides, and sulfatides—but are not covalently linked to the glycan layers.[6] Cord factor is responsible for growth in serpentine cords in vitro and is a virulence factor in vivo.[7] These cell wall moieties (lipoarabinomannan, trehalose dimycolate and its sulfated derivatives) have multiple effects on mycobacterial virulence: they inhibit phagosome maturation and fusion with lysosomes,[6,8] reduce cell surface expression of key host antigen presentation proteins and costimulatory molecules, thus diminishing the presentation of mycobacterial antigens by infected macrophages,[9] and even modulate macrophage survival and apoptosis.[10]

EPIDEMIOLOGY

Tuberculosis and HIV infection are each responsible for approximately 1.8 million deaths worldwide each year (which includes ~400,000 deaths due to dual infections, especially in resource-limited settings).[11] The World Health Organization (WHO) estimates that approximately one third of the world's population is latently infected with M. tuberculosis with approximately 9 million new cases occurring each year. Although more than 90% of cases occur in the developing world with significant overlap among symptomatic HIV infected individuals,[12] 10 to 15 million individuals in the United States are infected with M. tuberculosis, mostly with latent tuberculosis infection.[13,14]

The long-term secular decline in tuberculosis incidence that followed the development of successful antituberculous chemotherapy was disrupted in the 1980s by a decline in the support of tuberculosis control programs, as well as the interrelated challenges of the HIV epidemic, troubling social trends producing growing populations of homeless and incarcerated individuals, and the rising incidence of drug-resistant tuberculosis infections.[15–17] These processes often acted in synergy to produce epidemics of tuberculosis, frequently involving multiply drug-resistant strains, among vulnerable populations in hospitals, correctional facilities, residential care facilities, and homeless shelters.[18,19] The number of reported U.S. tuberculosis cases in 1992 (~26,000) roughly equaled those of 1982, erasing a decade of progress in tuberculosis control. An intensification of tuberculosis control measures, including greater emphasis on intensive initial empiric therapy, sensitivity testing of clinical isolates, and reliance on directly observed therapy, has helped to regain control of the tuberculosis epidemic, and by 2010 only 11,181 new cases were reported.[20]

Tuberculosis in the United States is primarily an urban disease with 75% of the new cases occurring in the 99 metropolitan areas that have populations of more than 500,000. Increasingly, in the United States tuberculosis is found among immigrants and minority groups. Approximately 80% of reported cases in 2010 occurred in Asian, Hispanic/Latino, and African American residents in roughly comparable numbers, whereas whites accounted for only 16% of cases. The nationwide incidence of tuberculosis was 3.6/100,000 overall, with marked variation by ethnicity and immigrant status: the incidence was highest among Asians and Pacific Islanders (22.5/100,000) and was lowest among non-Hispanic whites (0.9/100,000). In 2010 only 40% of cases occurred in native-born individuals, and in many states over 70% of cases occurred among foreign-born individuals. Dual infection with HIV was reported in approximately 10% of cases.[20] In Europe, similar patterns of increased tuberculosis have been seen among immigrants from high tuberculosis incidence countries.[21–23] In addition, relatively high rates of extrapulmonary tuberculosis and drug-resistant tuberculosis have been observed among these immigrant populations.[24,25]

Although the proportion of extrapulmonary disease has nearly tripled from 7.6% to 21% of reported cases of tuberculosis in the United States over the past 40 years, the relative incidence of genitourinary infections among all forms of extrapulmonary tuberculosis has gradually declined.[26] Regional lymph node infections remain the most commonly encountered form of extrapulmonary tuberculosis—genitourinary disease, once common,[27] has declined to 6.5% of extrapulmonary tuberculosis cases in the United States over the past 20 years or so, a frequency roughly comparable to that of tuberculous meningitis.[26] Similar low rates of genitourinary tuberculosis have been reported recently from both low incidence (France)[28] and high incidence countries (Nepal).[29] As outlined later and in Figure 27.1, extrapulmonary tuberculosis is the result of hematogenous spread from a pulmonary site of primary infection. Thus, genitourinary tuberculosis is observed in two clinical settings: commonly, as a late manifestation of earlier clinical or subclinical pulmonary infection and, rarely, as part of the multiorgan infection seen with disseminated (miliary) tuberculosis.

Statistics from the prechemotherapy era indicated that approximately 3% of unselected autopsy patients and 26% of those dying of tuberculosis had evidence of genitourinary tract tuberculosis at autopsy.[30] This high rate of genitourinary disease has declined with effective treatment of pulmonary tuberculosis. Currently, it is estimated that significant genitourinary disease will develop in approximately 4% to 8% of non–HIV-infected individuals with pulmonary tuberculosis if adequate therapy is not instituted.[31]

Traditionally, genitourinary tuberculosis has been a disease of young to middle-aged adults with a slight male predominance.[27,31–34] Although genitourinary tuberculosis has been reported in children,[35–37] it is quite uncommon, and seen today in the rare young child with concomitant miliary disease[37] or in school-age children with somewhat more indolent clinical features similar to those seen in adults.[36] Approximately one quarter of the patients with genitourinary tuberculosis have a history of diagnosed tuberculosis

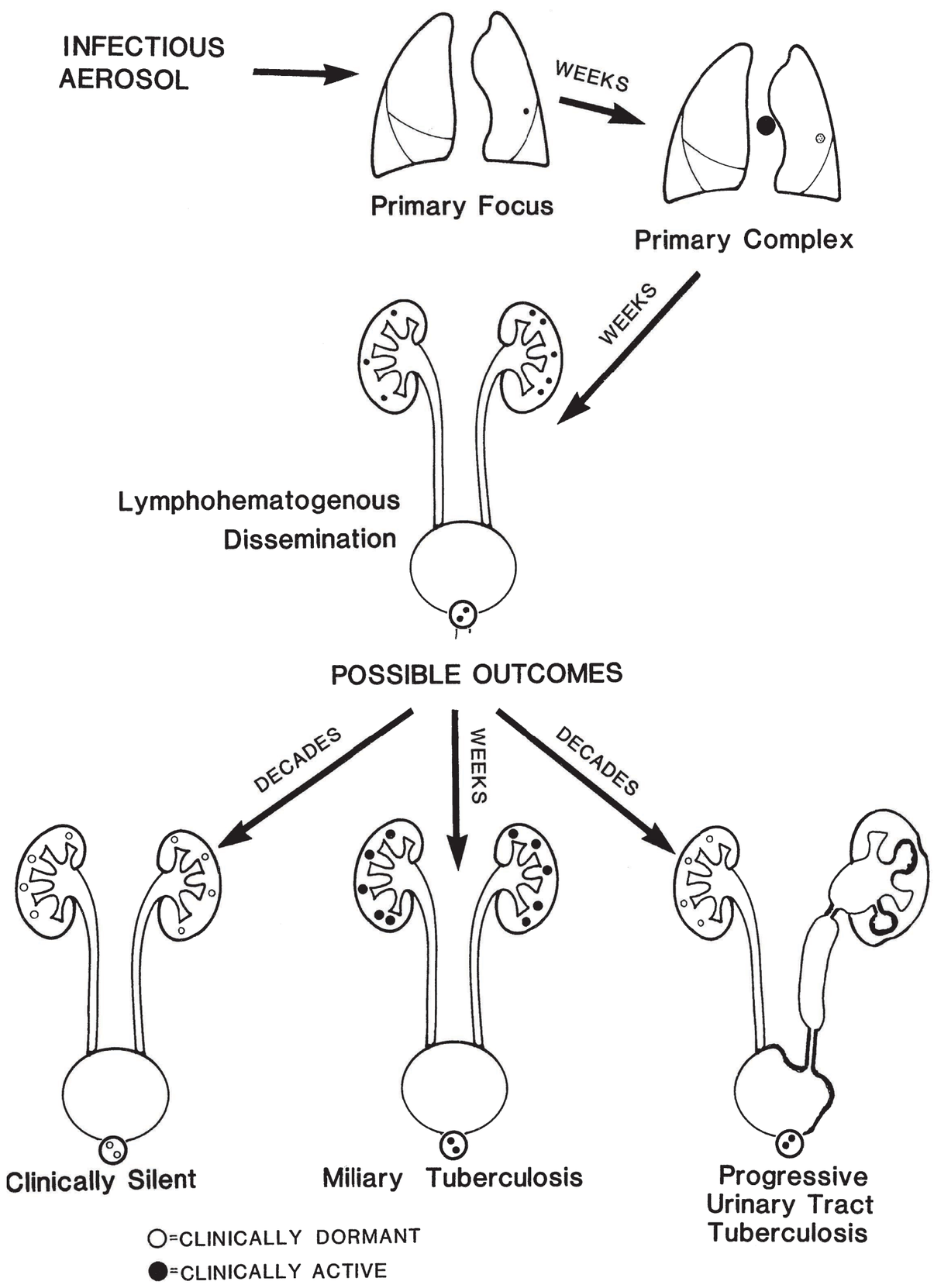


FIGURE 27.1 Schematic representation of the pathogenesis of urinary tract tuberculosis.

(usually of the lung). In an additional 25% to 50% of patients, changes compatible with old pulmonary tuberculosis can be found on chest X-ray films made at the time of diagnosis of genitourinary tract disease.[27,31–33]

Thus, in the non–HIV-infected individual a considerable interval exists between the onset of pulmonary infection and the diagnosis of active genitourinary tuberculosis. Considering patients with early clinical manifestations of primary tuberculous infection (i.e., erythema nodosum, pleurisy, or hilar adenopathy), the time lapse is most commonly 16 to 25 years; intervals exceeding 40 years have been well documented. If one looks at patients with reactivation pulmonary tuberculosis, the time lapse is usually about 4 to 8 years, but still may be as long as several decades.[31,32,38]

There are several epidemiologic implications of these observations. Decreasing the incidence of genitourinary tuberculosis requires the identification and treatment of persons with pulmonary infection prior to the development of extrapulmonary disease. Second, long after the incidence of pulmonary tuberculosis falls, the incidence of genitourinary disease will remain relatively stable, because a reservoir of patients with silent genitourinary tract infection will persist for decades after the incidence of new pulmonary infection falls.[24–26] Thus, the rise in tuberculosis cases that occurred in the late 1980s and early 1990s virtually guarantees an increase in extrapulmonary infections such as genitourinary disease in the next few decades, unless extensive case finding and effective treatment of these individuals are achieved. This task will be rendered particularly difficult because the burden of disease has fallen heavily on those with poorer access to skilled medical care—foreign born, the homeless, the incarcerated, migrant farm workers, and inner city ethnic minority groups.[16,17,20,39] Finally, it is clear that the age of the population with genitourinary tract tuberculosis, and other forms of extrapulmonary tuberculosis, reflects the average age at presentation of active pulmonary tuberculosis—significantly younger among immigrants, urban ethnic minority populations, and the disadvantaged, and over the age of 50 among other groups.[25]

In assessing epidemiologic factors that might predispose to tuberculosis, it is important to emphasize that fewer than 10% of persons with latent tuberculosis infection (reflecting a positive tuberculin skin test or in vitro interferon-γ release assay) ever become ill from this infection.[40] Of this group,

approximately 3% to 5% have manifestations of genitourinary tract disease.[27] In addition to exposure to tuberculosis in high incidence countries, public health factors including crowding, homelessness, poverty, drug addiction, and incarceration, all amplified by the AIDS epidemic,[12] play a significant role in the spread of tuberculosis.

A minimum estimate of clinical tuberculosis in HIV-infected patients is approximately 15% worldwide[11] and 8.6% in the United States,[20] with a somewhat higher incidence (13.8%) in adults 25 to 44 years of age.[41] The annual rate of tuberculosis among tuberculin skin test positive HIV-infected individuals in the United States has been reported to be 35 to 162 cases per 1,000 person years, although in Africa the risk of clinical tuberculosis may be as high as 5% to 10% annually.[42] These variations reflect differences in the prevalence of tuberculosis and immunodeficiency in different population groups. Thus, the rate of tuberculous disease among HIV-infected, tuberculin positive individuals in the United States is roughly 10 times higher than that among comparable HIV-infected, tuberculin negative individuals,[43] and is approximately 80 to 370 times that of the general American population.[44] The continued refinement and availability of antiretroviral therapy combined with antituberculous therapy has altered the natural history of tuberculosis in HIV-infected individuals and, when combined with vigorous public health efforts to identify and monitor treatment, the prognosis of both HIV and tuberculosis in dual-infected patients has improved dramatically.[12,45–47] Dual therapy is often challenging due to drug interactions: for example, rifabutin is required instead of rifampin if a protease inhibitor is used. Fluconazole metabolism is increased in the presence of rifampin[48] and other agents, thus complicating care in these patients. Despite the need for care in constructing the therapeutic regimen, early diagnosis and appropriate therapy of both infections are critical to the patient's survival.[41,44,46,47]

Two epidemiologic patterns of mycobacterial infection are observed in HIV-infected individuals. Individuals from population groups with a low rate of endemic tuberculosis, such as gay men and those with posttransfusion HIV disease, primarily have difficulties with disseminated M. avium-intracellulare infection. In contrast, HIV-infected individuals who either belong to or interact with populations bearing a high rate of endemic tuberculosis (those in developing countries, immigrants from these countries, the homeless, intravenous drug users, prisoners, and, in the United States and other developed countries, the inner city poor) are primarily afflicted with disseminated M. tuberculosis.[44,45] Careful molecular epidemiologic studies have demonstrated that in HIV-infected individuals, outbreaks of tuberculosis resulted in either progressive primary disease or reinfection of individuals whose immunity had been attenuated by the effects of progressive HIV disease.[49,50]

In HIV-infected individuals tuberculosis is often the AIDS-defining illness, not infrequently occurring early in the course of HIV infection. In contrast to other AIDS-related opportunistic infections, the $CD4^+$ count is not a reliable indicator of tuberculosis risk among HIV-infected persons.[44] Extrapulmonary disease, often in conjunction with pulmonary disease, is common,[44] and the time course for the development of disseminated disease may be greatly abbreviated in these individuals. In this setting, genitourinary disease is less commonly seen as an isolated phenomenon, but rather as part of disseminated infection. The incidence of HIV infection among patients with extrapulmonary tuberculosis was significantly elevated in the initial phases of the HIV epidemic,[51] but at present the rate of extrapulmonary tuberculosis in dually infected individuals is comparable to that seen in HIV-uninfected patients.[26] Serial New York City data confirmed aggressive dual therapy led to a reduced rate of extrapulmonary tuberculosis in dually infected individuals.[45]

The coexistence of tuberculosis with HIV infection has already had major public health consequences on the control of tuberculosis. Because HIV infection both increases the burden of infectious tubercle bacilli and obscures the symptoms (owing to the impaired inflammatory response of these immunosuppressed individuals), these people are highly efficient transmitters of tuberculosis. The expected consequence of this is the potential for a marked increase in the occurrence of secondary cases, and even epidemics, in contacts of these individuals, particularly in medical settings, crowded living conditions, prisons, and shelters for the homeless. Those populations most at risk for both HIV infection and tuberculosis, and the coexistence of these two infections, are the same populations with the highest incidence of drug-resistant tuberculosis. The result of this concordance of events is that the tuberculosis that can be amplified by the HIV epidemic includes a high potential for drug-resistant disease, particularly in the former states of the Soviet Union and more generally in resource-limited settings, and can complicate both the management of individual patients and the public health strategies that must be taken to protect the community.[11]

One additional epidemiologic consideration is the possibility that urine from patients with urinary tract tuberculosis could transmit tuberculosis to household members. Vasquez and Lattimer[52] reported a doubling of the incidence of tuberculin positivity among children of parents with active urinary tract tuberculosis without active pulmonary disease. Other observers have been unable to confirm this finding. Our policy has been not to isolate persons with isolated urinary tract tuberculosis but to consider their urine potentially infectious and to maintain contact precautions when handling it.

PATHOGENESIS OF TUBERCULOSIS

Systemic Aspects

The host–pathogen interaction in tuberculosis involving a slowly proliferating pathogen that resists host microbicidal mechanisms stands in stark contrast to conventional bacterial disease, where despite the pathogen's rapid proliferation, the host's resources (complement fixation, opsonization, phagocytosis, and ready lysis within phagocytic cells)

are formidable. The pathogenesis of tuberculosis reflects the balance of intrinsic mycobacterial virulence and the host immunologic response. The classic response in tuberculosis, the formation of granulomas, is ordinarily protective for the host by limiting the proliferation and spread of M. tuberculosis, but may be pathogenic as well, because it may lead directly to tissue injury in the form of caseation.[53–55] Thus, the clinical manifestations of tuberculosis represent not only the consequences of mycobacterial proliferation but also host reparative and destructive responses. In the absence of immunosuppression, the lifetime risk of symptomatic M. tuberculosis infection among latently infected individuals is only ~10%. Similarly, the risk of developing extrapulmonary disease, such as genitourinary tuberculosis, is rather low. An increased understanding of the molecular mechanisms responsible for host defense against mycobacterial infections has led to a growing appreciation that human susceptibility to mycobacterial infection may be attributable in significant measure to host genetic factors, as well as to the intrinsic virulence of M. tuberculosis isolates.[56–58]

Tubercle bacilli are inhaled as small particle aerosols and gain direct access to the alveoli.[53] Presently, ingestion of M. tuberculosis with primary localization of disease in the intestinal tract or oropharynx is rare. The small aerosol inoculum multiplies slowly and is phagocytosed by polymorphonuclear leukocytes, pulmonary macrophages, and dendritic cells. Mycobacteria interact with respiratory epithelium,[59] alveolar surfactant proteins,[60] and both the classic[61] and alternate complement systems,[62] but interactions with macrophage Toll-like receptors (TLRs 2, 4, and 9)[63–65] play a critical role in initiating the host immune response. TLRs are pattern-recognition proteins, expressed on macrophages and dendritic cells, which serve as innate immune receptors.[66] Each TLR binds one or more of a variety of microbial products (endotoxin [lipopolysaccharide], bacterial DNA, flagellin, mycobacterial lipoarabinomannan, etc.) and transduce inflammatory signals culminating in the activation of NF-κB and transcription of tumor necrosis factor α (TNF-α) and interferon-γ.[67] Following opsonization by C3, M. tuberculosis binds to phagocytic cell surface complement receptors and is phagocytosed.[62] Mycobacteria suppress the intracellular calcium flux that normally accompanies phagocytosis and inhibits macrophage activation and phagolysosome maturation.[68,69] M. tuberculosis can also directly adhere to, infect, and translocate across alveolar epithelial cells and endothelial cells,[70–72] facilitating access to lung interstitium and the pulmonary microcirculation, enhancing early dissemination to extrapulmonary foci.

The host response to mycobacterial infection has been called the IL-12–interferon-γ axis.[56,58] Macrophage activation via TLR signaling and other early events is associated with secretion of TNF-α and IL-12 and the related cytokines IL-18 and IL-23[65,73] as well as by activation of NO synthase 2, leading to the synthesis of reactive nitrogen intermediates.[65] The cytokines program resting T lymphocytes toward an inflammatory Th1 response. Activated Th1 lymphocytes secrete as their dominant cytokines interferon-γ as well as TNF-α, and this in turn activates macrophages and enhances their mycobactericidal activity. TNF-α also triggers apoptosis of infected macrophages, which may inhibit mycobacterial replication (see later). The use of TNF-α antagonists (e.g., etanercept, infliximab, and adalimumab) as disease-modifying agents in the treatment of rheumatoid arthritis and other inflammatory diseases confirms a central role for TNF-α in the host response against mycobacterial infection. These therapies have been associated with rapidly progressive tuberculosis, impaired granulomatous reactions in tissue biopsies, and a high rate of extrapulmonary disease.[74]

In the initial stages of primary infection, resting macrophages have a limited ability to lyse mycobacteria, and the bacillary titer rises despite entrapment within macrophage and granulocyte phagosomes and lysophagosomes. Some bacilli can even escape from these organelles and replicate freely within the cytoplasmic compartment.[75] Macrophage-mediated killing of intracellular M. tuberculosis requires the L-arginine-dependent generation of reactive nitrogen intermediates, such as nitric oxide, and this capability is greatly enhanced following macrophage activation by interferon-γ and TNF.[65,76] Thus, infected macrophages program T cells toward a Th1 response which in turn augments macrophage-mediated mycobacterial killing. Foamy macrophages are characteristically seen in caseating granulomata and offer a protected locus of mycobacterial persistence; the disruption of these macrophages helps to recycle M. tuberculosis to the extracellular milieu.[77] T cell-infected macrophage interactions are more complex because several cytolytic T cell effector populations are generated which can lyse infected macrophages.[78,79] In addition to the expansion of conventional peptide-specific $CD4^+$ and $CD8^+$ $\alpha\beta$ T-cell receptor-expressing populations,[79] T cells with a double negative phenotype (CD4-, CD8-) expressing $\gamma\delta$ T-cell receptors recognize mycobacterial phospholipid antigens presented by MHC-like molecules (e.g., CD-1) and lyse infected macrophages via Fas–FasL interaction.[80] These phospholipid antigens are also recognized by $CD8^+$ $\alpha\beta$ T-cell receptor-expressing cytotoxic T lymphocytes, triggering perforin-mediated cytotoxicity, and by natural killer (NK) T cells, which possess NK markers as well as $\alpha\beta$ T-cell receptors.[81] In at least some instances, immune lysis of infected macrophages appears beneficial to the host. Perforin-mediated lysis of infected macrophages reduces M. tuberculosis viability by 50% in vitro,[81] and may be important in vivo in reducing the number of infecting tubercle bacilli. The cytotoxic granules that contain perforin also contain granulysin, a lipid-binding protein that has potent mycobactericidal activity in the presence of perforin.[82] In contrast, Fas-mediated lysis of macrophages does not affect mycobacterial viability, but may be important in reducing antigen presentation and dampening the immune response. The immune lysis of heavily infected macrophages may facilitate the phagocytosis of released mycobacteria by additional activated macrophages. These newly recruited cells have a lower bacillary

burden and may be more effective at killing their intracellular bacilli, or serve as target cells for cytotoxic T lymphocytes.

In addition to their direct cytotoxic activities, the activated lymphocytes secrete a variety of cytokines, including interferon-γ, migration inhibitory factor (MIF), granulocyte-macrophage colony-stimulating factor, TNF-α, IL-12, and inhibitory cytokines such as interleukin-4 (IL-4)[83] and IL-10.[84,85] IL-4 production undermines the host Th1 response, and triggers tissue fibrosis,[86] a characteristic finding in chronic tuberculosis. In HIV-infected individuals coinfected with M. tuberculosis, immunosuppressive cytokines (e.g., IL-10) produced by macrophages/monocytes diminish the T-lymphocyte response in vitro, suggesting that Th2-like activity contributes to uncontrolled, systemic spread in these patients.[87] Macrophages are recruited to infiltrate the area of mycobacterial growth to form granulomas and mature into epithelioid cells by the macrophages' elaboration of TNF[88]; this process requires NK T cells.[89] In spite of macrophage activation, the killing of intracellular mycobacteria by human macrophages is often incomplete.[69]

The outcome of early tuberculous disease covers a spectrum from granuloma formation with efficient containment and healing to slowly progressive disease at the site of the primary pulmonary infection, or to clinically significant systemic spread of disease. Mycobacterial dissemination is actually the rule rather than the exception (Fig. 27.1). Although most bacilli are contained within macrophages initially, their continued proliferation disrupts the macrophages and the bacilli return to the extracellular environment. Most are engulfed again, but some bind to respiratory epithelial cells and ultimately translocate to the microcirculation[71,72] or are carried in the lymphatic drainage and produce regional lymphadenitis. Alternatively, some viable mycobacteria may reach regional lymph nodes while entrapped within dendritic cells or migratory macrophages. Progressive infection within the lymph node contaminates efferent lymph, and when sequential lymph node barriers fail, thoracic duct lymph delivers mycobacteria to the venous blood, seeding the pulmonary bed as well as extrapulmonary sites, such as the skeletal system, lymph nodes, and, most frequently, the kidneys.

Thus, limited hematogenous dissemination due to low grade bacillemia can occur early in the process of granuloma formation when the number of mycobacteria is small, and most organisms are found intracellularly within the macrophages comprising the granuloma. Small granulomas rapidly form at the metastatic foci because mycobacterial immunity is evolving or is already established at the time of dissemination. Although the bacilli may remain viable, the granulomas may remain clinically silent for decades.

Granuloma formation may itself contribute to the pathogenesis of severe tuberculosis.[53] Granulomas are active lesions with continued ingress of immune T lymphocytes and monocytes.[54,90] Shortly after microscopic granulomas become well established, polymorphonuclear leukocytes and monocytes enter the lesion.[91] The resultant phagocytosis is accompanied by exocytosis of lysosomal contents with local tissue destruction. This leads to a characteristic local necrotic process, known as caseation. Macrophage disruption returns the mycobacteria to the extracellular environment, where their proliferation accelerates.[53] Communication of the caseating granuloma with the bronchial tree restores favorable metabolic conditions, and mycobacterial titers can increase by several logarithms. This highly infected material can spread endobronchially to produce additional foci of pulmonary tuberculosis or excavate into a pulmonary vessel, leading to intense bacillemia. Such severe hematogenous dissemination commonly is responsible for miliary tuberculosis rather than limited extrapulmonary disease. In miliary tuberculosis, the systemic features of illness overshadow the asymptomatic renal involvement.

Pathogenesis of Renal Tuberculosis

Local factors play a significant role in the evolution of clinically significant renal tuberculosis. The small silent renal granulomas resulting from silent hematogenous dissemination are typically found bilaterally in the renal cortex and arise from capillaries within and adjacent to glomeruli (Fig. 27.2).[92] A glomerular location is not surprising in view of their high rate of perfusion (increased likelihood of bacillus delivery during sparse bacillemia) and their favorable oxygen tension. Such cortical granulomas usually remain dormant for decades. In some patients, however, bacillary

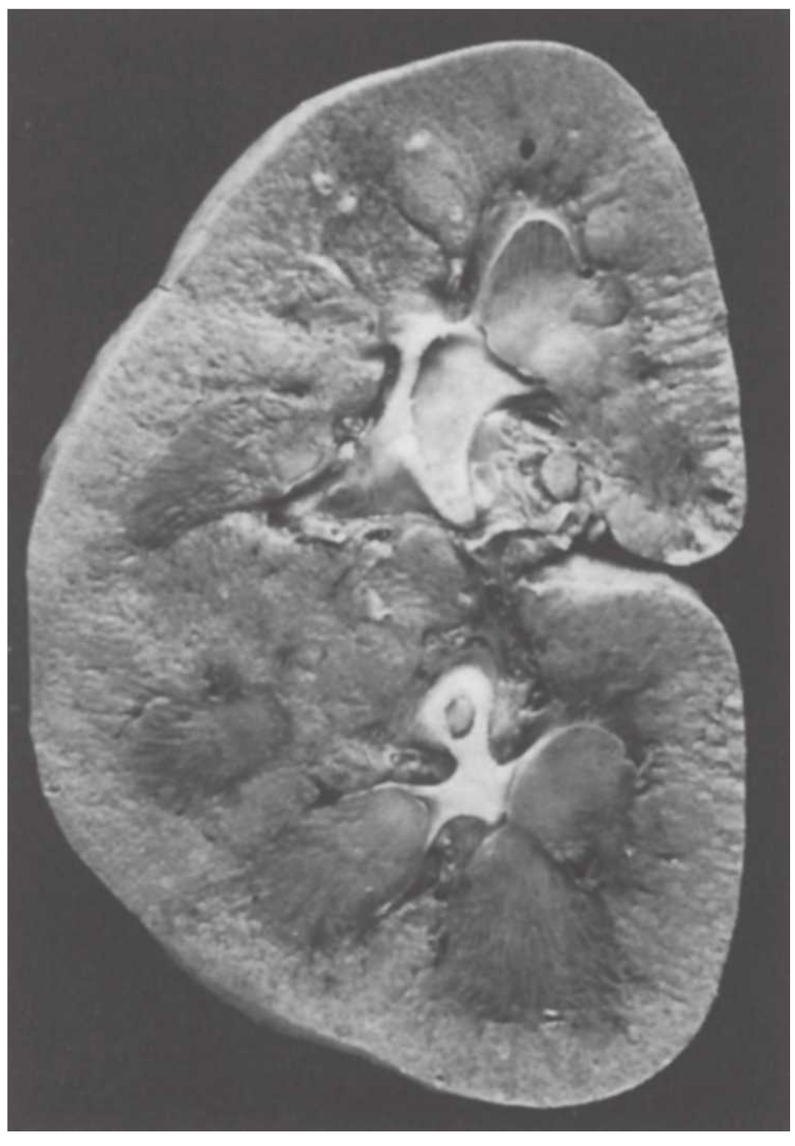


FIGURE 27.2 Early renal tuberculosis. Three small granulomas are visible in the cortex. The adjacent papillary tip is involved as well. (From Kollins SA, Hartman GW, Carr DT, et al. Roentgenographic findings in urinary tract tuberculosis. *Am J Roengentol Radium Ther Nucl Med.* 1971;121:487, with permission.)

proliferation within the glomerular capillary leads to capillary rupture and delivery of organisms into the proximal tubule. Clinically important renal tuberculosis, therefore, is usually initially localized to the medulla. This is likely caused by entrapment of mycobacteria and infected macrophage debris within the loop of Henle[92] and the known impairment of phagocyte function associated with the hypertonic environment found in the medulla.[93,94]

Analogous to progressive pulmonary disease, granulomas may enlarge in the medulla, leading to caseation and papillary necrosis. Such intraparenchymal granulomas may persist as mass lesions but commonly cavitate into the calyceal system. Despite bilateral hematogenous seeding of the kidneys, clinically significant disease is usually unilateral.[92] Communication of the caseating granuloma with the collecting system usually is responsible for the spread of bacilli to the renal pelvis, ureters, bladder, and accessory genital organs and is analogous to the endobronchial spread of infection seen in cavitary lung disease.[30] Lymphatic spread to contiguous structures also occurs in addition to epithelial infection by a luminal mechanism,[95] and direct hematogenous seeding of pelvic genital organs with clinical sparing of the kidney can occur occasionally.[30] In addition to the direct parenchymal destruction associated with advanced renal lesions, the fibrosis that accompanies the granulomatous process within the collecting system, such as infundibular strictures and renal pelvic kinking, adds an obstructive mechanism that may contribute significantly to progressive renal dysfunction.

PATHOLOGY

The caseating granuloma is the classic microscopic finding in essentially all forms of tuberculosis (Figs. 27.3 and 27.4). Medlar[92] characterized the early pathologic findings in renal tuberculosis by meticulously examining microscopic sections of kidneys from patients who died of pulmonary tuberculosis. Bilateral microscopic renal involvement is the rule, although the extent of involvement usually is asymmetric. Granulomas vary greatly in size, from lesions contained within a single glomerulus to large caseous abscesses, as well as in the apparent density of acid-fast bacilli.

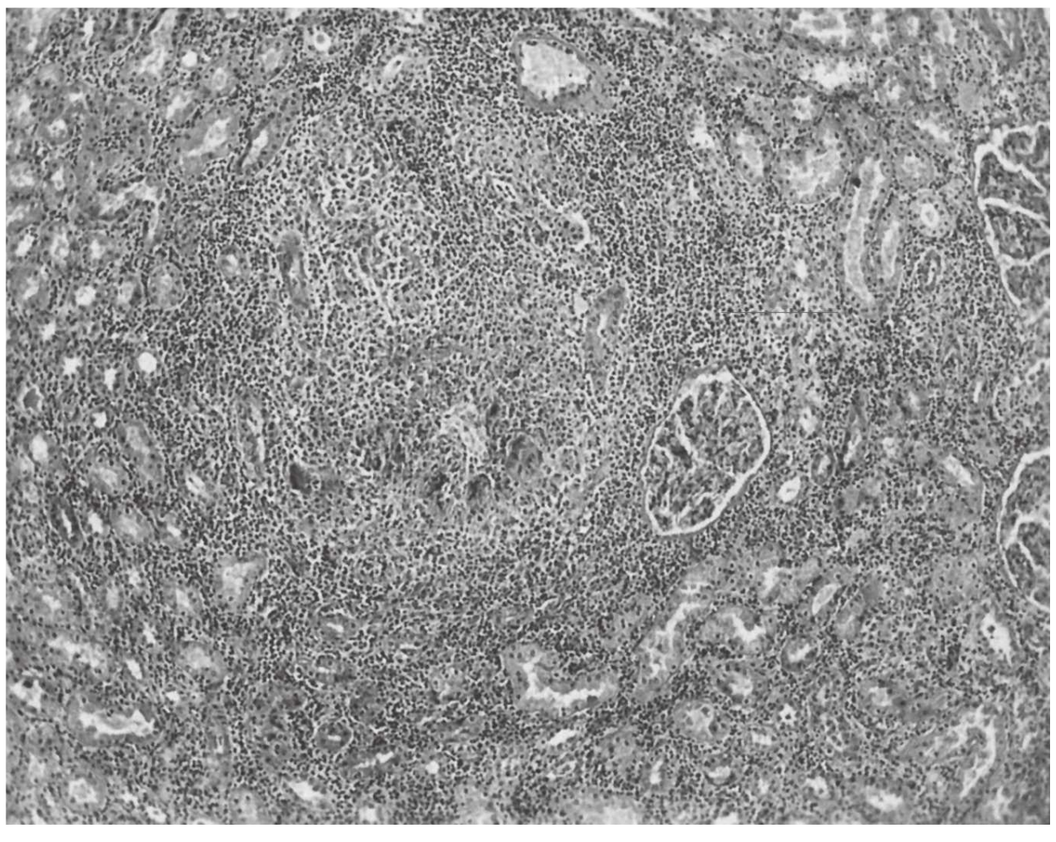


FIGURE 27.3 A caseating granuloma in the renal cortex. In addition to the necrotizing granuloma in the center of the field, a diffuse interstitial infiltrate of lymphocytes is seen. Some tubules are preserved (H&E, magnification ×79).

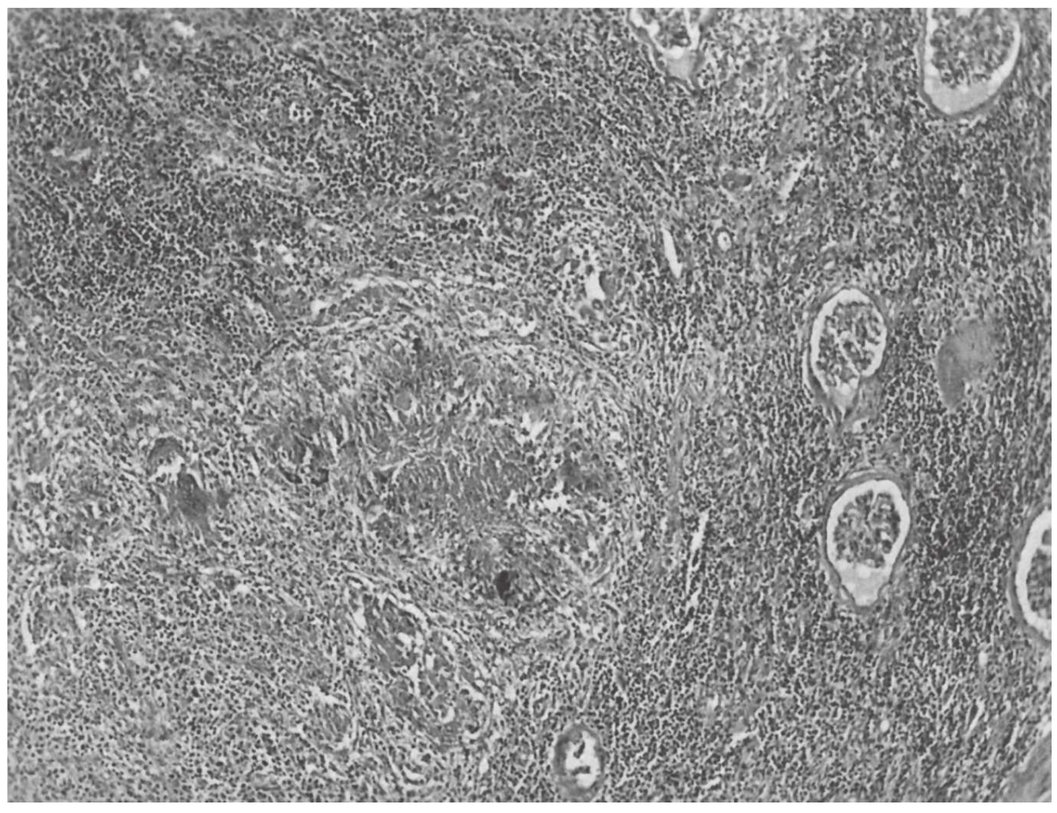


FIGURE 27.4 Renal cortical tissue showing destruction of tubules and a diffuse lymphocytic infiltrate with focal caseating granulomas. The glomeruli are spared (H&E, magnification ×79).

Most renal granulomas originate as vascular lesions in the cortex. Although glomerular lesions predominate, with foci within the capillary tuft, granulomas may develop within capillaries in relation to the convoluted or collecting tubules. Lesions within the collecting system per se are usually at the nadir of the loop of Henle or in the pyramidal collecting tubule, always draining a vascular granuloma (Fig. 27.2), and presumably developing in response to ulceration and discharge of these lesions into the collecting system. Focal sparing of tubules, glomeruli, or both within the granulomas is characteristic of renal tuberculosis (Figs. 27.3 and 27.4).

Clinically significant caseation progresses from the medullary collecting system lesions.[96] The enlarging medullary abscess extends to the papilla and commonly produces papillary necrosis. It may replace the medullary pyramid and persist as a parenchymal cavity, or tuberculoma, or discharge into the draining calyx. Several pyramids may be involved individually with a variable extent of destruction or may coalesce to destroy the bulk of the renal parenchyma (Fig. 27.5). Infection of the calyces, pelvis, and ureter is followed by stricture formation, so that caliectasis[97] and tuberculous pyonephrosis ("caseocavernous renal tuberculosis") are common in advanced disease. The end-stage kidney is nonfunctional ("autonephrectomy") and destroyed by the combined necrotizing and obstructive processes. Calcification in advanced lesions is common and may be focal or generalized, which produces a "putty" or "cement" kidney.

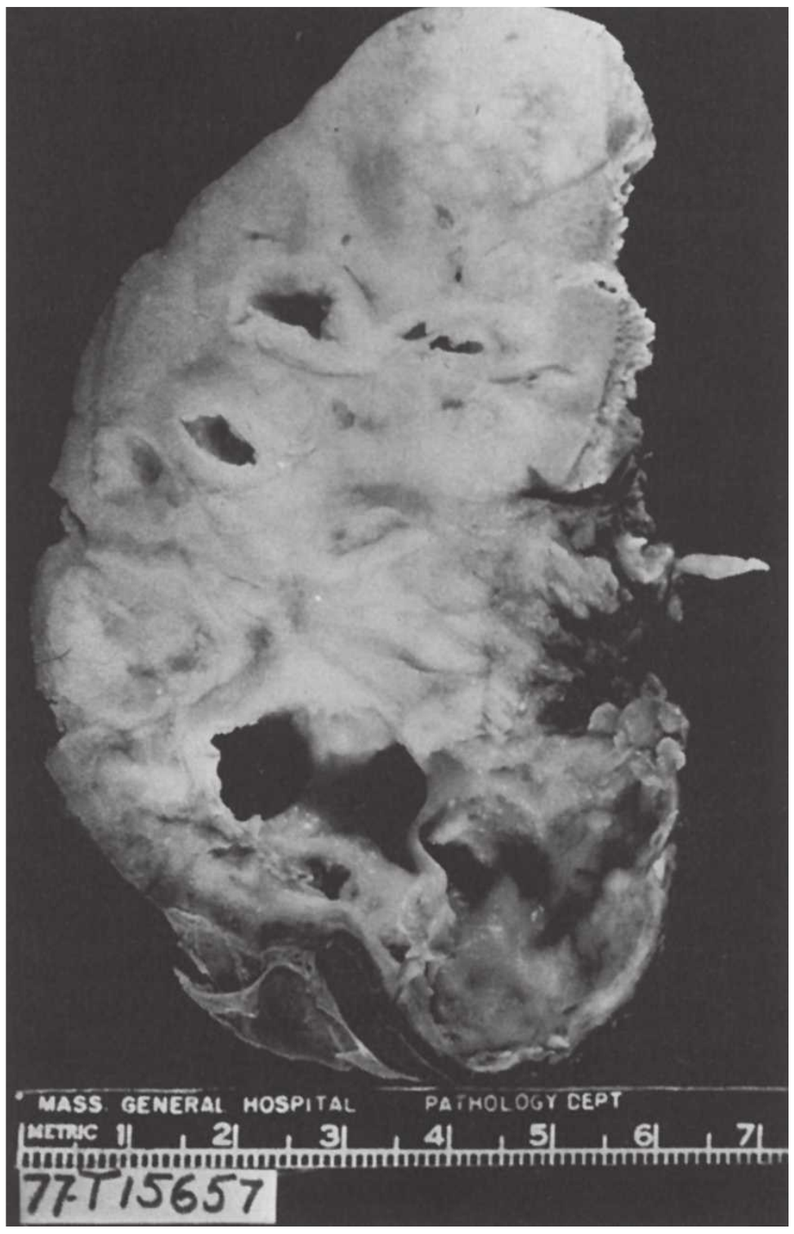


FIGURE 27.5 Renal tuberculosis with replacement of most of the parenchyma by caseous necrosis. There is cavitation of pyramids.

CLINICAL FEATURES

Urinary tract tuberculosis is notorious for its insidious mode of presentation, with approximately 20% of cases diagnosed unexpectedly at operation or autopsy. As many as 20% to 56% of patients with active urinary tract tuberculosis detected on the basis of an abnormal urinalysis or culture deny either constitutional symptoms of tuberculosis or symptoms referable to the urinary tract.[31–34,98,99] One measure of the frequently occult nature of urinary tract tuberculosis comes from Lattimer's classic report[99] wherein 18 of 25 physicians with renal tuberculosis were diagnosed only after developing far-advanced cavitary disease. If physicians with ready access to medical care have renal tuberculosis overlooked, then the problem is only compounded in the highest risk populations—inner city minority groups, immigrants from the developing countries of the world (particularly those undergoing social disruption), and the indigent elderly—all of whom have relatively poor access to medical care.

Roughly 75% or more of patients with urinary tract tuberculosis present with symptoms suggesting urinary tract inflammation. Such symptoms resemble those of conventional bacterial urinary tract infection—dysuria, urgency, frequency, mild or moderately severe back or flank pain, hematuria, nocturia, and pyuria. Renal colic, owing to the passage of clots or stones, may be observed in as many as 10% of patients.[31,32] Severe pain localized to the kidneys is uncommon but has been reported.[100] Epididymal thickening reflecting tuberculous epididymitis should heighten suspicion of upper tract tuberculosis. Noteworthy for their infrequency are the constitutional symptoms usually associated with tuberculosis—fever, weight loss, night sweats, and anorexia. Fewer than 20% of non-HIV infected patients with tuberculosis restricted to the urinary tract have constitutional symptoms, and the presence of such constitutional symptoms should suggest the presence of active tuberculosis in other organs as well.[31–33,98]

As noted previously, the extent of renal dysfunction secondary to tuberculous infection can be quite variable, from small focal areas of infection and scarring unassociated with any functional impairment to gross parenchymatous destruction and complete loss of function. As in any other form of tubulointerstitial nephritis (see Chapter 35), patients with renal tuberculosis may be subject to dehydration because of a concentrating defect, a tendency to lose salt, or both.[101] However, any patient with these findings should be evaluated for possible concomitant tuberculous adrenal disease (Addison disease), particularly when constitutional symptoms are present.[102,103]

Early diagnosis and therapy offers the best hope of limiting renal function loss resulting from parenchymatous infection and destruction. It is also important to identify patients experiencing renal functional loss due to hydronephrosis secondary to obstruction induced by the tuberculous process. Here the second element of the pathologic process induced by tuberculosis, sclerosis, exerts its effects. Strictures, usually either at the ureteropelvic junction or at the lower end of the ureter, can result in hydronephrosis and loss of renal function. Obstruction and hydronephrosis can develop during therapy because such sclerotic strictures are frequently part of the healing process. The clinician must be alert to this possibility, because correction of such obstruction is the best way to preserve renal function in patients with tuberculosis.[31,32,97,104–109]

There are three other major complications of renal tuberculosis: hypertension, superinfection with conventional bacteria, and nephrolithiasis. In 1940, Nesbit and Ratliff[110] reported that hypertension could be cured by the removal of a tuberculous kidney, an observation subsequently confirmed by other authors.[111–114] Subsequent data suggest that this is an uncommon event. First, hypertension may not be more common in patients with renal tuberculosis (<5% of those with tuberculous kidney infection are hypertensive) than in the general population. Second, surgical cure of hypertension in these patients appears to be the exception rather than the rule.[31,32,111] Renal vein renin sampling may be useful in predicting the outcome of surgery for patients with hypertension and renal tuberculosis.[115] Reversible renovascular hypertension due to direct involvement of the renal artery by tuberculous vasculitis has been rarely observed.[116]

Both nephrolithiasis and bacterial superinfection of a urinary tract rendered anatomically abnormal by the tuberculous process are not uncommon. Nephrolithiasis has been

reported in 7% to 18% of patients with renal tuberculosis, and superinfection has been reported in 12% to 50% of patients with urinary tract tuberculosis.[32]

The delivery of large numbers of M. tuberculosis into the urine of patients with renal tuberculosis is the major cause of tuberculous infection of the ureters and bladder. In both loci, scarring and contractures are the major results of tuberculous infection, again the not-so-benign effects of "healing." The result is a small, contracted bladder with greatly thickened walls. There are three functional consequences of this process: a small bladder capacity, incomplete emptying and thus a predisposition to secondary bacterial infection, and, most serious of all, vesicoureteral reflux.[117,118]

The incidence of genital infection in association with urinary tract tuberculosis is very different in the two sexes. In men, such dual involvement is relatively common. Indeed, genital disease may lead to the recognition of extensive urinary tract infection as noted previously. Epididymitis, with or without orchitis, presenting as a scrotal mass or discomfort, is the most common manifestation of male genital tuberculosis. The majority of such patients are free of constitutional complaints.[98,99,119] The importance of this form of tuberculosis is underlined by the report of Ferrie and Rundle[119] that 75% of their patients with tuberculous epididymoorchitis already had evidence on pyelogram of tuberculosis involving the bladder, ureters, kidneys, or all of these organs at the time they presented with their epididymal disease. Epididymitis can present decades after apparently adequate therapy for renal tuberculosis.[120]

Tuberculous prostatitis, an uncommon locus of genitourinary disease, may present with a mass lesion (mimicking prostatic carcinoma), pain, or both, rather than systemic symptoms in most patients and is frequently associated with urinary tract disease (Fig. 27.6). The prostate, like the epididymis and testes, can be infected either by means of the hematogenous route or more directly from infected urine.[121]

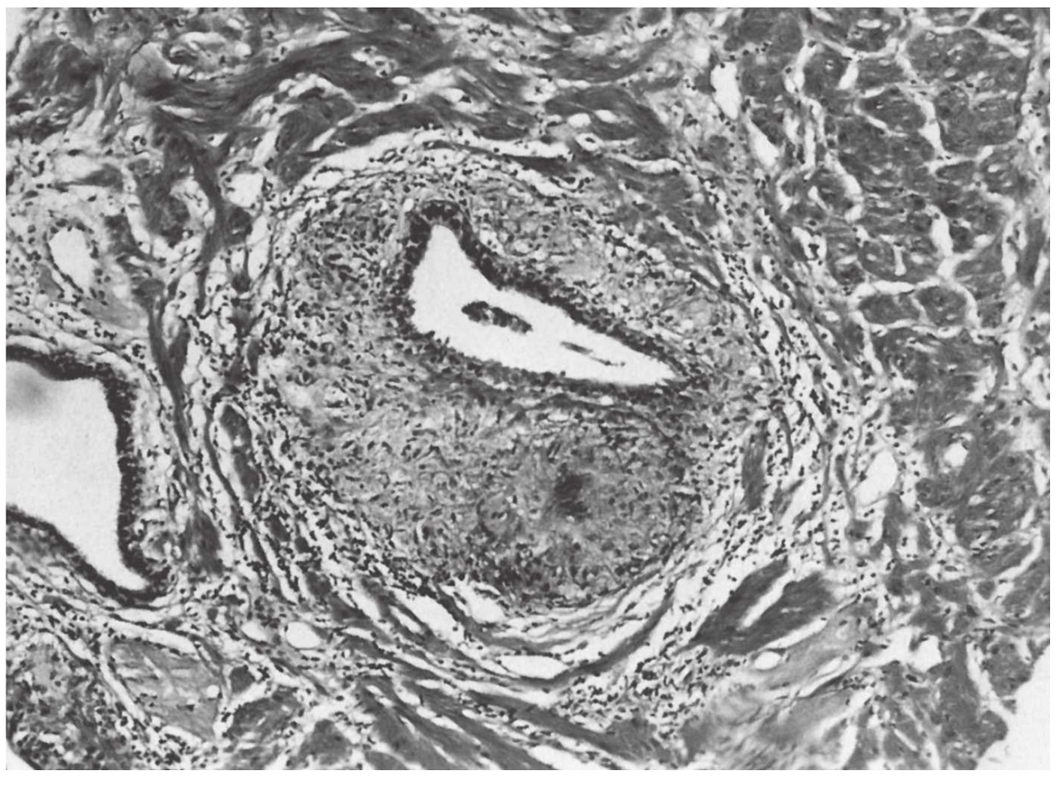


FIGURE 27.6 Prostatic tissue with epithelioid granulomatous reaction surrounding a duct (H&E, magnification ×125).

Viable organisms frequently persist in the prostate long after other parts of the genitourinary system have been sterilized.[31,121] Persistent prostatic infection is presumably related to the same difficulty in delivering effective antimicrobial therapy to the prostate that is responsible for persistent conventional bacterial prostatitis.

Urethral and penile tuberculosis are both quite uncommon but may present with strictures, fistulous tracts, and ulcerating or papulonecrotic skin lesions. Both these forms of tuberculous infection may reflect seeding from infected urine, spread from a contiguous lower genitourinary source such as the prostate, or from direct hematogenous seeding.[104,121] Primary penile tuberculosis has been reported to occur owing to the direct inoculation of M. tuberculosis into a wound following ritual circumcision, after the use of contaminated surgical instruments, sexual contact with women with tuberculosis of the female genital tract, or from contaminated clothing.[104,122,123]

In contrast, tuberculosis of the female reproductive tract is quite distinct from urinary tract tuberculosis. The incidence of renal disease in women with genital tuberculosis is less than 5%, which is little different from that found in patients with pulmonary or skeletal tuberculosis.[124] The explanation for this difference between men and women is clear: Whereas involvement of the male genital tract usually occurs from direct extension or from infected urine, the female genital tract is almost always infected hematogenously, with seeding of the fallopian tubes and then secondary extension from this site. Thus, the major manifestations of female genital tuberculosis—infertility, secondary amenorrhea, vaginal bleeding, and pelvic pain—are quite separate from the manifestations of urinary tract tuberculosis.[31,32 124,125]

Given its insidious nature, routine laboratory studies are quite important in raising the possibility of genitourinary tuberculosis (see later). By far the most useful screening test is urinalysis. As emphasized by Simon and associates,[31] virtually the only time when a urinalysis is normal and a urine culture is positive for M. tuberculosis is in the patient with miliary dissemination, in whom the urinary tract seeding is a recent and perhaps insignificant event. In contrast, essentially every patient with established urinary tract tuberculosis has an abnormal urinalysis with pyuria, hematuria, or both. The old clinical teaching that the asymptomatic patient with pyuria, particularly with an acid urine and a urine culture that fails to reveal conventional bacterial pathogens, must be considered as having tuberculosis until proved otherwise remains true today.[31–33,98,99] Although there are other causes of "sterile pyuria," such as Chlamydia trachomatis or invasive fungal infection, tuberculosis must be excluded in patients with these findings. An abnormal urinalysis should be followed by the placement of an intermediate-strength tuberculin test (5 TU). Virtually every patient with urinary tract tuberculosis who is not receiving systemic steroids, anti-TNF-α therapy, or who has not been rendered anergic by such debilitating conditions as advanced malignancy or HIV infection has a positive tuberculin test. In contrast, fewer

than 20% of patients with urinary tract tuberculosis have abnormalities on tests that measure systemic illness (i.e., anemia, changes in white blood cell count, low serum albumin, etc.).[31,32] Similarly, azotemia at presentation is quite infrequent, because severe bilateral obstructive uropathy is uncommon. Approximately two thirds of patients with urinary tract tuberculosis have evidence of old or current tuberculosis on chest roentgenography.[14,31,32]

ATYPICAL MYCOBACTERIAL INFECTION

Genitourinary infection caused by atypical mycobacterial organisms is quite rare. A Taiwan university hospital recently reported a series of such patients[126]: surprisingly, underlying metabolic disease including chronic renal disease and diabetes mellitus was frequent whereas the incidence of HIV infection or steroid use was quite low. Patients presented with typical refractory lower tract urinary symptoms but had a high rate of systemic symptoms, including fever, and came to medical attention soon after the onset of symptoms, in contrast to patients with M. tuberculosis, who had a low rate of systemic complaints and often presented with persistent symptoms. A variety of nontuberculous mycobacteria was recovered by urine culture. M. avium-intracellulare accounted for 33% and the rapidly growing M. abscessus and M. fortuitum as well as M. gordonae caused another 40% of cases. Destructive and obstructive urinary tract disease, as seen with M. tuberculosis, was common.

In addition, a few cases of prostatic or epididymal infection, or both, owing to M. kansasii have been reported.[127,128] Disseminated disease due to this organism has also been reported in immunocompromised patients, with hematogenous seeding associated with a granulomatous reaction in the kidney and isolation of M. kansasii in the urine.[129] This form of genitourinary infection was more prominent in the early years of the HIV epidemic and currently in resource-limited settings with high rates of advanced HIV-associated immunosuppression.[130]

Diagnosis

The isolation of M. tuberculosis by urine culture is the definitive diagnostic test in renal tuberculosis. Early morning urine specimens are preferred over 24-hour urine samples,[14] because mycobacterial viability falls with prolonged exposure to acid urine.[131] In order to detect the low rate of bacilluria, three to five specimens should be submitted. Samples are routinely decontaminated by limited exposure to acid or alkaline solutions and then concentrated by centrifugation. Neither direct nor amplified nucleic acid hybridization probes are licensed for rapid diagnosis of urinary M. tuberculosis given their borderline sensitivity (~70%) compared to traditional culture,[132] although newer real-time polymerase chain reaction (PCR) methodology may prove as sensitive and specific as culture for the identification of urinary M. tuberculosis.[133]

Cultures are established on standard solid mycobacterial media, either egg-based (Löwenstein-Jensen) or agar-based (Middlebrook 7H10). These media also contain aniline dyes, such as malachite green, to inhibit the growth of bacterial contaminants. The transparent agar medium facilitates early visualization of microcolonies by approximately 1 week. Newer automated liquid based (Middlebrook 7H12) radiometric or colorimetric culture techniques have yields comparable to culture on solid media, with considerably more rapid recovery times.[134]

Microbiologic identification is based on colonial morphology, growth rate and optima (37°C, carbon dioxide-enriched atmosphere), absence of pigment production, accumulation of niacin, reduction of nitrate, and absence of significant catalase activity.[14] Commercial oligonucleotide reagents are available for the rapid speciation of primary isolates by nucleic acid hybridization techniques.

Other rapid diagnostic techniques have been developed but are not licensed yet for routine clinical use. These include high performance liquid chromatography to identify the spectrum of mycolic acids in the bacterial cell (which is useful after 7 to 10 days of culture), enzyme-linked immunosorbent assay (ELISA) techniques to detect mycobacterial protein antigens, and the detection of tuberculostearic acid by gas chromatography and mass spectroscopy.

In retrospective analyses of patients with renal tuberculosis, urine cultures were reported to be positive in ~70% to 90% of cases.[27,31–33] In patients with negative cultures despite optimal processing of multiple samples, the diagnosis is often reached by the recovery of M. tuberculosis from other sites (e.g., sputum or surgical specimens) in the setting of abnormal urinalyses and imaging, together with a positive tuberculin reaction. Some culture-negative patients have enclosed intraparenchymal granulomas which have not yet drained into the collecting system. When tissue specimens are submitted for mycobacterial culture, they are best macerated using sterile sand with a mortar and pestle, because automated homogenization methods may heat samples excessively and kill any mycobacteria present.[131]

Successful mycobacterial isolation provides an opportunity to perform drug susceptibility testing in addition to confirming the diagnosis of genitourinary tuberculosis. Drug susceptibility testing is strongly recommended on initial isolates from all patients, but is critically important in evaluating patients who previously received chemotherapy and in patients epidemiologically linked to known cases of drug-resistant tuberculosis.[14]

Radiology

Radiologic evaluation has long played a central role in the diagnosis and long-term management of patients with renal tuberculosis.[135,136] There is excellent correlation between the pathology of renal tuberculosis and the corresponding abnormalities seen by excretory urography.[137] Even plain films of the abdomen are valuable, as genitourinary

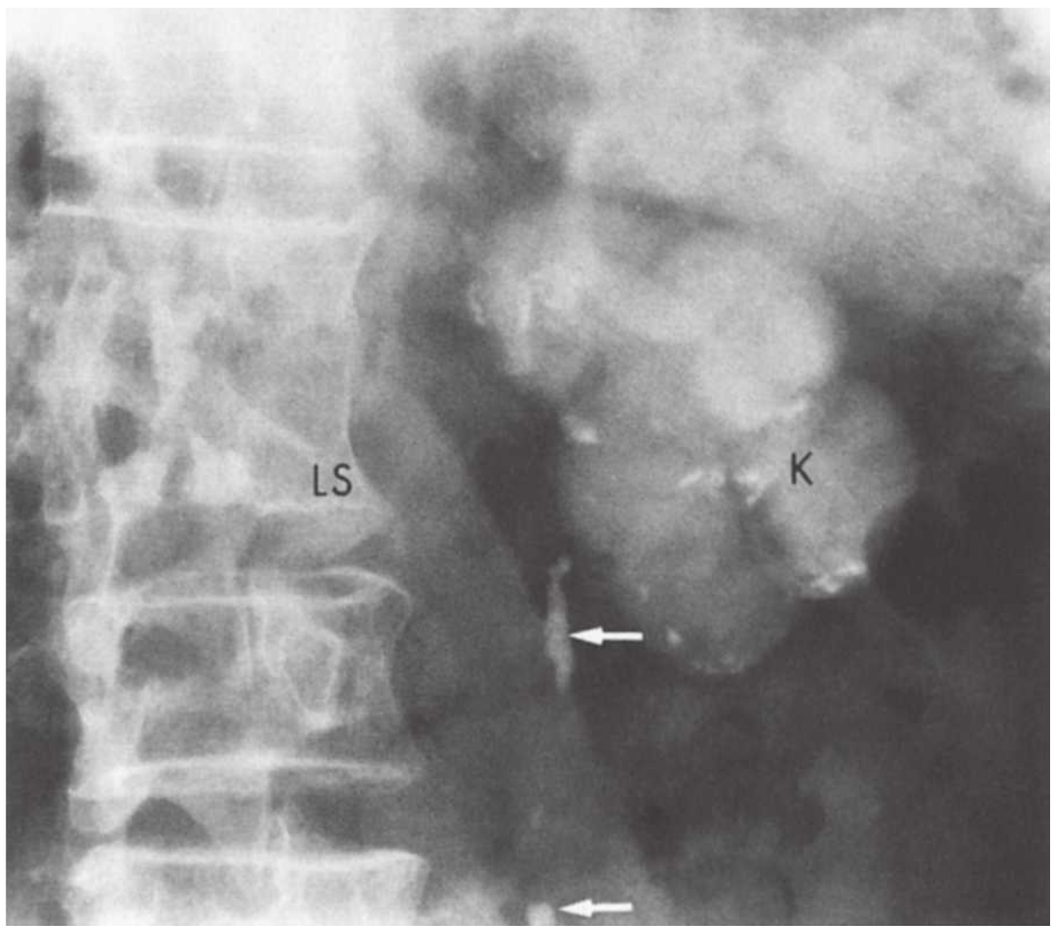


FIGURE 27.7 Plain film of the abdomen shows a left autonephrectomy with calcification of the kidney parenchyma and the left ureter (*arrows*). There is tuberculous involvement of the lumbar spine, resulting in fusion of the intervertebral disc space. *K,* kidney parenchyma; *LS,* lumbar spine.

calcifications (present in up to 50%)[138] as well as other extrapulmonary foci of mycobacterial disease (vertebral, mesenteric lymph node, adrenal glands) may be present (approximately 10%) (Figs. 27.7 and 27.8).[138] Chest radiographs show evidence of tuberculosis in 50% to 75% of patients with active renal disease.[31,32,139] In the remainder, the primary pulmonary granuloma, responsible for hematogenous spread, heals and may no longer be detectable by radiograph, but the metastatic renal granulomas progress to cause local destruction.

Excretory urography including nephrotomography traditionally has been the standard diagnostic imaging technique. In recent years, computed tomography (CT) with contrast, particularly helical CT, has provided increased delineation of renal parenchymal abnormalities, although contrast administration (with risks of allergy and nephrotoxicity) is still required, and the total radiation dose exceeds that of traditional excretory urography. Cross-sectional imaging including CT, magnetic resonance imaging (MRI), and sonography, provide a framework for assessing the renal parenchyma, adrenals, bladder, and genital organs that complements excretory urography.[140,141]

The earliest stage of renal involvement, the cortical granuloma (Fig. 27.2), is associated with positive urine mycobacterial cultures but negative excretory urograms. The spread of infection to the medulla and the evolution of cavitary disease in the papillae represent the earliest changes detectable radiologically (Fig. 27.9). Papillary granulomas caseate and rupture into the collecting system. The resulting small communicating cavities may be single or multiple but

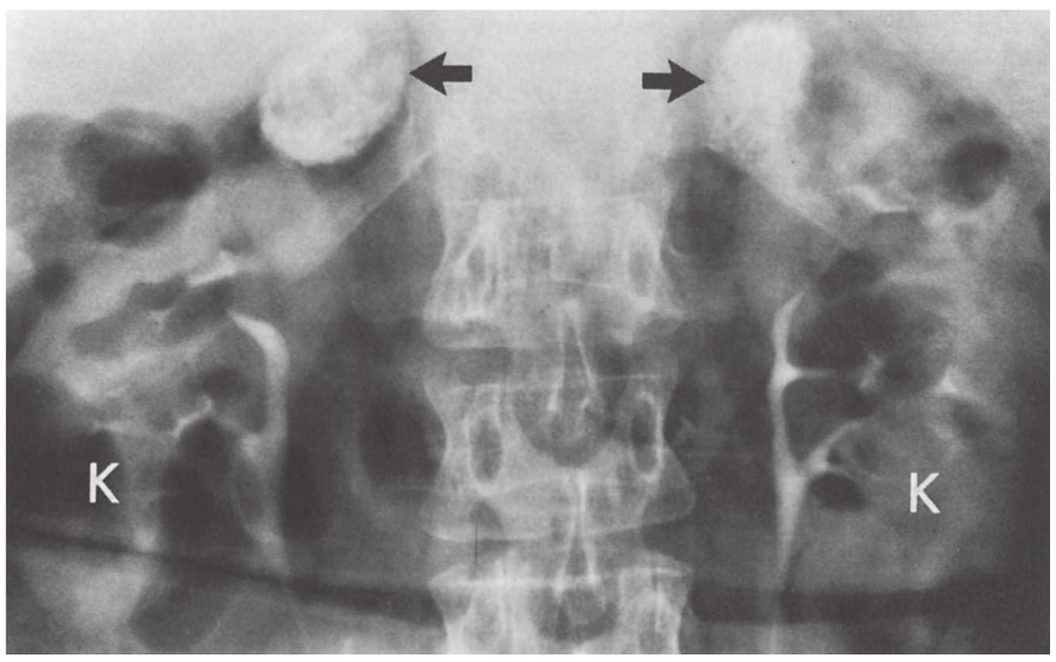


FIGURE 27.8 Tuberculous calcification of both adrenal glands (*arrows*). Intravenous urogram demonstrates the normal kidneys. *K,* normal kidneys.

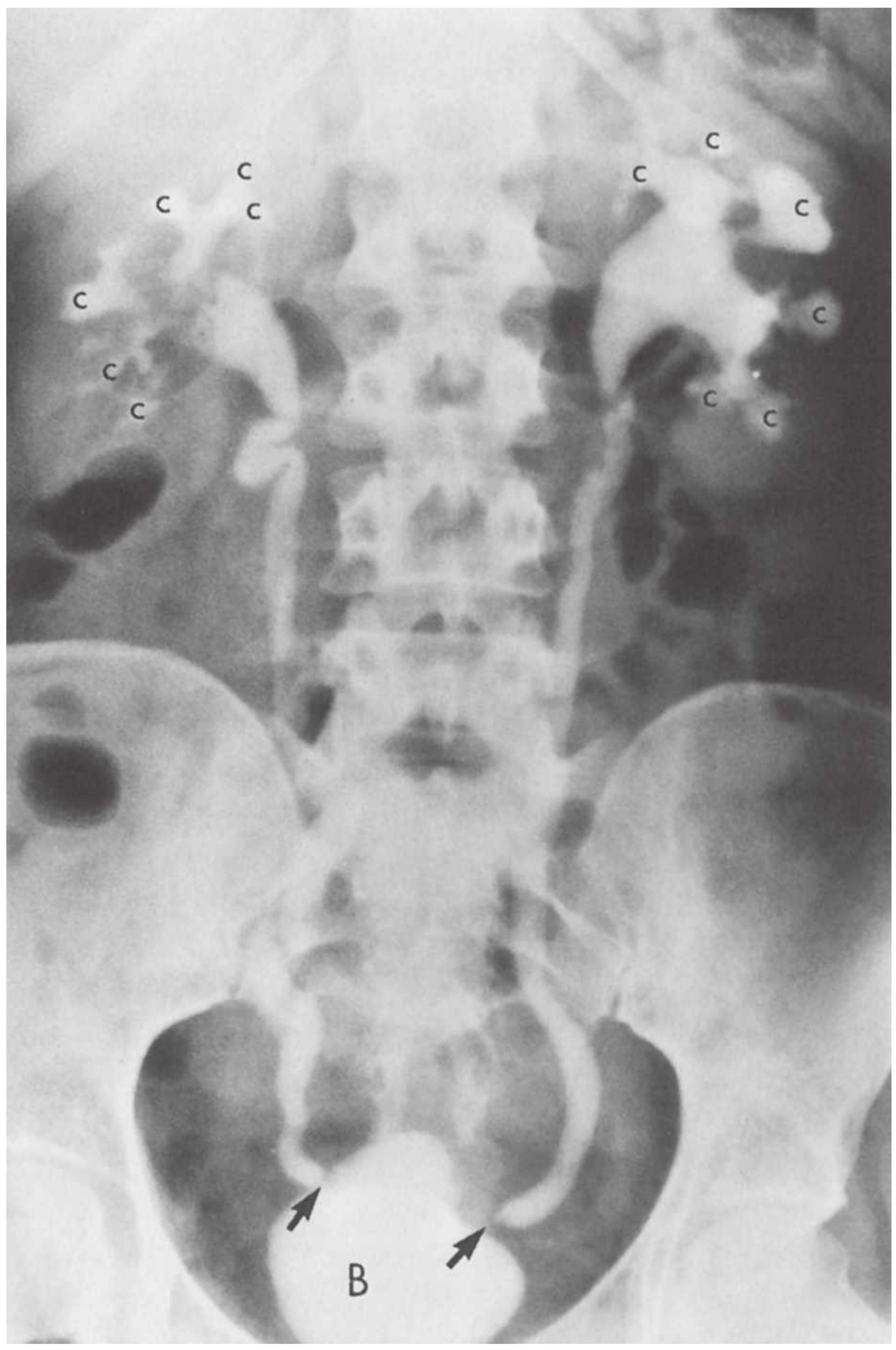


FIGURE 27.9 Intravenous urogram in a patient with active urinary tuberculosis, demonstrating papillary necrosis diffusely involving all the calyces in both kidneys. There is a small irregular bladder and narrowing of both distal ureters (*arrows*). *B,* bladder; *C,* calyces.

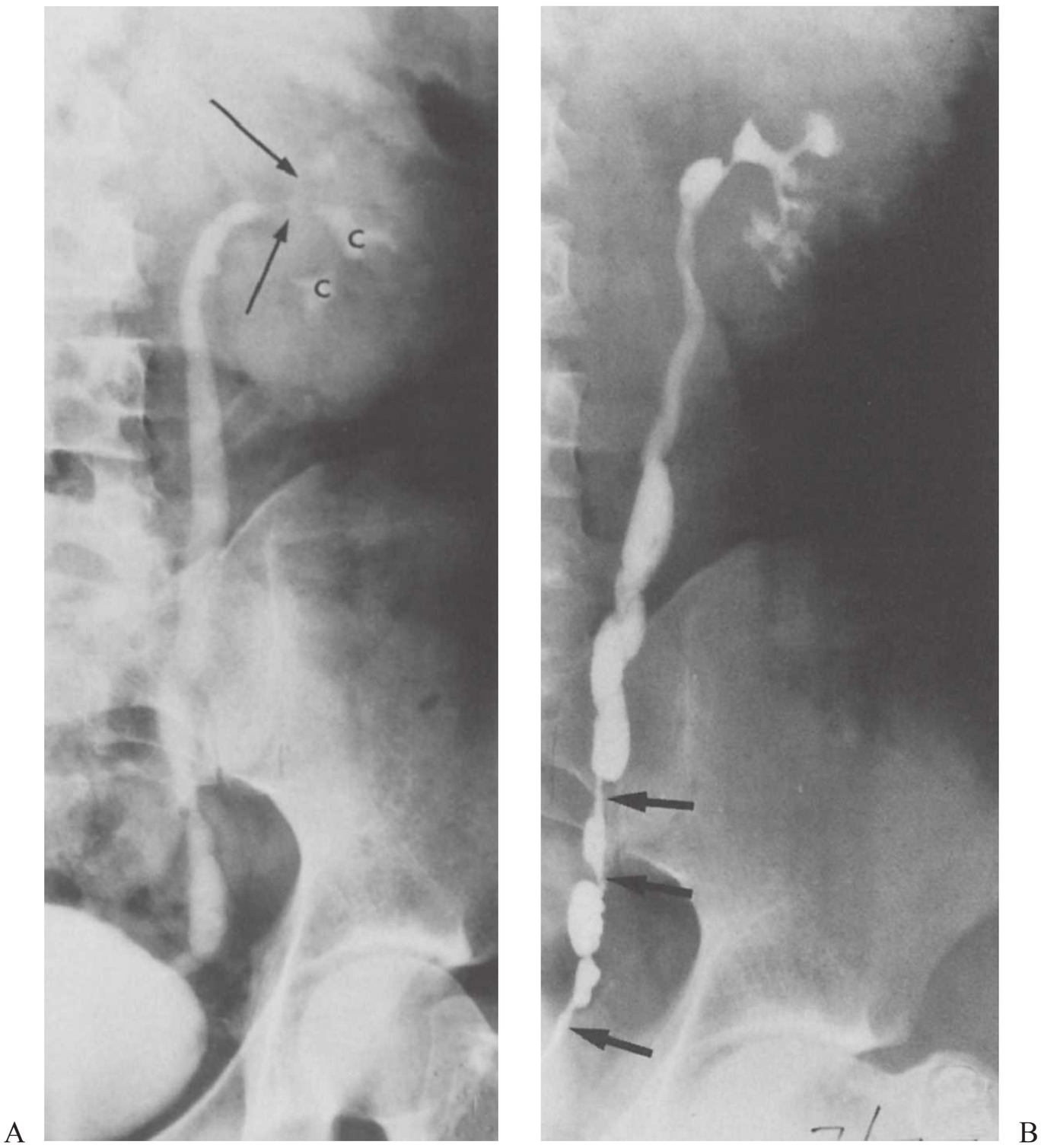
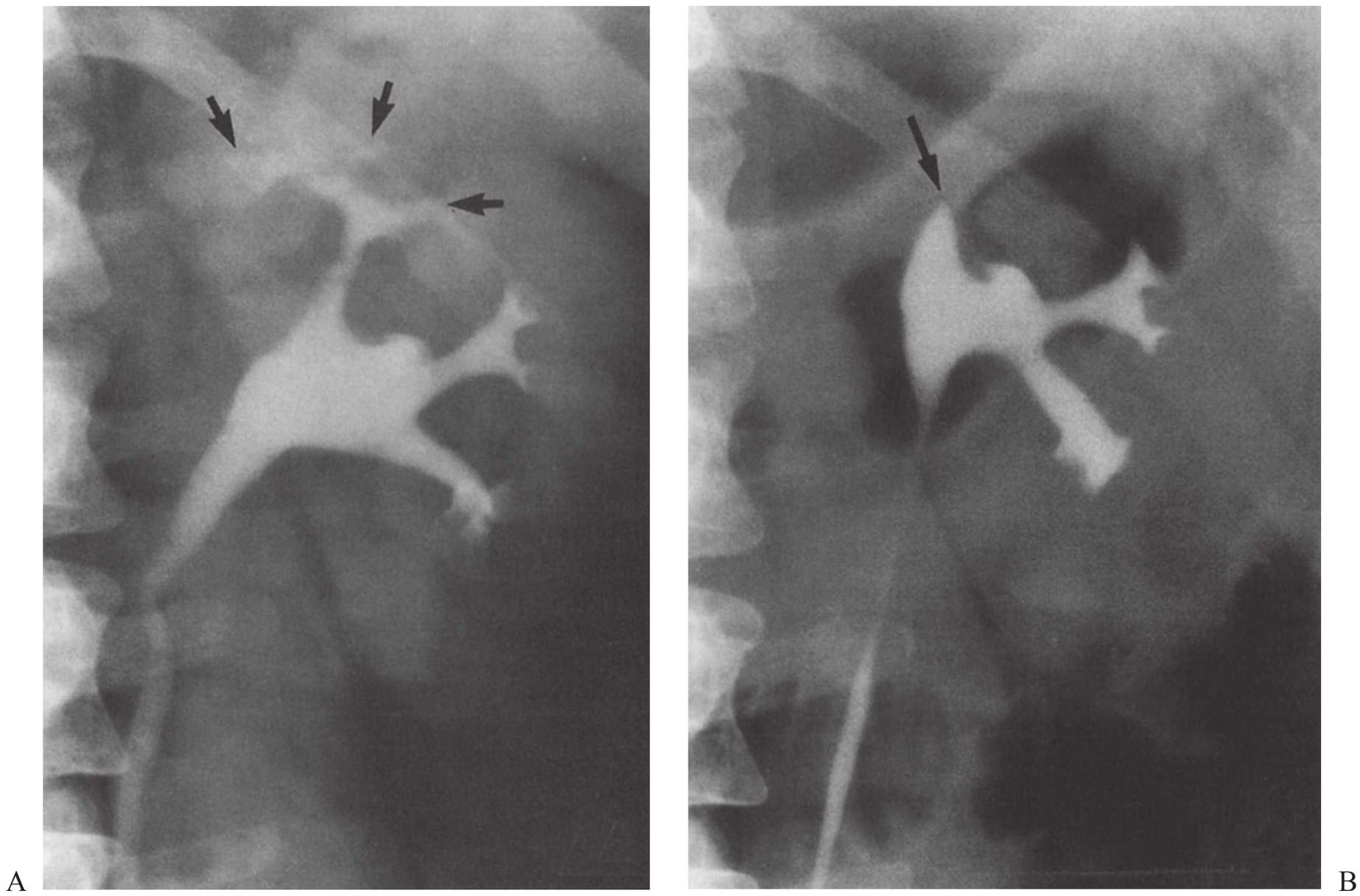


FIGURE 27.10 **A:** Intravenous urogram shows tuberculous narrowing of the left renal pelvis and upper pole infundibulum (*arrows*) and papillary necrosis of the lower pole calyces. The left ureter is moderately dilated and irregular in contour. **B:** Left retrograde pyelogram 6 months later, documenting fibrotic narrowing of the distal ureter (*arrows*) during antituberculous therapy. Tuberculous ureteritis commonly heals by cicatrization and may lead to severe obstruction of the collecting system. *C*, calyces.

are usually unilateral and may mimic papillary necrosis of other etiologies. With time, they enlarge in an irregular fashion, with shaggy margins, and may progress to involve the entire medullary pyramid. A consequence of papillary cavitation is spread of infection to the uroepithelium and submucosa of the draining calyx. The resulting fibrotic reaction leads to stenosis and even complete stricture of the calyceal infundibulum. Thus a medullary cavity may be excluded from the collecting system and an abscess, or localized tuberculous pyonephrosis, ensues. These lesions may be difficult to distinguish from a truly noncommunicating caseous parenchymal cavity. Advanced medullary disease may lead to renal cortical scarring as well. Amorphous calcification is frequently seen (Fig. 27.7) and may progress to outline the entire granuloma. Both the parenchymal tuberculous granuloma and the noncommunicating pyonephrosis may be confused with primary mass lesions; CT,[140,141] ultrasonography,[141,142] and, in selected cases, magnetic resonance[141,143] or even positron emission tomography (PET)-CT[144] may be helpful by confirming the nonneoplastic, cystic, and avascular properties of the mass.

The discharge of caseous material infects the renal pelvis, ureter, and bladder as well. Renal pelvic involvement is manifested by obstructive changes involving portions of the kidney due to stenosis or kinking of the pelvis or the entire organ due to ureteropelvic junction stenosis (Figs. 27.10 and 27.11). Thus poor visualization by excretory urography may be segmental or involve the entire kidney. Ureteral disease initially presents as mucosal irregularity, together with diffuse dilatation or narrowing due to inflammation or edema (Figs. 27.9 and 27.10).[145] There may be a single focus or multiple areas of ureteral involvement. In addition to an irregular border, the combination of multiple strictures and accompanying segmental dilation leads to a beaded or corkscrew configuration (Fig. 27.10). Unlike nonspecific strictures, tuberculous lesions may extend for several centimeters and are usually present together with ipsilateral renal disease. In some instances, intramural fibrosis leads to a rigid "pipestem" ureter. Calcification and even calculi may be present as well. Ureterovesical junction involvement may produce a stricture responsible for ureteral obstruction or a patulous, rigidly dilated orifice associated with vesicoureteral reflux.

Bladder involvement similarly begins with focal mucosal irregularity and progresses to produce a small, contracted trabeculated bladder with intramural thickening (Figs. 27.9 and 27.12). Occasionally, calcification of the bladder wall may occur.



A B

FIGURE 27.11 Intravenous urograms 14 years apart. **A:** Early change of renal tuberculosis with papillary necrosis of the upper pole calyces (*arrows*). **B:** Fourteen years later, there has been complete stenosis of the left upper pole infundibulum (*arrow*) as the tuberculous process heals by scarring.

The excretory urogram has long been the radiologic standard for evaluating patients with renal tuberculosis because of its capacity to record focal calyceal abnormalities, including cavitation, scarring, and obstruction, and to survey the collecting system in equal detail simultaneously. Sonography cannot record erosive calyceal abnormalities or distinguish advanced caseous disease from focal pyonephrosis. Sonography may, however, play a role in monitoring the possible development of hydronephrosis.[141,142] CT studies, particularly when intravenous contrast reagents are administered, have become the procedure of choice for diagnosing and assessing renal tuberculosis (Fig. 27.13). Thus, in early or focal disease, obstruction of a single major calyx or a group of minor calyces may be observed; tuberculous involvement of the renal pelvis may be visualized as either dilatation owing to ureteropelvic junction obstruction or diffuse pelvic contraction. With advanced disease, small, atrophic kidneys, often with one or more low density areas, are observed.[140] Calcifications of the genitourinary system and extrarenal intra-abdominal disease can likewise be observed at the same time.[140,141,146] Sonographic and CT studies are particularly useful in patients with advanced disease when there is nonvisualization of the affected kidney by excretory urography. Angiography has been used in the past for the rare patient when isolated focal disease caused by obstruction or cavitation has mimicked a primary renal mass,[147] but MRI has largely supplanted the need for arteriography.[141,143] The obliterative arteritis that accompanies progressive caseation is responsible for the avascular appearance of the granulomatous mass with pruning and obliteration of the interlobar arteries.[147] As noted above, tuberculous arteritis of the main renal artery is exceedingly rare.[116]

Clinical Management

The advent of effective chemotherapy has revolutionized the clinical management of urinary tract tuberculosis, although the recent increase in drug-resistant infection, amplified by the AIDS epidemic, threatens this success. Whereas in the prechemotherapeutic era extirpative surgery was the only hope of controlling infection, today medical cure is the rule. There is a continuing need for surgical intervention, but now it is primarily for the correction of anatomic abnormalities caused by scarring rather than for the removal of infected tissues. The two goals, then, in the management of urinary tract tuberculosis are the conservation of tissue and function (both with medical treatment and surgical relief of obstruction resulting from tuberculous scarring) and antimycobacterial cure.

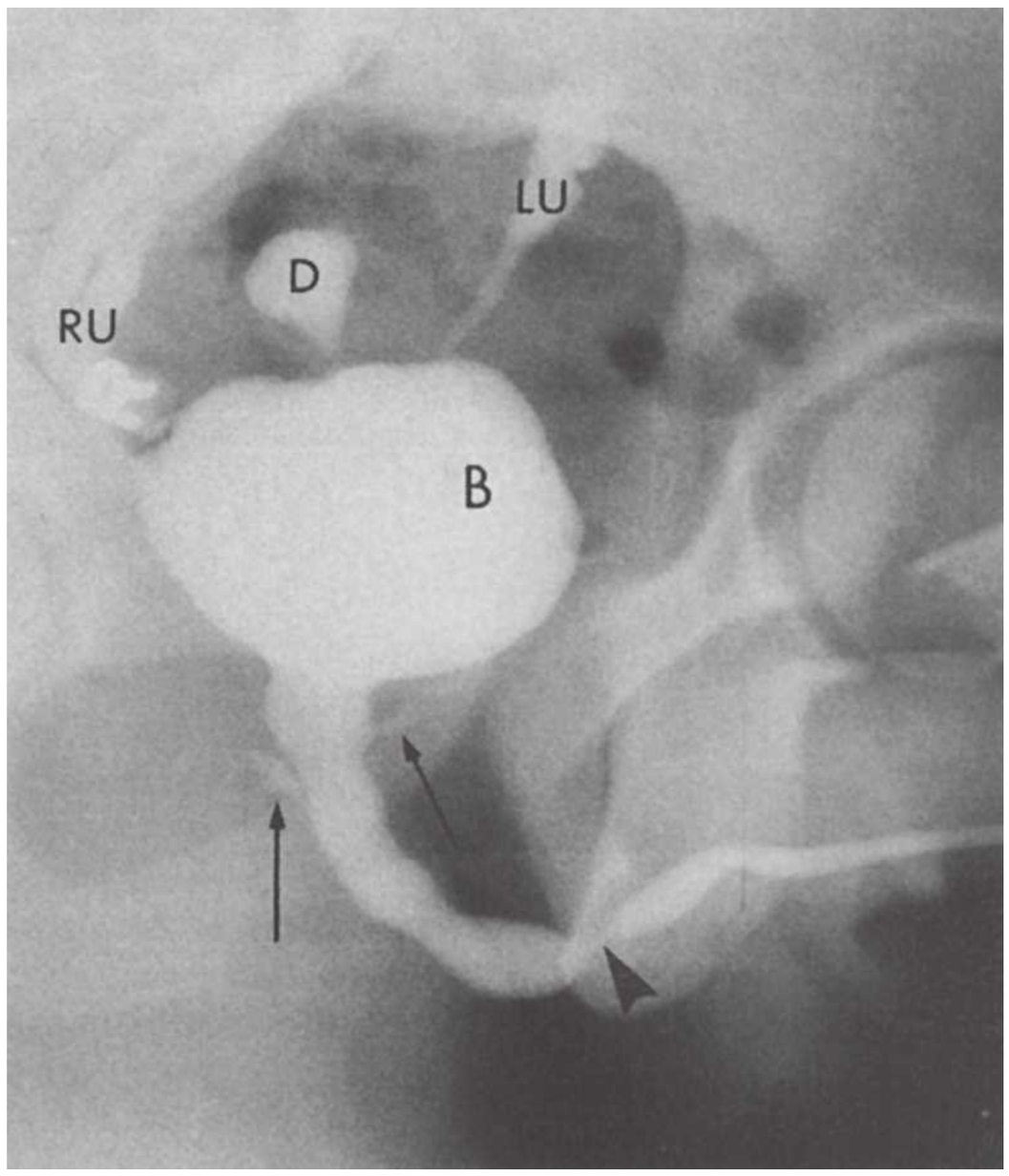


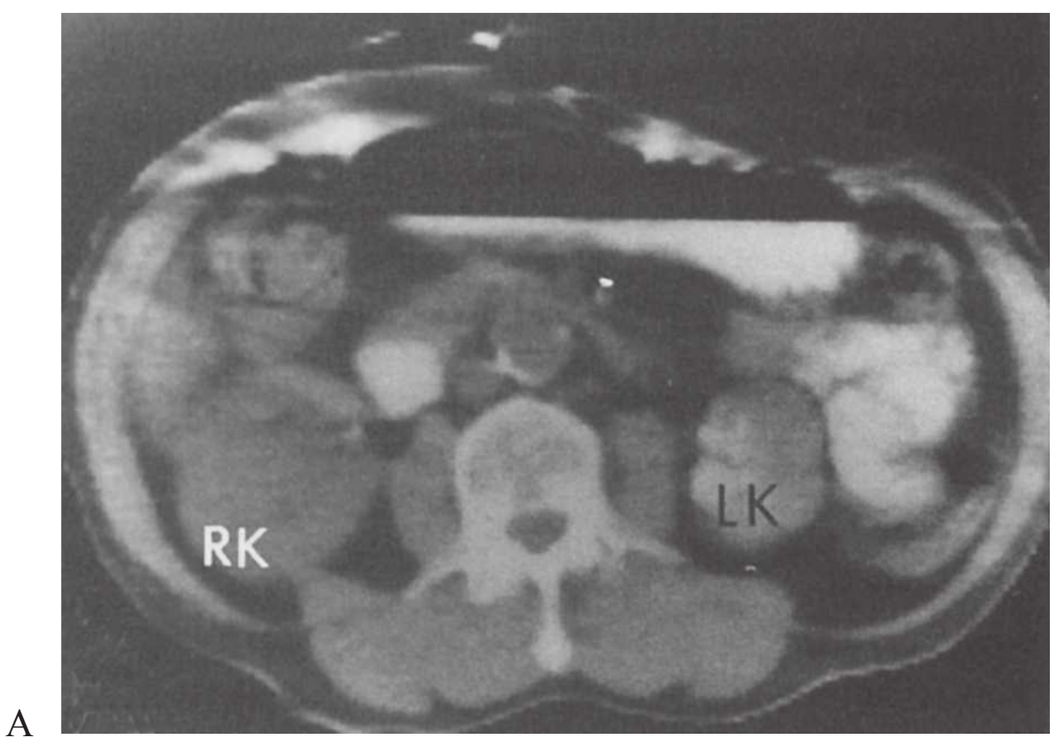
FIGURE 27.12 Voiding cystourethrogram in a young man with left renal tuberculosis, showing reflux into duplicated ureters of a normal right kidney and into an abnormal single left ureter, with a concomitant distal stenosis. The bladder is markedly reduced in volume and there is a diverticulum at the dome. There is reflux of contrast into the prostatic gland (*arrows*) secondary to tuberculous prostatitis. There is a stricture of the midbulbous urethra (*arrowhead*), which is an unusual site for tuberculous involvement. *B,* bladder; *D,* diverticulum; *LU,* left ureter; *RU,* duplicated ureters.

CHEMOTHERAPY OF URINARY TRACT TUBERCULOSIS

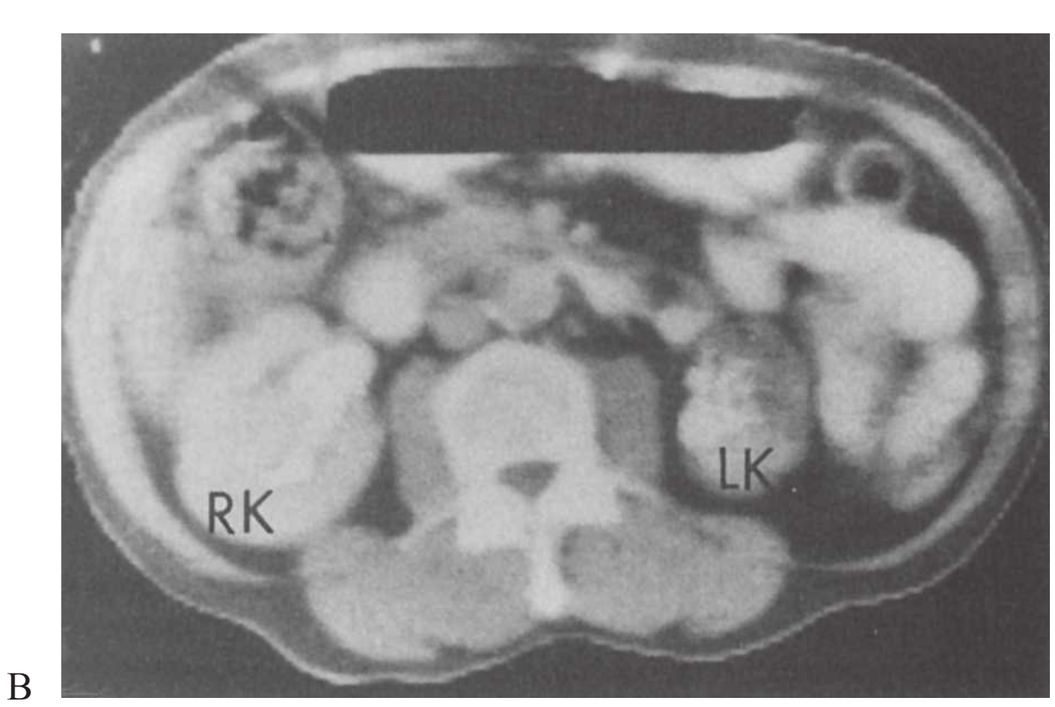
The chemotherapeutic approach to tuberculosis is based on the following general principles (Table 27.1).[15,148,149]

1. Mycobacterium tuberculosis may persist in a viable form while multiplying slowly or even intermittently. It is believed that there are three populations of organisms that must be considered when treating patients with active tuberculous infection.[150] The largest number, and fortunately the most easily treated, are those that are extracellular, as within a cavity, where the pH is either neutral or alkaline. Because this group of organisms is actively multiplying, this is the population most easily treated with two or more bactericidal drugs. Also, because it is quantitatively the largest population, drug resistance is most apt to emerge within this population if an inappropriate therapeutic program is employed. A much smaller population of slowly or intermittently multiplying organisms is found at an acid pH within macrophages. Finally, there are a variable number of organisms exhibiting slow or intermittent multiplication at a neutral pH within closed caseous lesions.

   Curative therapy requires eradication of all three populations of organisms. Because of pH constraints, differing abilities to penetrate at different sites, and inherent effects on the tubercle bacillus, each of the available antituberculous drugs is more or less effective for these different populations of M. tuberculosis. Rifampin and related agents (rifabutin and rifapentine) are the only drugs that are bactericidal for all three populations of M. tuberculosis. Isoniazid (INH) is bactericidal both for the actively growing organisms in cavities and for those slowly multiplying within macrophages. Streptomycin and the other injectable aminoglycosides are bactericidal only for the actively replicating extracellular organisms. Pyrazinamide is bactericidal only

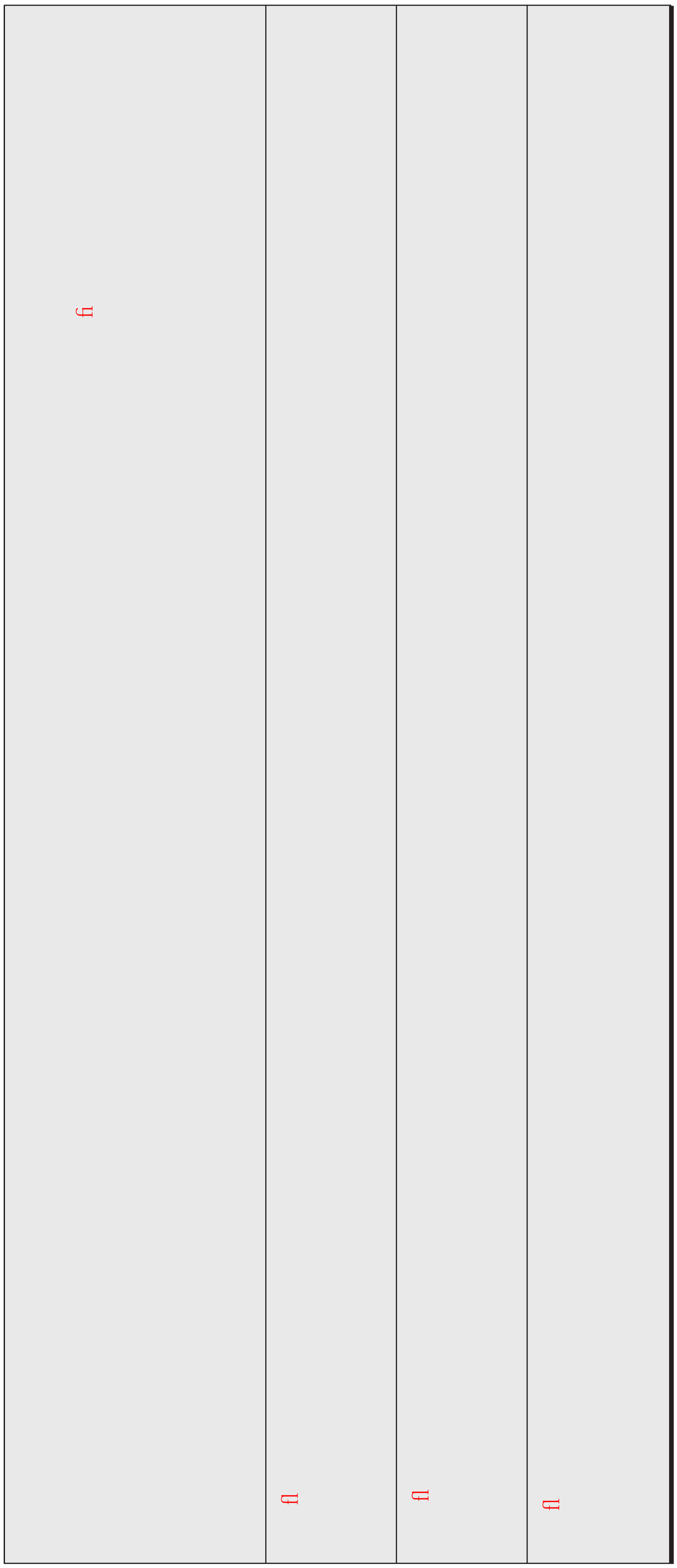


A



B

FIGURE 27.13 Computed tomography scans **(A)** before and **(B)** after the intravenous administration of contrast. There is a calcified nonfunctioning left kidney characteristic of a tuberculous autonephrectomy in a patient with old pulmonary tuberculosis. The right kidney is normal. *LK,* left kidney; *RK,* right kidney.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for intracellular organisms. All other drugs currently licensed for the treatment of tuberculosis are bacteriostatic. The fluoroquinolones— levofloxacin, moxifloxacin, and gatifloxacin —are bactericidal for M. tuberculosis in vitro (minimum bactericidal concentration of 2 μg/mL, in the setting of peak blood levels >4 μg/mL) and achieve excellent concentrations intracellularly. They are utilized in the treatment of patients who are intolerant of first line agents, or infected with isolates resistant to one or more first line antituberculous agents.[149] The primary role of bacteriostatic antituberculous drugs is to inhibit the development of mutants resistant to simultaneously administered bactericidal agents.

2. The spontaneous development of drug-resistant mutants of M. tuberculosis occurs at a rate of approximately 1×10^{-6}. The probability that a single organism would be resistant to two drugs simultaneously approximates the product of the probabilities of resistance to each drug alone ($1 \times 10^{-6} \times 1 \times 10^{-6} = 1 \times 10^{-12}$). Therefore, a major determinant of the number of drugs necessary to treat tuberculous infection is the number of organisms harbored by the individual. In the case of urinary tract tuberculosis without active infection at other sites, an organism burden of approximately 1×10^{7} is likely. Therefore, a minimum of two drugs to which the patient's isolate is susceptible is required for therapy. Use of only one effective drug, given the rate of mutation to drug resistance and the number of organisms present, would lead to not only clinical failure but also the selection of resistant organisms.
3. The epidemiology of drug-resistant tuberculosis is rather complex and reflects variations in resistance rates among different patient groups based on tuberculosis treatment history, ethnicity, and socioeconomic determinants. The rate of isoniazid monoresistance in the United States was largely unchanged between 1993 (4.1%) and 2005 (4.2%). INH resistance rates were elevated among both U.S. and foreign born Asian/Pacific Islanders, foreign born blacks, and U.S. born Hispanics. Resistance tracked with a history of tuberculosis, failure to complete timely antituberculous therapy, and a history of incarceration.[151] Interestingly, HIV serostatus did not confer an increased risk of INH resistance. Multidrug resistance (MDR-Tbc, INH + rifampin resistance) was identified in 1.3% of isolates in 2010, with rates of 1% in previously untreated patients and ~5% in previously treated individuals. Nearly all MDR-Tb isolates (89.4%) occurred in foreign born individuals.[20]
4. Because of the persistence of drug-resistant tuberculosis in the United States in recent years and the substantial rate of drug resistance in resource-limited settings, drug susceptibility testing should be performed routinely on all patient isolates, rather than just on isolates from high-risk patient groups. Because the most common cause of the development of de novo drug resistance is failure of compliance by the patient, directly observed therapy ("DOT") in which public health workers directly administer the therapy to the patient is often central to successful therapy. Particularly in urban areas, this strategy of enforced antituberculous therapy compliance has proved to be quite successful in blocking the spread of tuberculosis, particularly drug-resistant tuberculosis.[15,149]

The standard of care for urinary tract tuberculosis due to drug-sensitive organisms in a compliant patient is a 6-month regimen, with four-drug initial therapy (INH, rifampin, pyrazinamide, and ethambutol) for 2 months, followed by a 4-month course of INH and rifampin for 4 months[15,149]; ethambutol therapy may be discontinued when sensitivity data becomes available and confirms drug susceptibility. Patients not tolerating pyrazinamide should receive a 7 month continuation phase regimen of INH and rifampin (plus one or more of the other drugs listed until susceptibility testing results are known). More prolonged courses of therapy are indicated for any patient slow to respond to one of the standard regimens, those with miliary or central nervous system disease, those with significant immunosuppression (e.g., AIDS, organ transplant recipients, etc.), and children with multiple sites of involvement (including the skeleton).[148,149] Prolonged therapy for prostatic tuberculosis and advanced renal parenchymal disease is often recommended as well.[152]

HIV-associated tuberculosis is managed similarly to that observed in the HIV-negative population (although our preference is to prolong therapy for an additional 3 to 6 months in those with AIDS), with the following modifications to increase the probability of success: directly observed therapy for all patients with HIV-related tuberculosis; the substitution of rifabutin for rifampin in individuals receiving anti-HIV protease inhibitors because of the risk of drug interactions that affect the efficacy of treatment of both the HIV and the tuberculosis; and monitoring the responses to antituberculosis treatment to individualize the appropriate duration of antituberculosis therapy.[149] HIV-infected individuals initiating antituberculosis therapy soon after starting antiretroviral therapy may experience fever, lymphadenopathy, and exacerbation of symptoms which is now recognized as the immune reconstitution inflammatory syndrome.[149,153] This is seen primarily with advanced immunodeficiency (i.e., low CD4 counts) and can be treated by brief courses of systemic steroid therapy.[149,153]

The management of drug-resistant disease is determined by the results of in vitro susceptibility testing. Three agents including at least one of the front-line bactericidal agents (INH, rifampin, and pyrazinamide) to which the isolate is susceptible, are prescribed. INH resistant isolates may be treated with rifampin, pyrazinamide, and ethambutol, with fluoroquinolone therapy added if there is extensive disease. Rifampin monoresistant isolates are recognized primarily in HIV-infected individuals and require prolonged courses of therapy because rifampin is the cornerstone of highly active

short course antituberculosis regimens: treatment with INH, pyrazinamide, and ethambutol is recommended, with added fluoroquinolone therapy for extensive disease. Dual INH/rifampin resistance requires prolonged multidrug therapy including a fluoroquinolone and an injectable aminoglycoside in addition to two second line agents. The American Thoracic Society guidelines[149] outline treatment requirements for drug-resistant tuberculosis; expert infectious disease consultation should be sought for detailed clinical guidance.

Two types of genitourinary tract tuberculosis are particularly difficult to treat—"the autonephrectomized" kidney destroyed by tuberculosis and now presenting as a nonfunctioning, avascular, calcified, caseous mass, and prostatic tuberculosis. In both these circumstances, delivery of antituberculous therapy to the site of infection is fraught with difficulty. Indeed, as observed in the following, some authorities believe that all end-stage tuberculous kidneys should be removed surgically, and Dutt and Stead[152] noted that tuberculous abscesses must be surgically drained in any patient undergoing short-course therapy. As far as prostatic tuberculosis is concerned, we have observed patients who had been rendered culture negative with 2 years of therapy for urinary tract tuberculosis who, a few years later at the time of an incidental prostatectomy, were shown to harbor living M. tuberculosis at this sequestered site.

SURGICAL MANAGEMENT OF URINARY TRACT TUBERCULOSIS

In the prechemotherapy era, surgical ablation of infected foci was the only therapy available for renal tuberculosis. Without surgery, the 5-year survival rate for patients with renal tuberculosis was 15% to 42%, but with surgery, 10-year survival rates approached 50%.[154] With modern chemotherapy, urinary tract tuberculosis should be routinely curable, but surgical intervention is still required at times.[106–109] Surgical intervention is required for the relief of strictures, particularly ureteral strictures, which can result from the scarring process and may develop in nearly half of patients with renal tuberculosis.[106] Thus, ureteral stenting, sometimes preceded by balloon dilatation, ureteral reimplantations, and, in some cases, relief of intrarenal obstruction to urine flow are important aspects of the modern function-conserving approach to urinary tract tuberculosis.[106–109] Obstruction present at the time of diagnosis is usually addressed several weeks after initiating intensive antituberculous therapy, although percutaneous nephrostomy and stent placement to relieve severe obstruction can be performed acutely. The administration of corticosteroids together with antituberculosis chemotherapy to reduce the risk of progressive ureteral scarring and subsequent surgical intervention has long been discussed[31] but this practice remains unproven. Because ureteral strictures can develop on therapy, the possible need for surgical intervention continues long after cultures for M. tuberculosis turn negative, and close follow-up with periodic radiologic assessment is mandatory (see the previous discussion). Less commonly, patients whose bladders have been badly scarred by the tuberculosis process have such poor bladder function that bladder augmentation or even urinary diversion may be necessary to deal with unbearable urinary frequency, inadequate emptying, or both.[155,156]

Nephrectomy for the nonfunctioning end-stage tuberculous kidney,[152] once considered controversial, has gradually come to be recommended more routinely.[147,149,150] Nephrectomy is indicated for persistent microbiologic failure despite adequate chemotherapy, refractory pain, bacterial superinfection, severe symptomatic stone disease, or definite secondary hypertension. In patients with elevated surgical risk by virtue of age, underlying illness, etc., conservative management may be considered, but more generally nephrectomy is offered in this situation.

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CHAPTER 28

Epidemiology, Diagnosis, and Therapy of Acute Kidney Injury

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EPIDEMIOLOGY OF ACUTE KIDNEY INJURY

Acute kidney injury (AKI) is a complex syndrome associated with several etiologic factors. AKI occurs in a variety of settings with clinical manifestations ranging from a minimal elevation in serum creatinine (SCr) to anuric kidney failure.[1–3] The incidence of AKI with and without need for dialysis has been progressively increasing in the last 15 years[4] (Fig. 28.1) and is more pronounced in older hospitalized patients. Data from the USRDS shows that 1.6% of patients age 66 and older who were continuously enrolled in Medicare inpatient/outpatient had the diagnosis of AKI[4,5] (Fig. 28.2). The increasing incidence can also be explained by the growing awareness by the medical community about AKI as a main risk factor for mortality and an important contributor for chronic kidney disease (CKD).

The reported incidence of AKI is widely variable in different regions of the world (Table 28.1). In the developed world AKI is seldom a community-acquired disease; the condition develops primarily in hospitalized patients. In these regions the incidence of hospital-acquired AKI exceeds that of community-acquired AKI by 5 to 10 times, having an estimated yearly incidence of 0.15% to 7.2%.[6] In the developed world more than 20% of AKI cases occur in the intensive care unit (ICU) setting. On the other hand, in the developing world a number of cases of AKI are found in rural areas. The demographics, etiologies, and outcomes of AKI in rural settings differ from those in more developed areas. In rural regions AKI is caused predominately by snake, spider, caterpillar, or bee envenomations, or by specific infections such as leptospirosis, tetanus, or severe malaria. Those patients are managed by primary caregivers who have limited resources.

The age of AKI patients is another difference between developed and developing countries. Elderly patients predominate in the developed world whereas in the developing world AKI is generally a disease of the young. Children are more often affected in developing countries, constituting more than 15% of patients in some studies. Age differences might partially account for differences in reported survival rates between the developed and developing countries. Paradoxically, patients in developing countries might have a better chance of survival. Here younger patients develop AKI as a result of a single disease (e.g., leptospirosis or malaria) rather than from multiple organ failure, but are more prone to complications secondary to poor nutrition and resource availability (Table 28.1).

The first step for the nephrology community to better understand and quantify the clinical importance of AKI was to develop a uniform definition. Before 2004 acute renal failure (ARF) had no accepted definition resulting in more than 30 different definitions reported in the literature.[7] Consequently, epidemiologic studies used different clinical and physiologic endpoints making it difficult to compare the results between studies (Table 28.1). This lack of a uniform definition yielded discrepancies in AKI incidence, prevalence, and outcomes in various clinical settings. The reported incidence ranged from 1% to 31% and mortality from 28% to 82%.[8–10] The formation of the Acute Dialysis Quality Initiative (ADQI) group in 2000 was the beginning of a process to establish consensus and evidence based guidelines in ARF. In 2004, ADQI formulated the Risk, Injury, Failure, Loss, and End-stage Kidney (RIFLE) classification for ARF.[7] The RIFLE classification system provides three grades of severity for AKI based on the maximal increase in SCr or decrease in urine output from the baseline condition: injury—risk (class R), injury (class I), and failure (class F)—and two outcome classes (loss and end-stage renal disease [ESRD]) (Table 28.2).

The clinical predictive ability of the RIFLE classification has been extensively validated in large general and ICU populations.[8,11–15] In a single center retrospective cohort study, including 5,383 patients admitted during a 1-year period, the incidence of AKI was 67.2%. In that cohort AKI was associated with an increased risk for hospital mortality compared to those who never developed AKI. The higher incidence that might have been considered unusual before the RIFLE era was confirmed in other studies that applied the criteria.[8,11–15]

The ADQI group, associated with representatives of nephrology societies (ASN, ISN, and NKF) and the European

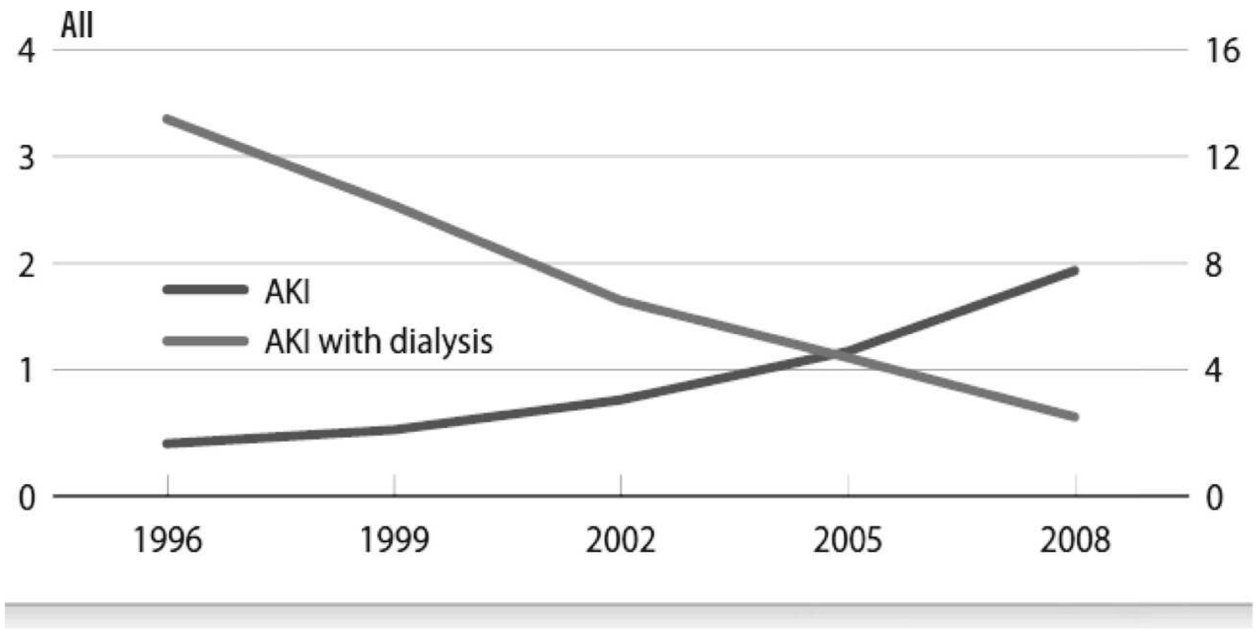


FIGURE 28.1 Hospitalizations for acute kidney injury, with or without dialysis. (Modified from Collins AJ, Foley RN, Herzog C, et al. Excerpts from the US Renal Data System 2009 Annual Data Report. *Am J Kidney Dis.* 2010;55(1 Suppl 1):S1–420, A426–427.)

Society of Intensive Care Medicine, created the Acute Kidney Injury Network (AKIN) as an independent collaborative network intended to facilitate international, interdisciplinary, and intersocietal collaborations.[16] One of the tasks proposed by the AKIN was to further refine the AKI definition. In 2007, a modified version of the RIFLE classification was published, also known as the AKIN classification (Table 28.2).[17] The terms Risk, Injury, and Failure were replaced by stages 1, 2, and 3, respectively. An absolute increase in creatinine of at least 0.3 mg per dL was added to stage 1. Patients starting renal replacement therapy (RRT) are automatically classified as stage 3, regardless of their SCr or urine output. The outcome categories Loss and ESRD were eliminated. Another difference between RIFLE and the AKIN classification is the 48-hour time frame within which the diagnosis of AKI is made, "AKIN criteria . . . change in creatinine should occur within 48h."[17] However, after the diagnosis is established, staging should be applied with no time frame constraint. Additionally, the glomerular filtration rate (GFR) criteria were eliminated.

In the RIFLE and AKIN classification systems patients are classified based on the worst category achieved. This is intended to describe the change or trend in AKI severity over time. Several studies in the ICU population have validated the concept verified in clinical practice that patient outcome progressively worsens with the maximal severity of AKI achieved.[8,12,14,18–20] Over 71,000 patients were included in published studies with the RIFLE classification system; these studies showed a stepwise increase in relative risk (RR) for death going from Risk (RR: 2.40) to Injury (RR: 4.15) to Failure (6.37).[21] Osterman and Chang[22] performed a retrospective analysis of a database of 41,972 patients admitted to ICU. AKI based on RIFLE occurred in 35.8% of patients: 17.2% Risk, 11% Injury, and 7.6% Failure. Patients with Risk, Injury, and Failure had a hospital mortality of 20.9%, 45.6%, and 56.8%, respectively, compared to 8.4% among non-AKI patients. Abosaif et al.[23] retrospectively applied the RIFLE classification in order to evaluate its sensitivity and specificity to predict renal and patient outcomes in 183 critically ill patients with AKI. Mortality rate in the ICU (60 days, 74.4%) and 6-month mortality rate (86%) were significantly greater in the RIFLE-Failure group compared with all groups. Cruz et al.[15] conducted a prospective multicenter study in 19 ICUs in northeastern Italy. Of 2,164 ICU patients who were admitted during the study period, 234 (10.8%) developed AKI whereas 3.3% were treated with RRT. Of the AKI patients, 19% were classified as Risk, 35% as Injury, and 46% as Failure. Overall, ICU mortality was higher among those in RIFLE class Failure (49.5% vs. 20% in R, 29.3% in I).

Hoste and colleagues[14] performed a retrospective single-center study on 5,383 patients admitted during a 1-year period in seven ICUs. AKI occurred in 67% of ICU admissions, and 12% reached a maximum RIFLE class of Risk, 27% Injury, and 28% Failure. Interestingly, among the patients that reached a level of Risk, 56% progressed to either Injury or Failure. Patients with maximum RIFLE class Risk, Injury, and Failure had hospital mortality rates of 8.8%, 11.4%, and

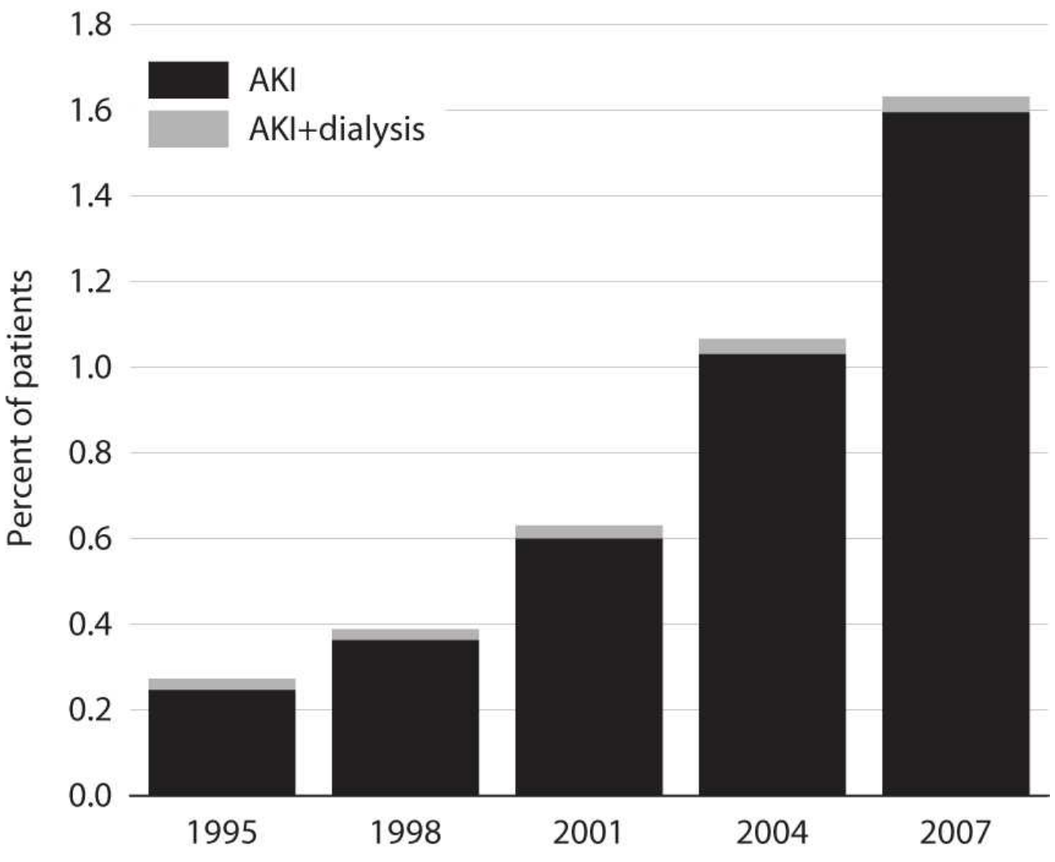


FIGURE 28.2 General Medicare patients age 66 and older continuously enrolled in Medicare inpatient/outpatient, surviving and without end-stage renal disease. (From Collins AJ, Foley RN, Herzog C, et al. United States Renal Data System 2008 Annual Data Report. *Am J Kidney Dis.* 2009;53(1 Suppl):S1–374.)

28.1 Epidemiologic Population Studies of Acute Renal Failure

Country	Definitions of ARF	Incidence (pmp)	Reference
USA	Increase in serum creatinine of 0.5 mg/dL (44.2 mol/L) in patients with baseline serum creatinine <1.9 mg/dL (168.0 mol/L) Increase in serum creatinine >1.0 mg/dL (88.4 mol/L) in patients with baseline serum creatinine 2.0–4.9 mg/dL (176.9– 433.2 mol/L) Increase in serum creatinine of 1.5 mg/dL (132.6 mol/L) in patients with baseline serum creatinine >5 mg/dL (442.0 mol/L)	1% of all hospital admissions All ARF was acquired out of hospital	251
Kuwait	Unknown	95	252
England	Serum creatinine >500 mol/L (5.7 mg/dL) Need for RRT	175 22	253
France	Unknown	104	254
Spain	Sudden increase of serum creatinine >177.0 mol/L (2.0 mg/dL) or sudden increase in serum creatinine >50% when prior renal function was normal or mild CKD was present Need for RRT	209[a] 57	255
Scotland	Serum creatinine 300 mol/L (3.4 mg/dL) Serum creatinine 500 mol/L (5.7 mg/dL) Need for RRT	620 102 50	256
USA (African Americans)	Serum creatinine 2 mg/dL (176.8 mol/L) without renal disease	5/1,000[b] hospital admissions	257
Australia	Need for RRT and mostly critically ill	135	258
England	Serum creatinine 300 mol/L (3.4 mg/dL)	486	259
Scotland	Need for RRT	203	260
South India	Unknown	336	261
Brazil	Increase in serum creatinine of at least 0.5 mg/dL (44.2 mol/L), admission serum creatinine >1.4 mg/dL (123.8 mol/L) for men or >1.3 mg/dL (114.9 mol/L) for women, and a normal serum creatinine level at admission, but presenting an increase during hospitalization	325/3,684 renal evaluations[c]	262
England	Serum creatinine 500 mol/L (5.7 mg/dL) or need for RRT Multiorgan ARF Single-organ ARF	380 125	263

[a]50% of ARF occurred before admission to hospital, 50% developed in hospital.
[b]Represents 79% of all ARF cases per year in one hospital.
[c]53% community-acquired ARF, 47% hospital-acquired ARF.
ARF, acute renal failure; CKD, chronic kidney disease; pmp, per million people; RRT, renal replacement therapy.
From Lameire N, Van Biesen W, Vanholder R. The changing epidemiology of acute renal failure. Nat Clin Pract Nephrol. 2006;2(7):364–377.

28.2 RIFLE and AKIN Classification Systems

Stage	RIFLE	Serum Creatinine Criteria	Urine Output Criteria
1	R	Increase to ≥150%–200% from baseline (AKIN and RIFLE)[a] or increase in serum creatinine >0.3 mg/dL (AKIN)	Less than 0.5 mL/kg/h for more than 6 h
2	I	Increase in serum creatinine >200%–300% from reference	Less than 0.5 mL/kg/h for more than 12 h
3	F	Increase in serum creatinine >300% from reference	Less than 0.3 mL/kg/h for 24 h or anuria for 12 h

[a]Within 48 hours for AKIN criteria, within 7 days for RIFLE criteria.

26.3%, respectively, in contrast to 5.5% in non-AKI patients. RIFLE classes were still associated with hospital mortality after adjusting for multiple covariates (baseline severity of illness, case mix, race, gender, and age). These findings showed that patients with RIFLE-Risk are indeed at significant risk of progression to more severe AKI. Patients with RIFLE class Injury or Failure incur a significantly increased length of stay and an increased risk of in-hospital mortality compared with those who do not progress past Risk or those who never develop AKI.

The increasing severity of illness in critically ill patients with AKI is one of the contributors to the persistently high mortality rate associated with this syndrome. Observational studies suggest that critically ill patients with AKI are increasingly older, have more comorbid diseases, have a higher incidence of septic, and have greater severity of illness and organ failure scores. Two severity of illness scoring systems are widely used: the Acute Physiology and Chronic Health Evaluation (APACHE) score and the Sequential Organ Failure Assessment (SOFA) score.[24,25] Although patients developing AKI have shown a significant decrease in mortality rate in the last decade,[25–27] the persistently high mortality in patients with multiorgan system failure remains a challenge. This emphasizes the need for early assessment and intervention in all cases of AKI. It is apparent that AKI is associated with substantial morbidity, mortality, and cost.

The financial costs of AKI are high. Fischer et al.[28] performed a multicenter analysis in 23 Massachusetts hospitals for a 2-year period (1999 to 2000). They identified 2,252 records of patients hospitalized with uncomplicated ARF. Patients hospitalized with uncomplicated ARF incurred median direct hospital costs of $2,600, median hospital length of stay (LOS) of 5 days, and mortality of 8%. Dialysis was independently associated with significantly greater hospital costs and LOS for patients with uncomplicated ARF ($P < 0.05$) compared to patients with other commom medical diagnoses.[28]

The cost of RRT for patients with AKI is high; however, information on the costs of the three dialytic techniques for AKI is minimal. In a Canadian ICU, the cost of dialysis was $3,486 to $5,117 (Canadian) per week for continuous renal replacement therapy (CRRT).[29] For intermittent hemodialysis (IHD), major costs include the need for supervision by a trained dialysis nurse, which can become an economic issue if IHD is performed on a frequent or daily basis. For CRRT, major costs include disposables and replacement fluids. Most investigators have found that CRRT costs are somewhat greater than IHD.[30,31] An evaluation of total hospital costs[32] showed that from the start of RRT to hospital discharge patients on CRRT total cost was US$57,000 more than that for those on IHD. A recent cost analysis of the of RRT for patients with AKI estimated that mean adjusted total costs were U$1,342/week for IHD compared to U$3,486/week for CRRT[30] and no difference was found in the outcome, renal recovery at hospital discharge. However, there was a nonsignificant statistical trend toward enhanced renal recovery in the CRRT group despite a significantly lower mean arterial pressure and a trend toward higher Acute Physiology and Chronic Health Evaluation (APACHE) II scores. Considering that nonrecovery of renal function would adversely affect quality of life, a modality that enhanced the rate of renal recovery would offer an important advantage, even if there were no difference in survival across modalities.

Parameters for Acute Kidney Injury Diagnosis

Serum Creatinine

Therapeutic interventions are generally based on an evaluation of clinical data and diagnostic information. In AKI the importance of timing of diagnosis was poorly appreciated as therapeutic interventions have generally been lacking, and the small number of studies reported have failed to improve

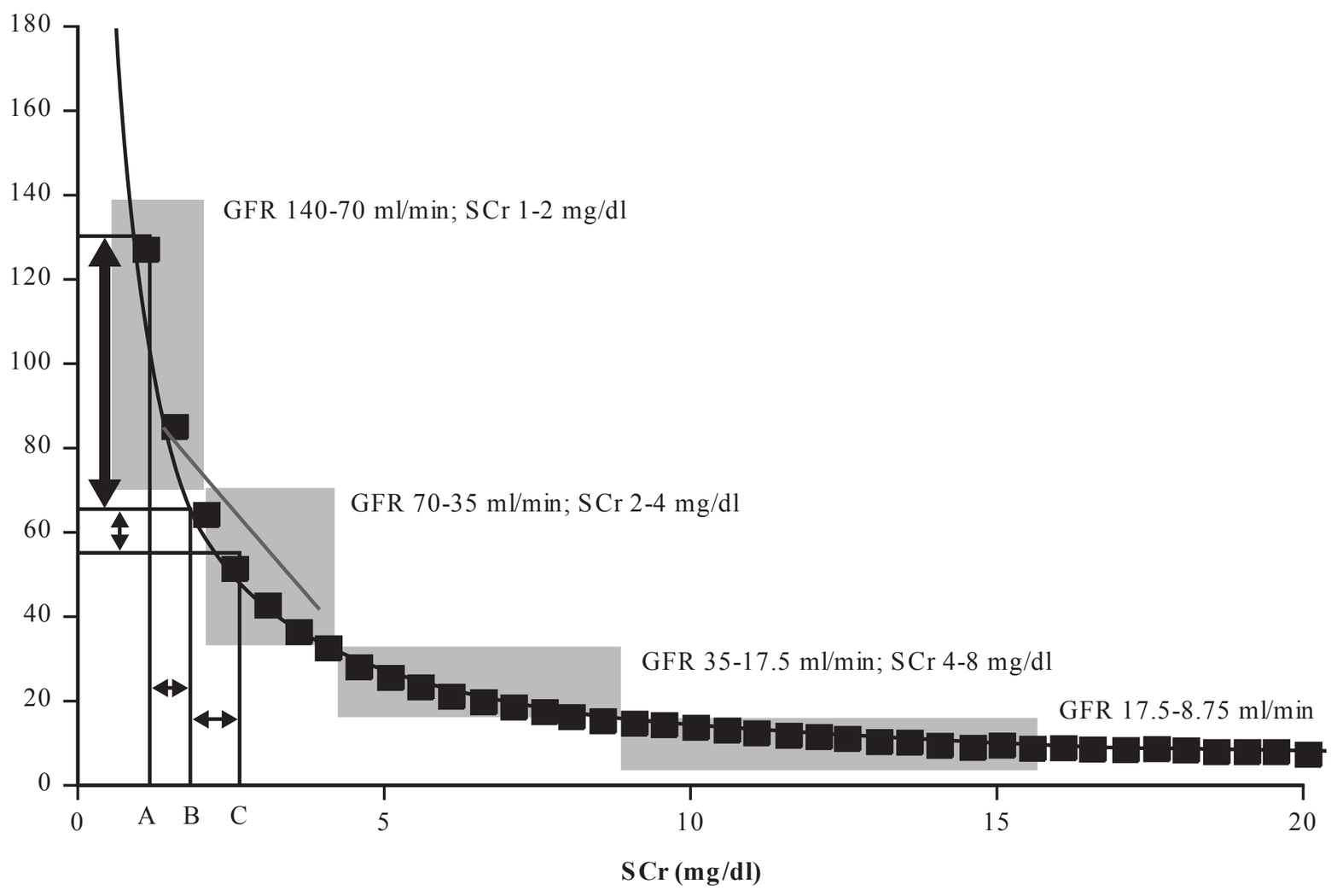
outcomes. In the last decade, the concept of interventions based on "windows of opportunity" coupled with targeted therapy became evident in other ischemic events such as acute chest pain syndromes and stroke. The importance of finding early diagnostic information in AKI has since been highlighted and the development of technology has facilitated the search for new biomarkers of kidney injury.

The current criteria for AKI diagnosis and classification, RIFLE and AKIN, are still based on SCr incremental concentrations and decreased urine output. Many characteristics other than renal function, such as age, muscle mass, catabolic rate, and race, influence SCr concentrations. In addition, SCr levels depend not only on renal elimination but also on creatinine generation and volume of distribution.[33] Given the exponential relation of SCr and GFR, significant decreases in GFR are reflected as small increases in SCr in the early phases of injury (Fig. 28.3).

In a steady state setting a reasonable approximation is that each time the GFR halves, the SCr concentration doubles. Thus, steady state GFRs of 100, 50, 25, 12.5, and 6.25 mL per minute are associated with increasing SCr concentrations ranging from 1 to 16 mg per dL; however, there is wide range depending on the level of extrarenal clearance that is accentuated as kidney function declines and may contribute to up to 40% of total clearance. AKI often occurs in a nonsteady state in which the three determinants of SCr concentration (production, volume of distribution, and renal elimination) fluctuate.[33] Computerized models derived from AKI patients demonstrate that several patterns of change in GFR occur during development and recovery from AKI. These GFR changes are poorly reflected by daily changes in SCr concentration.[33] Moreover, the rise in SCr that occurs in AKI is a post facto finding. In critically ill patients, a nonsteady state condition and the positive cumulative fluid balance enhances the insensitivity of SCr as a parameter of renal dysfunction.[34] Hoste et al. showed that in a group of recently admitted ICU patients with normal SCr the 1-hour urinary creatinine clearance revealed values lower than 80 mL/min/1.73 m^2 in 46.2% of the patients.[35] These data suggest that SCr is not a reliable tool to detect even moderate kidney dysfunction in AKI patients.

Blood Urea Nitrogen

Blood urea nitrogen (BUN) is also used as a parameter to evaluate renal function. However, elevations in BUN level are often, but not always, due to a decrease in GFR. Some factors enhance urea production, such as gastrointestinal bleeding, corticosteroid therapy, and high-protein diet. In conditions of decreased intravascular effective volume like decompensated heart failure, increases in BUN are not proportional to the rise in SCr level and fall in GFR. The usual BUN:SCr ratio is about 10:1 and the BUN and SCr increase by 10 to 15 and 1.0 to 1.5 mg/dL/day, respectively, in the absence of GFR. Increases in the basal metabolic rate that occur with fever or glucocorticoid administration enhance these daily rates. Although an increase in the BUN/



GFR, glomerular filtration rate; SCr, serum creatinine

FIGURE 28.3 Relationship between serum creatinine and glomerular filtration rate (GFR). Changes in serum creatinine represent smaller changes in GFR as renal functions decreases. Creatinine increase from A to B corresponds to a GFR decrease of GFR of 130 to 70 mL per minute, whereas from B to C the same delta creatinine corresponds to a decrease in GFR from 70 to 50 mL per minute. (Modified from Finn WF. The clinical and renal consequences of contrast-induced nephropathy. *Nephrol Dial Transplant*. 2006, 21(6):i2-i10.)

creatinine ratio has been used for many years to help to differentiate between prerenal and renal azotemia, BUN can increase independently from SCr in situations characterized by decreased glomerular perfusion pressure such as heart failure. Some studies have already shown elevations in BUN independently from SCr levels and demonstrated that these two parameters are not only a reflection of the severity of renal dysfunction, but rather the consequence of two distinct pathologic processes.[36–38] The activation of renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system is responsible for decreasing the glomerular perfusion pressure and GFR. The increment in vasopressin levels upregulates aquaporin-2 and urea transporter expressions and increases water and urea reabsorption. Urea, in contrast to SCr, is not secreted but reabsorbed by the renal tubules. The increased reabsorption of sodium and water, rather than the reduced GFR, enhances reabsorption of urea and increases BUN levels. Thus, BUN levels and BUN/creatinine ratio could be a more effective way to assess circulatory volume than GFR, which is regulated by the pressure difference between glomerular afferent and efferent arterioles.[39] In heart failure and possibly in other settings where underfilling is part of the physiopathologic process, the rise in BUN greater than any fall in GFR is a marker of the neurohumoral axis activation.[40]

In selected circumstances, it may not be clear if an elevated BUN:SCr ratio is due to an acute or chronic process. In this circumstance review of previous records is helpful.

Oliguria

Although the hydration status, osmolar excretion, as well as a large dose of diuretics will influence urine volume, and severe AKI can occur with normal urine output, the urinary flow rate also may provide helpful information about the cause of AKI. Sustained periods of anuria suggest urinary tract obstruction as the cause of AKI. Other rare causes of anuria include rapidly progressive glomerulonephritis, mechanical occlusion of renal blood flow, and diffuse renal cortical necrosis. Nonoliguric varieties of AKI are common, ranging from 25% to 80% of all cases of AKI with 33% nonoliguric at AKI diagnosis.[41–43] The nonoliguric state may be present in all types of AKI including those following surgery, trauma, hypotension, nephrotoxins, and rhabdomyolysis. Several factors may contribute to the devlopment of nonoliguric AKI: use of volume expansion, high-dose potent diuretic agents, high osmolar loads, and renal vasodilators. Another contributory factor is aggressive fluid resuscitation and improved supportive management of critically ill patients. However, nonoliguria may mask the underlying severity of AKI and lead to delayed recognition.

The definition of oliguria changed after the RIFLE and AKIN classification system. The AKIN group proposed the hourly measurement of urine volume, providing an opportunity to treat urine flow as a continuous rather than as an interval physiologic variable, with more time points for the diagnosis of oliguria and detection of AKI. Although fluctuations in this parameter can result from external influences, such as drug administration, the pattern of change can be detected earlier with more frequent observations. Experimental studies conducted in laboratory models of AKI, as well as clinical studies, have clarified the pathophysiologic aspects underlying the variations of urine flow rate in AKI.[44,45] In a study of 25 patients with predominantly renal ischemia-associated AKI, Rahman and Conger found that the urine flow rate strongly correlated with residual GFR.[45] In that study urine flow rate did not correlate with selected aspects of renal tubular function such as urine:SCr or the fractional excretion of sodium.

However, because of the difficulties in measuring and recording the hourly urine output, the AKIN oliguria criterion was evaluated in fewer studies than the SCr criterion. Of the studies evaluating the urine output criterion most were retrospective and used a modified definition of evaluating urine volume in 2 to 12 or 24 hours and shortening the time of observation to the first 24 hours of ICU admission or postoperative period. In a retrospective study Barrantes et al.[46] evaluated the outcomes of hospitalized patients classified by the AKIN criteria, using both the urine output (UO) and SCr criteria in the first 48 hours after ICU admission. Comparing patients that developed AKI using the UO, SCr, or both criteria, they found that UO criterion did not affect the AKI associated mortality. Joannidis et al.,[47] using the SAPS 3 database of ICU patients, assessed 24-hour urine volume for 48 hours. Patients with AKI defined by the lowest urine volume had higher mortality compared to non-AKI patients. In a prospective cohort of cardiac surgery patients, Haase et al.[48] subclassified the AKI patients based exclusively in the UO criterion (over a 48-hour period). Only those patients that reached a RIFLE-F or AKIN stage 3 had significantly longer ICU and hospital lengths of stay and a higher mortality rate (compared to non-AKI patients and those with maximum RIFLE-R/I and AKIN stage 1/2). In a systematic review,[21] the relative risk for death among studies that used both SCr and UO criteria was lower than in those using only the SCr criterion. Hoste and Kellum,[19] in a review including 10 studies, showed that patients in the RIFLE Risk class defined by the SCr criterion were more severely ill than those in the same class defined only by the UO criterion. Hoste et al.[14] also observed that patients in Failure based on the RIFLE GFR criterion had a slightly higher mortality than those in Failure based on the UO criterion. In a study by Cruz et al., RIFLE classes (using creatinine and UO criteria together) were the strongest predictor of ICU mortality in multivariable analysis.[15] In that study, the analysis was based only on the SCr criterion; the RIFLE class was sustained as an independent predictor of ICU mortality but with inferior statistical power. Based only on the UO criterion, RIFLE class did not emerge as an independent predictor.

These clinical observations, and a large body of experimental data, suggest the residual level of GFR is the primary determinant of urine flow in patients with AKI. The higher level of residual GFR in nonoliguric patients is

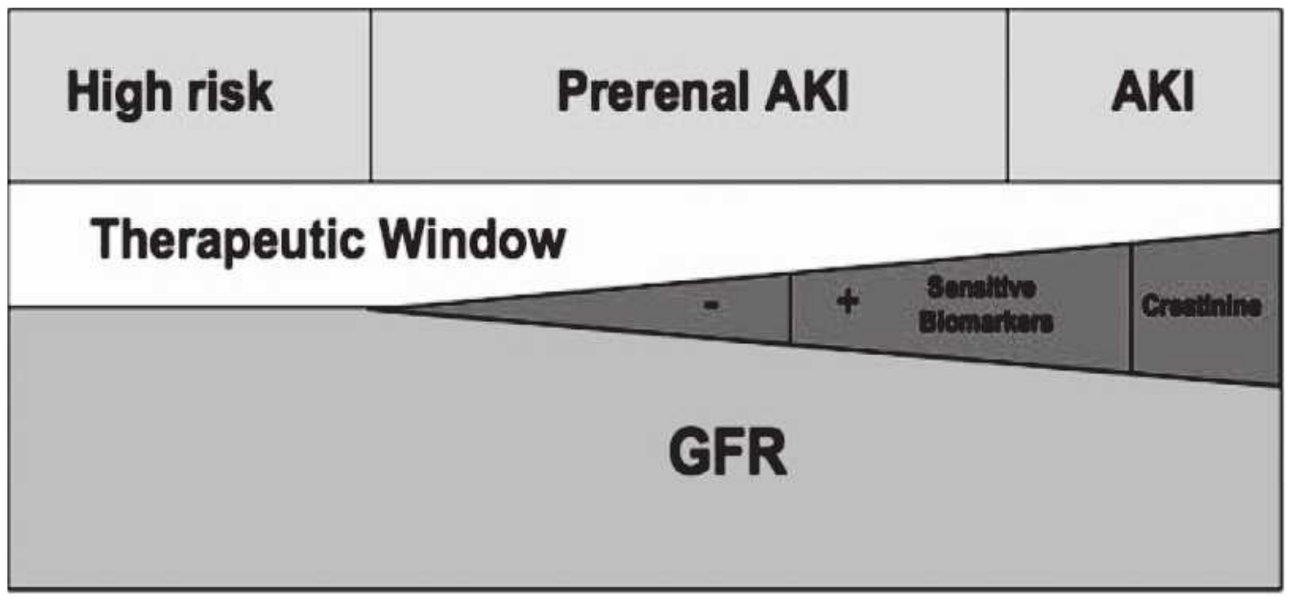


FIGURE 28.4 Time frame association for assessment of risk and early detection of acute kidney injury. Time runs along the *x*-axis, and the figure depicts a closing "therapeutic window" as injury evolves and kidney function worsens. Biomarkers of injury and function will begin to manifest as the condition worsens, but traditional markers of function (e.g., urea nitrogen and creatinine) will lag behind hypothetical "sensitive" markers of kidney injury. Mortality increases as kidney function declines. (From Himmelfarb J, Joannidis M, Molitoris B, et al. Evaluation and initial management of acute kidney injury. *Clin J Am Soc Nephrol.* 2008;3(4):962–967.)

compatible with improved survival and lower morbidity in these patients. However, one needs to be aware that there is a distinction between spontaneous nonoliguria from diuretic induced urine flow with respect to underlying GFR and nonoliguria does not automatically imply a higher GFR.

Biomarkers

The importance of early detection of AKI has been emphasized as an earlier diagnosis would provide a wider window to perform supportive and therapeutic interventions (Fig. 28.4). The recognition of the insensitivity to detect AKI by the most commonly used surrogates of kidney function (SCr and UO) has led to extensive efforts to identify alternative biomarkers for AKI diagnosis, including urine and serum biomarkers. Compared to the use of more sensitive biomarkers of kidney injury, SCr delays the AKI diagnosis by 48 to 72 hours.[49–52] Most of the studies have focused on the ability of these biomarkers to detect AKI earlier than the classical parameters, but biomarkers may be also useful to predict the course and prognosis of AKI (Fig. 28.5). Several promising candidates have emerged, demonstrating reasonable diagnostic performance for AKI up to 48 hours prior to a significant change in SCr.[53,54] Although the commercial platforms are becoming available for research use, the knowledge for the clinical application, utility, and diagnostic value of these early biomarkers remains to be validated. These new candidates are being tested in different AKI clinical scenarios. The different abilities to detect renal injury and estimate GFR are associated with their pathophysiology (Fig. 28.6). AKI biomarkers differ on the basis of how they

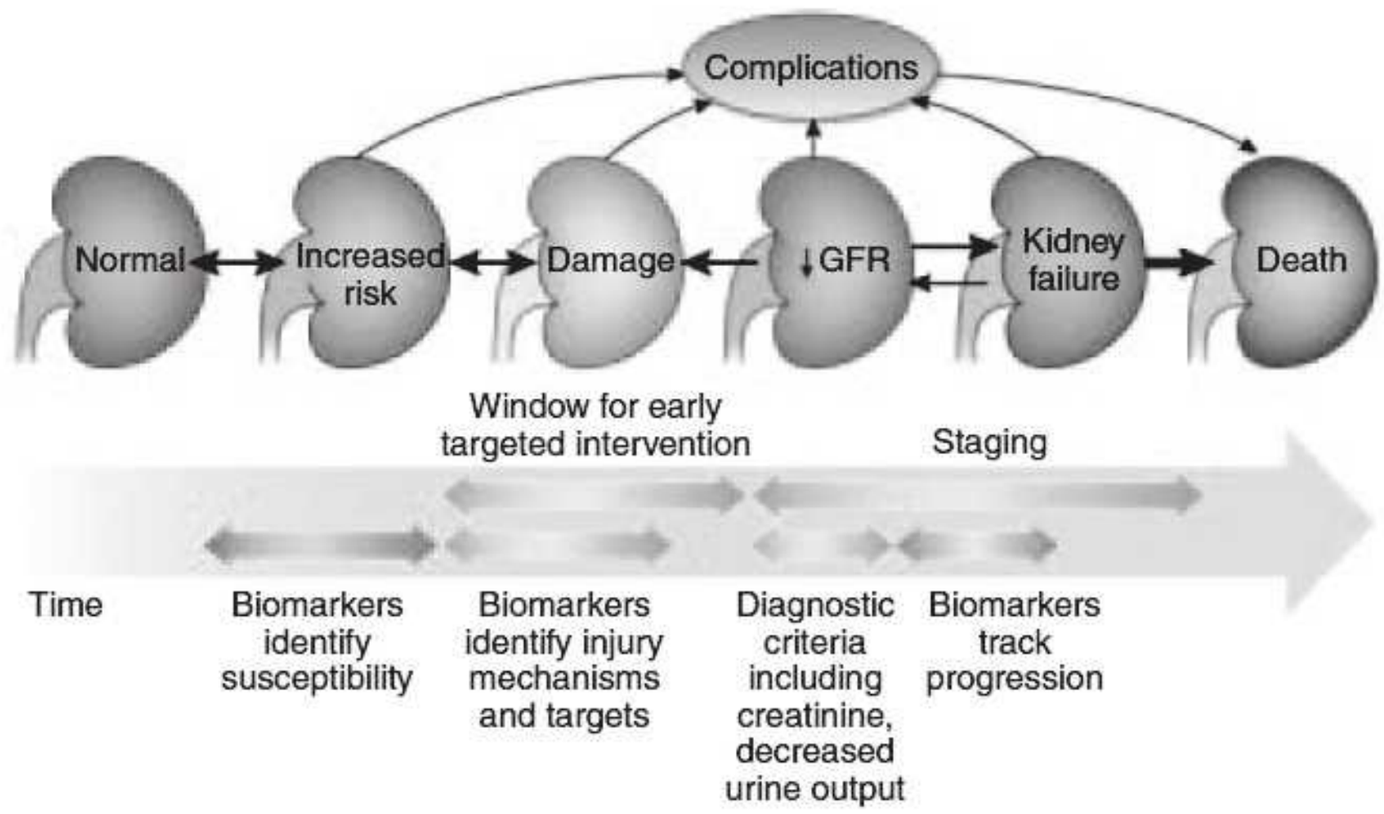


FIGURE 28.5 Conceptual framework for acute kidney injury. Surveillance could be initiated for high-risk individuals on the basis of clinical and biomarker criteria. Sequential assessment of biomarkers may permit identification of a window of opportunity in which kidney injury has been initiated but has not progressed to renal functional change. The duration of this window is inherently dependent on the type and site of injury and the nature and specificity of the biomarkers to determine the targets for intervention. Progression of kidney injury would be determined by development of functional changes staged on the basis of the severity of kidney injury. Biomarkers could further define progression, determine need for additional interventions, and predict prognosis. *GFR*, glomerular filtration rate. (Modified from Mehta RL. Timed and targeted therapy for acute kidney injury: a glimpse of the future. *Kidney Int.* 2010;77(11):947–949.)

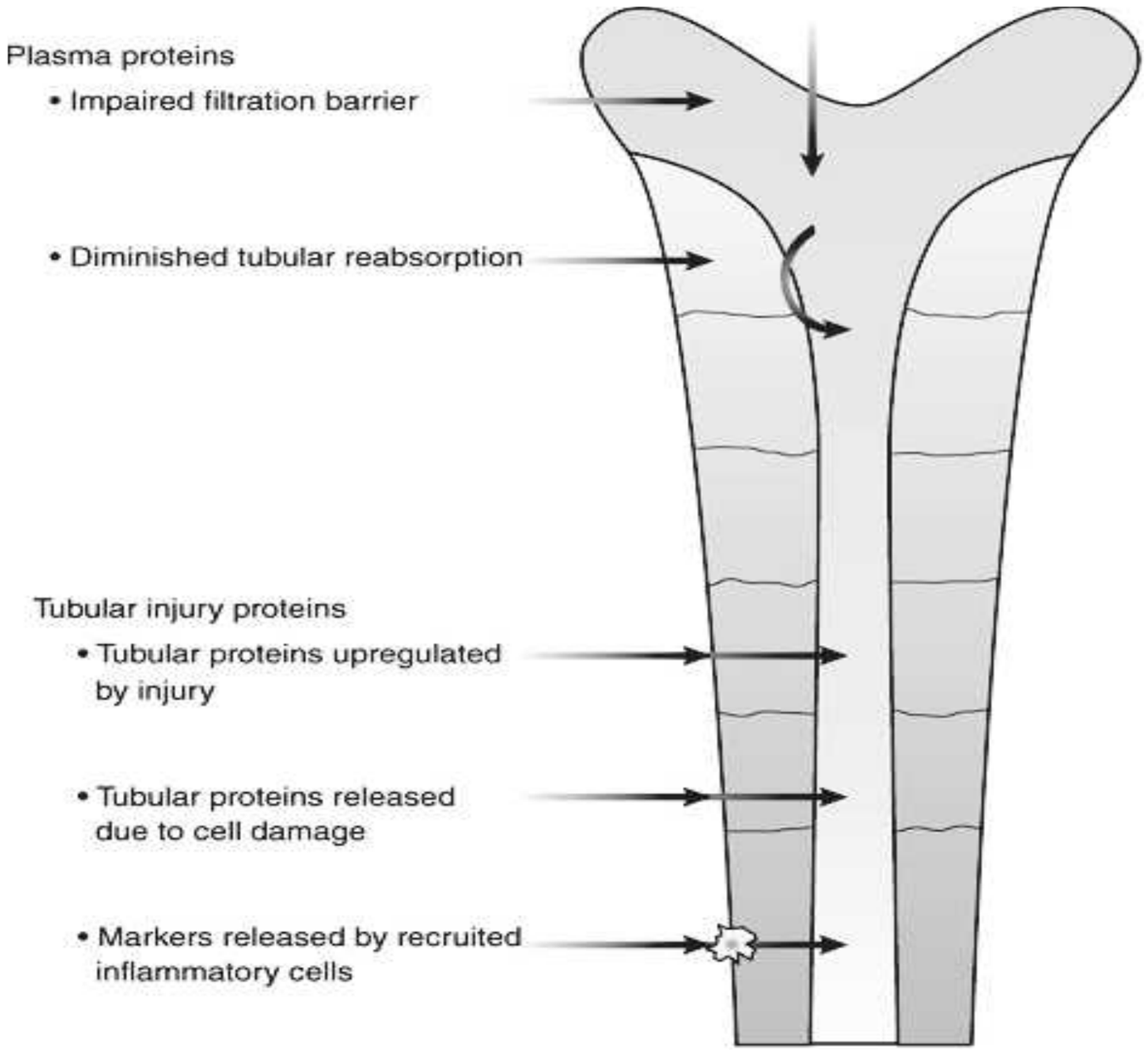


FIGURE 28.6 Pathophysiology of urinary biomarkers. Schematic representation of the mechanisms by which proteins escape into the urine. (Adapted from Briggs JP. The hunt for the perfect biomarker for acute kidney injury: back to gamma-trace? *Kidney Int.* 2008;74(8):987–989.)

enter the urine, either through filtration, upregulation, and secretion or through leakage from damage of a constitutive marker. The time sequence and magnitude of biomarker elevations are unique and may depend on the nature and severity of injury.

In addition to potentially facilitating an earlier diagnosis, these biomarkers will also allow for retesting preventive and therapeutic drugs that have failed in clinical trials that used SCr as a parameter to guide intervention. Another area of possible application for the new biomarkers is assisting in decisions to initiate RRT in patients with AKI. The main biomarkers being tested and some clinical data on their use are summarized below.

Cystatin C

Cystatin C is a low molecular weight protein produced at a constant rate by all nucleated cells. Because of its low molecular weight it is freely filtered at the glomerulus and reabsorbed and catabolized, but not secreted by the renal tubule. Plasma levels correlate with GFR and, unlike creatinine, are not significantly affected by age, gender, race, or muscle mass. In human studies, both pCyC and uCyC have been shown to predict AKI, although its superiority over SCr has not been a universal finding.[55–57] Recently, Herget-Rosenthal et al.[58] compared cystatin C with creatinine for the diagnosis of AKI in a series of 85 patients. Increases in serum levels of cystatin C were detectable 1 to 2 days earlier than comparable changes in SCr. In another study measuring plasma Cr (PCr) and pCyC in 444 adults on ICU admission, of whom 124 already had AKI on entry, pCyC moderately predicted death or RRT (area under curve [AUC] 0.61, 95% confidence interval [CI], 0.53–0.68) and performed similarly as PCr (AUC 0.60, 95% CI, 0.51–0.67).[59] In a cohort of 151 AKI patients from a small multicenter study, Royakkers et al.[60] used the RIFLE classification system to define AKI and compare the performance of sCyC and uCyC as early biomarkers for AKI. Urinary CyC had no diagnostic value during the days prior to AKI diagnosis by SCr (AUC <0.50). In addtion, sCyC and uCyC determined on the first day of AKI diagnosis were poor predictors for the need for RRT (AUC $= 0.66$).[60]

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kD protein, originally characterized in and secreted by neutrophils, that is bound to gelatinase. The physiologic role of NGAL in the kidney is unknown; however, it is believed to play a role in renal morphogenesis.[61] High throughput functional genomic studies have identified NGAL as one of the most upregulated transcripts in whole kidney tissue very early after acute injury. Downstream proteonomic studies using animal models have also revealed the 25-kD NGAL protein to be one of the earliest and most robustly produced proteins in the kidney after ischemic or nephrotoxic AKI.[62] Several clinical studies have suggested that urine NGAL expression may serve as an early marker of AKI.[63–66] In a clinical trial of 71 children who underwent cardiac surgery, urinary NGAL increased within 2 hours of cardiopulmonary bypass to a level of >50 μg per L in all 20 children who had

an increase in SCr of >50% (RIFLE Risk) and in only one of the 51 children who did not meet the RIFLE definition of AKI.[67] Urinary NGAL also was evaluated in adult patients who underwent cardiac surgery, with far less impressive results.[68] In a cohort of 81 such patients at a single institution, 16 developed AKI, as defined by RIFLE. Preoperative urinary NGAL levels were comparable among patients who did and did not develop AKI and were not significantly different immediately after surgery. However, within 1 hour after surgery, the urinary NGAL concentration began dropping in patients who did not develop AKI but continued to rise in patients with AKI, peaking at 3 hours and remaining elevated for 24 hours.[68] In one study evaluating urinary NGAL levels in emergency room patients, the AUC for NGAL to detect AKI (0.948) did not significantly differ from the curve for SCr (0.921). Nevertheless, there was very little overlap in NGAL values in patients with AKI and prerenal failure, whereas SCr values overlapped significantly in AKI patients and in those that reversed the condition within 48 hours.[64] Urinary NGAL was measured in hospitalized patients with established AKI at study inclusion and after 2 days. Of the 145 patients analyzed, 75 had intrinsic AKI, 32 had prerenal AKI, and 38 patients could not be classified. Urinary NGAL levels effectively discriminated between intrinsic and prerenal AKI (AUC 0.87). An NGAL level over 104 μg per L indicated intrinsic AKI (likelihood ratio 5.97), whereas an NGAL level of 47 μg per L made intrinsic AKI unlikely (likelihood ratio 0.2). A logistic regression analysis showed that NGAL independently predicted a composite outcome (worsening RIFLE severity class within 7 days, need for RRT, and in-hospital mortality) after correcting for demographics, comorbidities, creatinine, and RIFLE class. The authors concluded urinary NGAL was useful in classifying and stratifying patients with established AKI.[69]

Several studies have examined the clinical endpoint of RRT initiation using NGAL.[70] Urine or plasma/serum NGAL have been studied in various clinical settings including children and adults (Table 28.3). In a meta-analysis of studies that evaluated NGAL accuracy for diagnosis and prognosis, 1,948 patients from nine studies were included. The overall incidence of RRT was 4.3%, and the pooled analysis yielded an AUC of 0.782 (95% CI, 0.648–0.917) for discriminating patients who would receive RRT associated with AKI. For a cut-off in NGAL of 278 ng per mL, the sensitivity was 76% and specificity was 80%.[70] However, these studies included diverse patient populations, and the specimens and assays used in obtaining this estimate varied, making it difficult to translate the results to bedside clinical use.

N-acetyl-β-D-glucosaminidase (NAG)

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme (>130 kDa) that has been localized to lysosomes in several human cells including the renal tubules. The large molecular weight precludes glomerular filtration implying that urinary

28.3 NGAL for Prediction of Renal Replacement Therapy

Reference	Specimen	Population		RRT Endpoint	Results from Pooled Analysis
Cruz[268]	Plasma	ICU	Adults	15/301 (5%)	
Constantin[269]	Plasma	ICU	Adults	7/88 (8%)	
Wheeler[270]	Plasma	ICU	Pediatric	22/143 (15%)	
Nickolas[271]	Urine	Emergency room patients	Adults	12/541 (2%)	AUC 0.78 (95% CI, 0.65–0.92) Diagnostic odds ratio 12.9 (95% CI, 4.9–33.9) At cut-off 278 ng/mL, sensitivity 76%, specificity 80%
Koyner[272]	Plasma and urine	Cardiac surgery	Adults	7/72 (10%)	
Haase-Fielitz[273]	Serum	Cardiac surgery	Adults	4/100 (4%)	
Wagener[274]	Urine	Cardiac surgery	Adults	5/81 (6%)	
Wagener[275]	Urine	Cardiac surgery	Adults	8/426 (2%)	
Bennett[276]	Urine	Cardiac surgery	Pediatric	4/196 (2%)	

RRT, renal replacement therapy; ICU, intensive care unit; AUC, area under curve; CI, confidence interval.
Adapted from Haase M, Bellomo R, Devarajan P, et al. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis. 2009;54(6):1012–1024.

elevations are from tubular origin. Increased activity suggests injury to tubular cells or may reflect increased lysosomal activity. NAG catalyzes the hydrolysis of terminal glucose residues in glycoproteins and is the most active glycosidase found in proximal tubular epithelial cell lysosomes. Urinary NAG activity remains elevated during different kinds of active renal disease.[71] The diagnostic and prognostic ability of nine urinary biomarkers, including NAG, was evaluated in a cross-sectional study with 102 patients with established AKI and compared to 102 subjects without AKI.[72] The non-AKI subjects included healthy controls, ICU patients and subjects who underwent coronary angiography, whereas AKI patients were recruited at initial nephrology consultation. An age-adjusted analysis, using log-transformed biomarker values, showed NAG to be a significant predictor for RRT, mortality, and composite endpoint. The median normalized NAG level in AKI patients who underwent RRT was 0.06 U per mg Cr, versus 0.02 U per mg Cr in those who did not.

In another study including 635 patients presenting to the emergency room, urine NAG was not predictive of a composite outcome of nephrology consultation, ICU admission, RRT initiation, and mortality on multivariable analysis which included SCr and BUN.[64]

Kidney Injury Molecule-1 (KIM-1)

Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein with a cleavable ectodomain localized in the apical membrane of dilated tubules in acute and chronic injury. KIM-1 and its soluble ectodomain in urine (90 kDa) are believed to play a role in the regeneration processes after epithelial injury. It is undetectable in normal kidney tissue but expressed at very high levels in proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injury.[73] A small study in six patients with confirmed acute tubular necrosis (ATN) showed KIM-1 expression via immunohistochemistry on kidney biopsy. The same investigators subsequently examined urinary KIM-1 in 40 patients and found KIM-1 levels elevated to a higher degree in patients with ischemic acute tubular necrosis (ATN) compared to patients with contrast nephropathy, other forms of AKI, CKD patients, and normal controls.[74] In a cohort of adults undergoing coronary artery bypass graft (CABG), urinary KIM-1 levels were predictive of subsequent AKI (sensitivity 0.74, specificity 0.9, AUC 0.83) at 12 hours postsurgery. Other studies also suggest that urinary KIM-1 may be useful in identifying ischemic ATN.[49,75,76]

In a study by Liangos et al., the AUC for prediction of RRT or death for KIM-1 was 0.61 (95% CI, 0.53–0.61), comparable to that of SCr and UO.[77] On adjusted analysis, patients in the highest KIM-1 quartile had a 3.2-fold higher odds (95% CI, 1.4–7.4) for a composite outcome compared to patients with the lowest quartile. However, the result was no longer significant when adjusted for multiple factors. In another study by the same authors, KIM-1 was not a significant predictor for RRT, but was a significant predictor for mortality.[72]

Interleukin-18

Interleukin (IL)-18 also has been considered as a candidate biomarker for acute renal injury. IL-18 is a pro-inflammatory cytokine generated by caspase-1-mediated cleavage in injured proximal tubules and released into the urine.[78] It can also enter the urine by glomerular filtration. In animal models, IL-18 has been shown to exacerbate tubular necrosis, and neutralizing antibodies to IL-18 reduced renal ischemic injury in mice.[78] In humans urinary IL-18 levels were measured in 72 individuals, 14 with ATN, 8 with prerenal failure, 5 with urinary tract infections, 12 with CKD, 22 who received a kidney transplant, and 11 healthy control subjects. Patients with ATN had significantly higher urinary IL-18 levels as compared with control subjects and patients with other forms of kidney disease.[66] Similarly, patients who underwent transplantation and had delayed graft function had higher urinary IL-18 levels than patients with prompt graft function. Using samples collected on days 0, 1, and 3 of the Acute Respiratory Distress Syndrome (ARDS) Network trial, urine IL-18 levels of >100 pg per mL were associated with a 6.5-fold increased risk for development of AKI, defined by RIFLE.[52]

Many other factors beside GFR determine biomarker elevation: heterogeneity of clinical settings, underlying patient characteristics, severity of illness, and potential reasons for decreased renal function. It is more likely that a panel of biomarkers will provide a better understanding about the timing, nature, and the severity of an acute renal insult. Further work is clearly needed to inform the time course and performance of AKI biomarkers in various situations, to identify the pathways involved, to define clinical endpoints, including prerenal states, and to improve adjudication of biomarker data with respect to functional changes.[79]

DETERMINING REVERSIBILITY

Although epidemiologic studies have shown that even small increases in SCr are predictive of nonrenal outcomes, SCr remains an insensitive and delayed marker of AKI. A key unanswered question is whether reversible AKI or prerenal AKI is also associated with increased complications and worse outcomes. For decades the differentiation from intrinsic AKI and prerenal AKI was based on urine analysis and microscopy parameters, but could only be confirmed retrospectively by the reversibility of creatinine increase or improvent of urine output. In the era of the new biomarkers new paradigms may arise to answer this question.

Physiology of Reversibility

The traditional classification of AKI includes causes associated with a decrease in renal blood flow (Table 28.4), intrinsic renal parenchymal diseases (Table 28.5), or obstruction of urine flow (Table 28.6). Although obstructive AKI is usually easier to diagnose, prerenal and intrinsic renal causes can be difficult to distinguish in the clinical setting. Another

28.4 Causes of Reversible Acute Kidney Injury

Decreased Intravascular Fluid Volume
- Extracellular fluid loss—burns, diarrhea, vomiting, diuretics, salt-wasting renal disease, primary adrenal insufficiency, gastrointestinal hemorrhage
- Extracellular fluid sequestration—pancreatitis, burns, crush injury, nephrotic syndrome, malnutrition, advanced liver disease

Decreased Cardiac Output
- Myocardial dysfunction—myocardial infarction, arrhythmias, ischemic heart disease, cardiomyopathies, valvular disease, hypertensive disease, severe cor pulmonale

Peripheral Vasodilation
- Drugs—antihypertensive agents
- Sepsis
- Miscellaneous—adrenal cortical insufficiency, hypermagnesemia, hypercapnia, hypoxia

Severe Renal Vasoconstriction
- Sepsis
- Drugs—nonsteroidal anti-inflammatory agents, β-adrenergic agonists
- Hepatorenal syndrome

Mechanical Occlusion of Renal Arteries
- Thrombotic occlusion
- Miscellaneous (emboli, trauma [e.g. angioplasty])

28.5 Renal Causes of Acute Renal Failure

Renal Vascular Disorders
- Vasculitis
- Malignant hypertension
- Scleroderma
- Thrombotic thrombocytopenic purpura
- Hemolytic–uremic syndrome
- Disseminated intravascular coagulation
- Mechanical renal artery occlusion (surgery, emboli, thrombotic occlusion)
- Renal vein thrombosis

Glomerulonephritis
- Postinfectious
- Membranoproliferative
- Rapidly progressive glomerulonephritis (idiopathic, polyarteritis nodosa, systemic lupus erythematosus, Wegener syndrome, microscopic polyarteritis, Goodpasture syndrome, Henoch-Schönlein purpura)
- Drugs

Interstitial Nephritis
- Drugs (penicillin, sulfonamide, rifampin, ciprofloxacin, phenindiones, cimetidine, proton pump inhibitors [omeprazole, lansoprazole], azathioprine, phenytoin, captopril, thiazides, furosemide, bumetanide, allopurinol, nonsteroidal anti-inflammatory drugs including selective cyclooxygenase-2 inhibitors, 5-aminosalicylates)
- Hypercalcemia

Infections
- Nonspecific due to frank septicemia or systemic anti-inflammatory response syndrome
- Specific organisms (Legionella, Leptospira, Rickettsia, Hantavirus, Candida, malaria)
- Specific organ involvement (bacterial endocarditis, visceral abscess, pyelonephritis)

Infiltration
- Sarcoid
- Lymphoma
- Leukemia

Connective Tissue Disease

Tubular Necrosis
- Renal ischemia (prolonged prerenal)
- Nephrotoxins (aminoglycosides, radiocontrast agents, heavy metals, organic solvents, other antimicrobials)
- Pigmenturia (myoglobinuria, hemoglobinuria)
- Miscellaneous

way to approach AKI classification is to ascertain the possibility of reversibility.

Prerenal AKI has been accepted as a reversible form of renal dysfunction, caused by factors that compromise renal perfusion. The term has been used as part of a dynamic process that begins with a reversible condition, the prerenal state, and can progress to an established disease, ATN. Experimental models have largely informed our current understanding of the physiology of the kidney injury associated with prerenal failure. Before the onset of clinically evident prerenal azotemia, the kidney passes through a phase of remarkable compensation called pre-prerenal azotemia.[80] Three main steps are involved in this compensatory mechanism: (1) the cardiac output fraction that reaches the kidney; (2) plasma filtration by the glomerulus (filtration fraction); and (3) proportion of the glomerular filtrate that is reabsorbed by the tubules. Renal blood flow (RBF) depends on the tone of renal vascular resistance (RVR) in relation to systemic vascular resistance (SVR): if the RVR increases in relation to the SVR, the RBF decreases. At reduced levels

28.6 Causes of Postrenal Acute Kidney Injury

Intrarenal (Intratubular)
- Crystal deposition—uric acid, oxalic acid, methotrexate, acyclovir, triamterene, sulfonamides, indinavir, tenofovir
- Protein deposition—light chains, myoglobin, hemoglobin

Extrarenal
- Ureteral/pelvic
- Intrinsic obstruction—tumor, stone, clot, pus, fungal ball, papilla
- Extrinsic obstruction—retroperitoneal and pelvic malignancy, fibrosis, ligation, abdominal aortic aneurysm

Bladder
- Prostate hypertrophy/malignancy
- Stones
- Clots
- Tumor
- Neurogenic
- Medication

Urethral
- Stricture
- Phimosis

of cardiac output, intrarenal factors are triggered, increasing renal arterial vascular tone and, consequently, decreasing the RBF. In order to maintain the intraglomerular pressure, efferent arteriolar resistance increases, preserving the filtration pressure even when the pressure in the afferent arteriolar decreases to levels low enough to cease filtration. Augmented activity of the sympathetic nervous system, RAAS, and vasopressin secretion increases the amount of filtered fluid and Na^+ that are reabsorbed.

These three mechanisms, control of blood flow to the kidney, the filtration fraction, and amount of fluid and solutes reabsorbed by the kidney, are the components responsible for the kidney reserve. However, the efficiency of these mechanisms has limits imposed by structural changes and the severity of the insult. The reserve is diminished by the presence of underlying arterial and intrinsic renal diseases that interfere with the control of renal blood flow, filtration fraction, and reabsorbtion functions, as well as by drugs that interfere with the vascular or neural humoral control of these mechanisms. When these compensatory mechanisms are overwhelmed, a prerenal state is discernible.

The frequency of prerenal azotemia as a cause of AKI varies with the clinical setting. A prospective study by Hou et al.[81] found prerenal azotemia to be the single most common cause of AKI in a general medical-surgical hospital. Liano found that prerenal causes of AKI among the elderly accounted for 48% of community-acquired AKI and 58% of hospital-acquired AKI (Fig. 28.7).[82,83] Brivet observed that in critically ill patients, prerenal causes accounted for 17% of cases of AKI.[84] Prerenal forms of AKI also appear to be common causes of community-acquired AKI and constituted 70% of all such cases as reported by Kaufman and associates.[85]

The terms "reversible AKI" or "prerenal failure" refer to all these different conditions that vary considerably in pathophysiology and course, including intravascular volume depletion, relative hypotension, compromised cardiac output, or hepatorenal syndrome (HRS). Although these terms are usually defined as an elevation of SCr or a reduction of UO that is easily reversible with improved renal perfusion

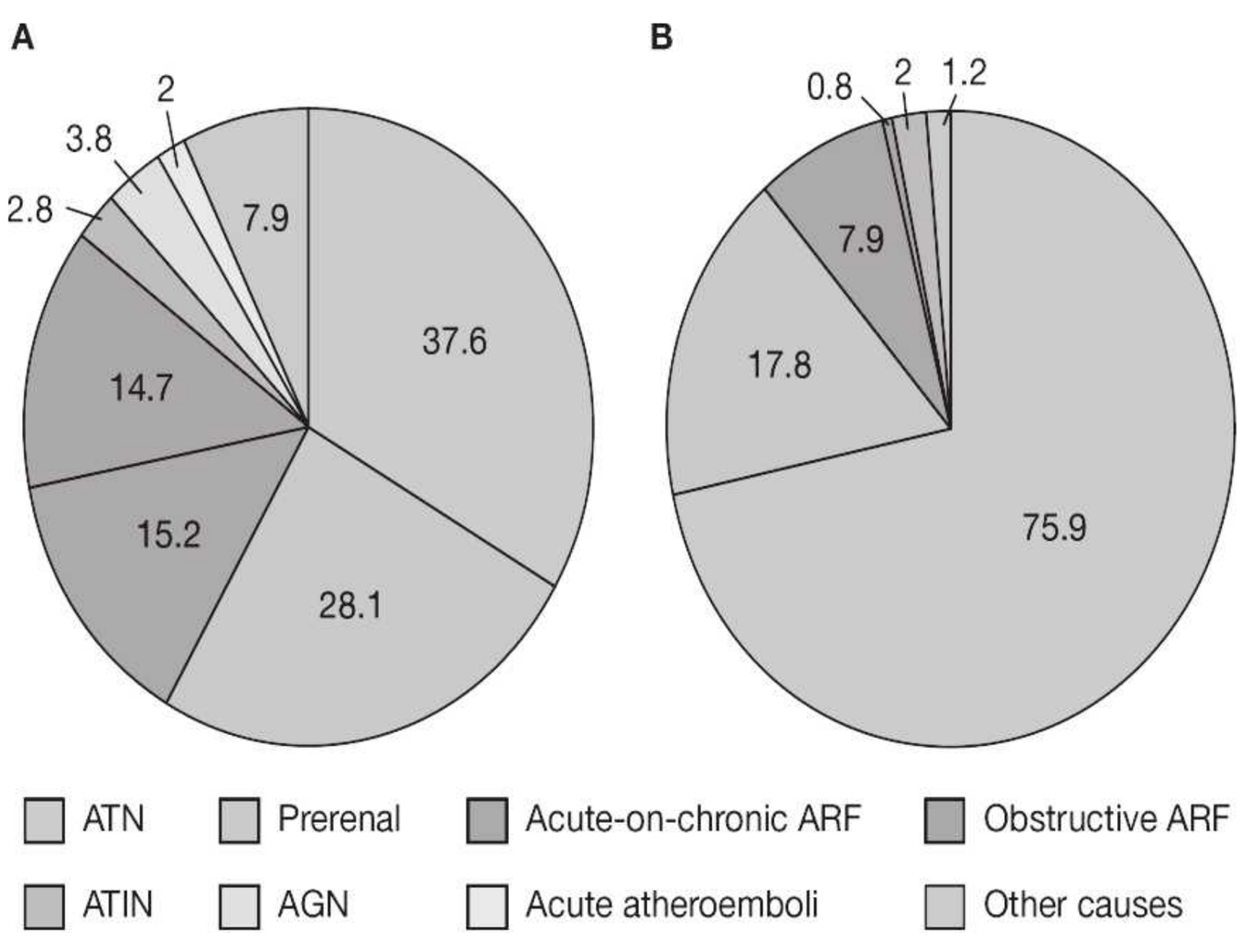


FIGURE 28.7 Percentage distribution of causes of acute renal failure in **(A)** non–intensive care unit (ICU) and **(B)** ICU settings. ICU patients are generally younger, less frequently afflicted by acute-on-chronic renal failure, and have significantly more acute tubular necrosis than the non-ICU group. *AGN*, acute glomerulonephritis; *ARF*, acute renal failure; *ATIN*, acute tubulointerstitial nephropathy; *ATN*, acute tubular necrosis; *ICU*, intensive care unit. (From Liaño G, Pascual J. Acute renal failure. Madrid Acute Renal Failure Study Group. *Lancet*. 1996;347(8999):479; author reply 479.) (See Color Plate.)

pressure, there is no agreement on the amount, nature, and duration of fluid resuscitation needed to eliminate a prerenal state. In most cases, the effect of fluid expansion or hemodynamic effect on renal function is retrospective and frequently evaluated by trial and error. The return of renal function to the previous baseline within 24 to 72 hours is considered to represent a prerenal or reversible condition. Diagnostic strategies have usually been based on demonstrating a fluid responsive change in renal function. The AKIN group recommended the "exclusion of urinary tract obstructions or . . . easily reversible causes of decreased urine output" and application of the diagnostic criteria "following adequate resuscitation when applicable."[17] This modification in the criteria was intended to exclude transient changes in creatinine or UO due to volume depletion or other easily reversible causes of renal hypoperfusion. Although ideally created for prospective application, most studies seeking to validate the criteria were retrospective. In those studies, the exclusion of urinary tract obstruction and prerenal failure are impossible to verify. One small retrospective study attempted to evaluate the outcome of patients that met the adequate resuscitation criteria in comparison to those patients in which data on fluid challenge was not available.[86] Detailed fluid challenge information was available in 123 patients who met AKI criteria. They found that the association of AKI with in-hospital mortality was still significant even when the appropriate fluid challenge requirement was discarded.

Several clinical scenarios are often associated with a potentially reversible or prerenal form of AKI. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with volume depletion, hypoalbuminemia, an edematous disorder, advancing age, underlying chronic renal failure, or recent diuretic use are all contributing factors for prerenal AKI.[87] A similar reversible form of AKI can complicate angiotensin-converting enzyme (ACE) therapy in the presence of decreased RBF from severe bilateral renal artery stenosis, renal artery stenosis in a solitary kidney, and other high-renin, high-angiotensin II states (i.e., edematous states and volume depletion disorders). In these cases ACE inhibition, with a resultant decrease in both renal perfusion pressure and efferent arteriolar constriction, can precipitously decrease GFR. About one third of patients with severe congestive heart failure experience an abrupt rise in SCr concentration following ACE inhibitor therapy.[88] In the setting of heart failure, this increase in SCr following ACE inhibition tends to be mild and readily reversible on discontinuation of the drug.

Although laboratory tests to distinguish reversible prerenal conditions from ATN have been used, these diagnostic parameters present frequent exceptions and the distinction between prerenal and renal causes are frequently not accurate (Fig. 28.8). The plasma (P) urea/creatinine ratio, urine (U) osmolality, U/P osmolality, U/P creatinine ratio, urinary Na level, and fractional excretions of Na (FE_{Na}) are the most frequently used tests. Serum U/P creatinine ratio

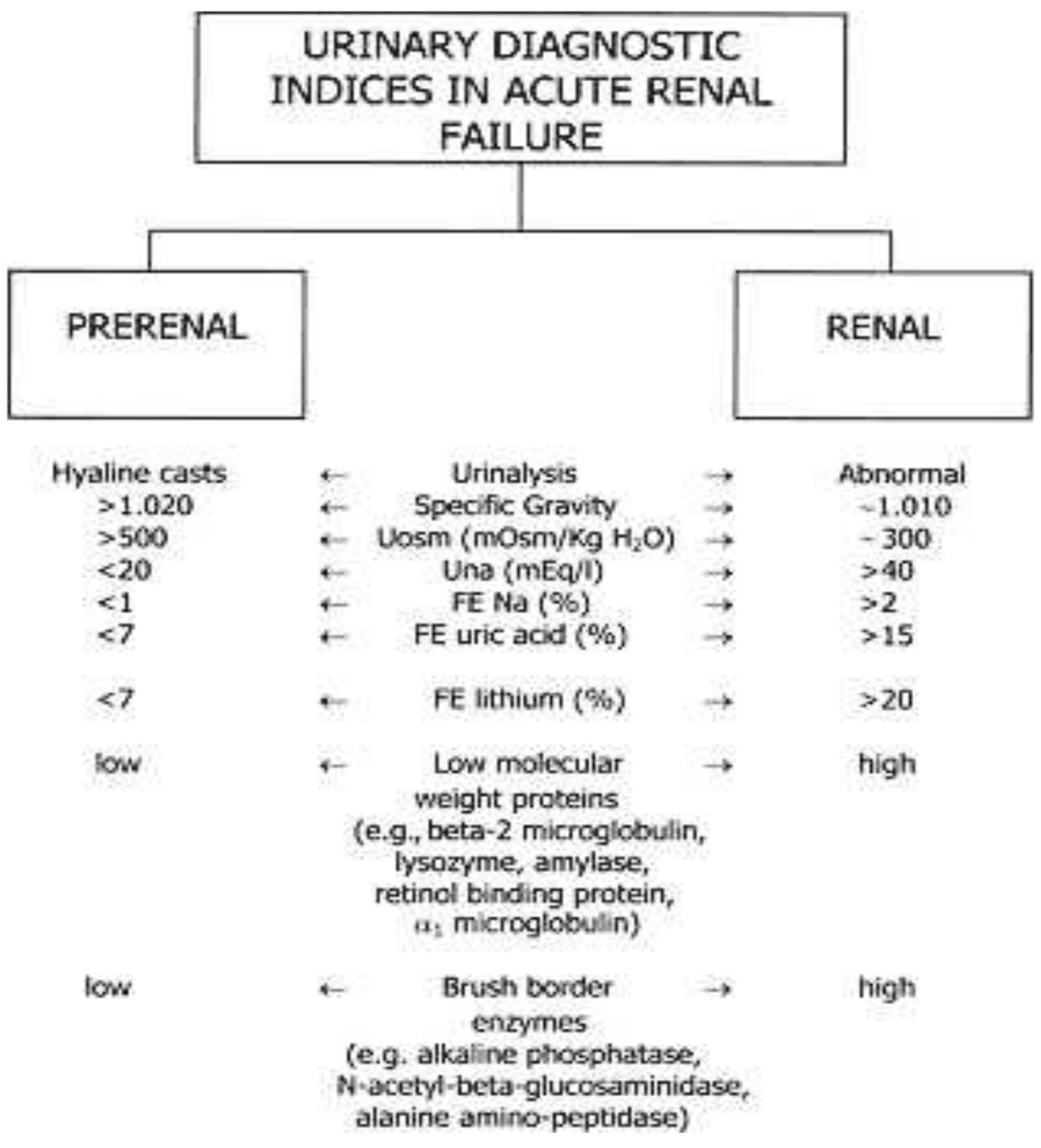


FIGURE 28.8 Urinary diagnostic indices to differentiate between prerenal and renal causes of acute kidney injury.

helps to identify whether the oliguria is a result of water reabsorption (U/PCr > 20) or loss of tubular function (U/PCr <20). In reversible states the reabsorption of sodium is increased, not only from the increase in proximal tubular reabsorption of water, but also by the increase in aldosterone level secondary to hypovolemia. The frequent use of diuretic therapy limits the value of FE_{Na}. The fractional excretion of urea (FE_{UN}) can be helpful in these cases. FE_{UN} relates inversely to the proximal reabsorption of water; urea reabsorption leads to a decrease in FE_{UN} and an increase in the BUN/creatinine ratio. Carvounis et al.[89] found that FE_{UN} has a high sensitivity (85%), a high specificity (92%), and a high positive predictive value; in that study a FE_{UN} less than 35% was associated with a 98% chance of reversible failure. Still, there are limitations for the use of FE_{UN}. In osmotic diuresis and with the use of mannitol or acetazolamide, the proximal tubular reabsorption of salt and water is impaired, so there can be an increase in FE_{UN} even in states of hypoperfusion. The same can occur when a patient is given a high protein diet or presents with excessive catabolism. Urinary osmolality is also used to evaluate the urinary concentration ability, a function that becomes impaired in the early process of tubular dysfunction. A value greater than 500 mOsm per kg indicates that tubular function is still intact, although there are also some considerations about this index; a low protein diet or low protein absorption by intestinal edema can impair the concentration ability of the urine and show a low osmolality even in reversible states.

There are some promising new biomarkers for AKI that may be helpful in distinguishing between reversible and established AKI.[64,69,90] During the reversible state, the persistent vasoconstriction associated with metabolic changes and inflammation promotes the release of cell functional markers that can be detected in the blood and urine. However, at the current time, there are no specific markers representing reversible conditions.

Obstruction to Urine Flow

Obstruction of urine flow is generally considered a less common cause of AKI. In several series, obstructive uropathy is encountered in 2% to 10% of all cases in AKI.[83,84] However, obstructive uropathy is more common in selected patient populations, such as the very young or older men with prostatic disease, and patients with a single kidney or intra-abdominal cancer, particularly pelvic cancer. Obstructive uropathy is most frequently encountered in community- and hospital-associated AKI and is less common in ICU-related AKI.[82,83] For example, obstructive uropathy constitutes 20% to 40% of all community-acquired AKI. Finally, the cause of obstructive uropathy is often amenable to therapy. Thus, obstructive uropathy should be considered in each case of AKI.

Obstruction of urinary flow can occur anywhere from the kidneys to the urethral meatus. Certain points along this path are more susceptible to obstruction. The three points of narrowing along the ureter include the ureteropelvic junction, the crossing of the ureter over the area of the pelvic brim, and the ureterovesical junction. The cause of obstruction of urine flow can be classified as intrarenal or extrarenal (Table 28.6). Intratubular deposition of either crystalline or proteinaceous material can increase intratubular pressure, thereby decreasing effective glomerular filtration pressure. For example, intratubular precipitation of uric acid can cause tubule obstruction and AKI. Acute uric acid nephropathy is most often seen following chemotherapy for leukemias and lymphomas. In this setting, the liver converts the purine load generated by cytolysis into uric acid. The high filtered load of uric acid and tubular reabsorption combine to produce high tubular concentrations of soluble urate and uric acid. Acidification of tubular fluid converts urate to uric acid, which can crystalize and occlude tubular lumens.

Abrupt exposure of the kidneys to high filtered loads of other insoluble crystalline substances can also cause an intrarenal form of obstructive uropathy. For example, AKI associated with calcium oxalate crystalluria can accompany ethylene glycol ingestion, administration of the anesthetic agent methoxyflurane, chronic pancreatitis, and use of gastrointestinal lipase inhibitors.[91–94] Administration of high doses of methotrexate can be associated with AKI, possibly owing to intratubular precipitation of the insoluble 7-hydroxy metabolite of methotrexate.[95] Other crystalline substances that can potentially precipitate within renal tubules and lead to AKI include acyclovir, triamterene, sulfonamides, and protease inhibitors such as indinavir.[96]

Another cause of intratubular obstruction is the deposition of immunoglobulin light chains in plasma cell dyscrasias. Immunoglobulin light chains are low molecular weight proteins that are filtered through the glomerulus and reabsorbed into the proximal tubular epithelium by initially binding to a heteromeric receptor complex composed of megalin and cubilin.[97] Saturation of this receptor-mediated endocytotic process results in the presence of free light chains in the distal nephron and urine. Nephrotoxicity of the metabolism of monoclonal light chains causes tubulointerstitial nephritis and cast nephropathy (also known as "myeloma kidney"), resulting in AKI and progressive CKD from tubular obstruction.[97]

Extrarenal lesions are the most common cause of postrenal AKI and are listed in Table 28.6. Several factors determine renal response to extrarenal obstruction. The site, degree, and rapidity of onset of obstruction are all important. Without a complicating infection, substantial improvement in renal function can follow decompression of the urinary tract after several days of complete obstruction. In men, prostatic obstruction is by far the most common cause of postrenal AKI because of its critical location at the bladder outlet. Obstruction of the upper urinary tract is a less common cause of AKI because it requires simultaneous obstructions of both ureters or unilateral ureteric obstruction with either absence of or severe disease in the contralateral kidney. Intraureteric obstruction can be due to stone, released necrotic papillae, tumor, pus, blood clots, and fungal balls. Papillary necrosis can occur in the

setting of sickle cell disorders, chronic urinary tract infections, analgesic abuse, and obstructive uropathy. Extraureteric lesions producing obstruction include retroperitoneal fibrosis, adenopathy, and tumors. Retroperitoneal fibrosis is often idiopathic but may be encountered in response to retroperitoneal neoplasia as well as in the setting of some pharmacologic agents (methysergide, methyldopa, β-blockers), prolonged peritoneal dialysis, and some connective tissue diseases. It has been reported that a high frequency of AKI occurs because of prostatic carcinoma in males and pelvic carcinoma (predominantly cancer of the cervix) in females causing ureteric occlusion. Less commonly encountered causes of extrinsic ureteric obstruction include inflammatory bowel disease (predominantly right-sided obstruction), an inflammatory reaction resulting from a leaking abdominal aortic aneurysm, and the late stages of pregnancy.

Acute obstruction can also be related to use of pharmacologic agents with potential anticholinergic effects (e.g., tricyclic antidepressants, phenothiazines, antihistamines) and cold remedies containing α-adrenergic agents (e.g., phenylpropanolamine) often precipitate acute urinary retention by impairing detrusor function and enhancing bladder sphincter tone, respectively.

PROLONGED OR SUSTAINED ACUTE KIDNEY INJURY

A variety of renal disorders can lead to a prolonged or sustained AKI (Table 28.5). In hospitalized adults in whom reversible and obstructive causes have been excluded, AKI is often caused by ATN. By contrast, in an outpatient setting in which reversible and obstructive causes have been excluded, other renal parenchymal diseases more often cause AKI.

Three major categories of insults are associated with ATN: prolonged renal ischemia, nephrotoxins, and pigmenturia (myoglobinuria and hemoglobinuria). Patients with ATN frequently present with more than one insult and several experimental studies in animal models of AKI demonstrate that multiple renal insults such as fever, bacteremia, endotoxemia, relative hypotension, and aminoglycoside exposure contribute to decrements in renal function with resulting AKI. This is referred to as multifactorial AKI.

The most common predisposing factor in the development of ATN appears to be renal ischemia resulting from a functional or structural reduction in renal perfusion.[10,83,98] Sepsis, and particularly septic shock, has assumed an ever-increasing role as a major predisposing factor in the occurrence of ATN.[10,99] Nephrotoxins are involved in about 20% of all cases of ATN.[10,83,98] Contemporary nephrotoxins commonly encountered include the aminoglycoside antimicrobial agents, radiographic contrast materials, NSAIDs, and antineoplasic drugs (Table 28.7). A high proportion of patients with AIDS develop nephrotoxicity from drugs used to manage HIV.

28.7 Factors Predisposing the Kidney to Nephrotoxicity

Drug-Related	Patient-Related
Concentration of the drug through reabsorptive and secretive processes	Preexisting renal dysfunction
High number of transporters result in high intracellular concentrations	Dehydration
Large luminal membrane surface area	Diabetes mellitus
Large biotransformation capacity	Exposure to multiple nephrotoxins

Diagnostic Approach

There are numerous causes of AKI, some of which are amenable to specific therapeutic interventions. For these interventions to be effective they must be applied early in the course of the disease process, preventing further deterioration of the renal function as mortality and complications of AKI appear to be proportional to its severity.[19] For example, Chertow et al. showed a 6.5-fold increase in the odds of death for patients with a 0.5 mg per dL increase in SCr.[100] In pediatric patients with acute decompensated heart failure, Goldstein et al. found that a rise in SCr of 0.3 mg per dL or more was associated with a sevenfold increased risk of in-hospital death.[101] Additionally, several studies have shown that the change in severity stage of AKI (AKIN from RIFLE) is associated with an incremental risk for mortality.[14,19,21,102] An early diagnosis and accurate assessment of AKI severity is essential to develop approaches for earlier intervention, correct reversible factors, and mitigate the downstream effects of AKI.

Chart Review, History, and Physical Examination

Meticulous chart analysis to determine recent clinical events possibly associated with the development of AKI is fundamental (Fig. 28.9). A history with regard to prescription drugs, over-the-counter agents, and herbal preparations, as well as possible environmental exposure, is critical as nephrotoxins are frequently contributing factors to the development of AKI. The relationship between medication exposure and AKI may not be readily apparent in some cases, thus a detailed history should always be performed. The presence of signs and symptoms of sepsis or heart failure, and symptoms related to the genitourinary tract (urine

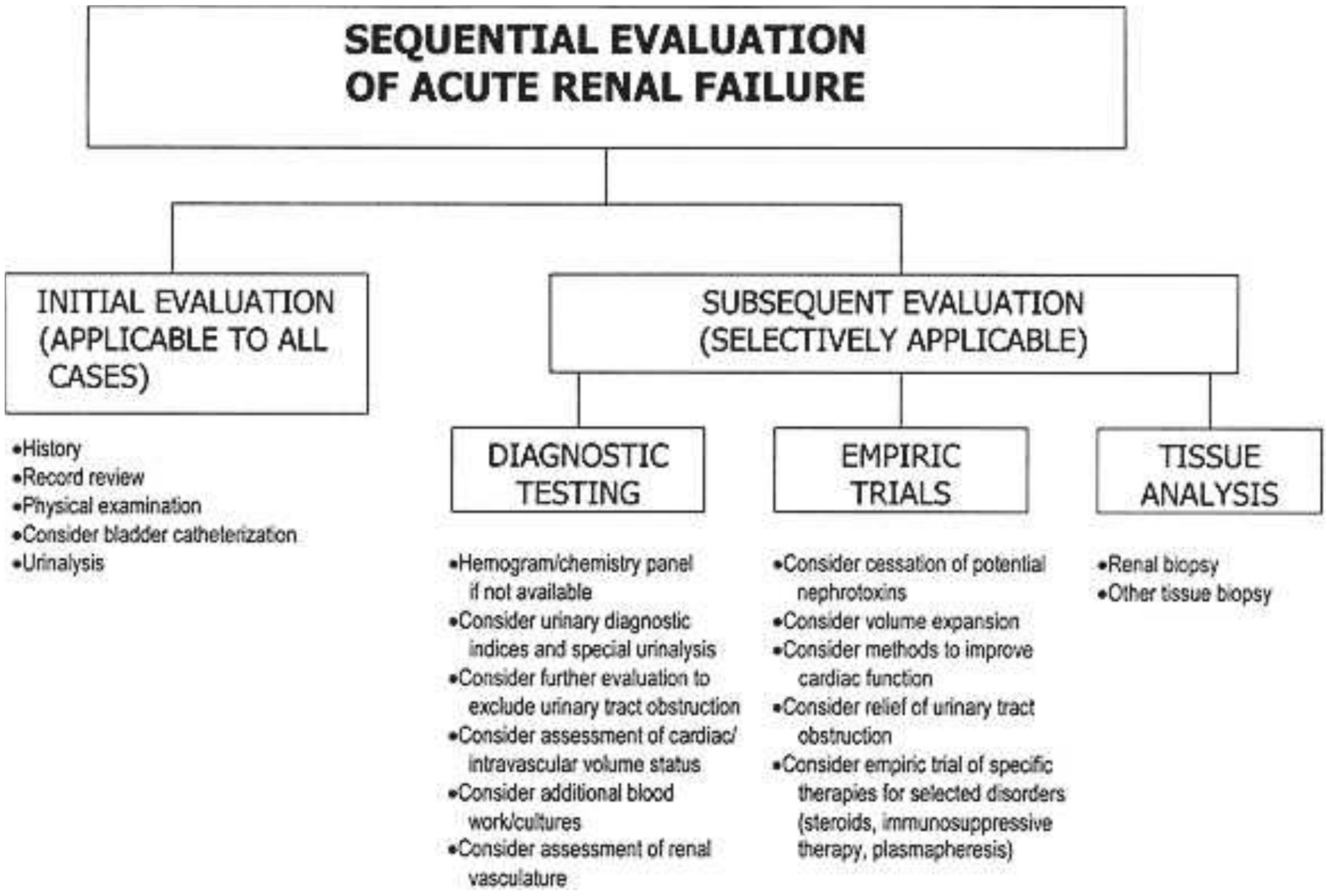


FIGURE 28.9 Suggested sequential diagnostic evaluation to determine the cause of acute kidney injury.

output, pyuria, dysuria, hematuria, and flank or abdominal pain) can provide helpful diagnostic information. Intense thirst, salt craving, orthostatic syncope, and muscle cramps often are symptoms of extracellular fluid volume depletion. Examination of serial vital signs, hemodynamic data, intake and output, and daily weight can provide important data regarding the cause of AKI. A weight change of greater than 0.25 to 0.50 kg per day indicates gain or loss of salt and/or water. Recording of serial renal functional data and correlation of any deterioration in renal function with clinical events such as those altering systemic hemodynamics and use of potential nephrotoxins often are of great diagnostic value. Physical examination can be of value in determining the presence or absence of prerenal and postrenal causes of AKI, as well as the presence of a systemic disorder that could result in a renal cause of AKI. The effect of either loss or sequestration of extracellular fluid volume on systemic hemodynamic responses depends on several variables, including the composition and rate of fluid loss and the underlying health state of the patient.

Physical examination must also include palpation for determining the state of peripheral circulation, renal size, and the possibility of abdominal aortic aneurysms. Palpation or percussion of the suprapubic area is necessary to detect bladder distention, and rectal and pelvic examinations are needed to detect prostatic and pelvic disorders. Examination of the skin may reveal palpable purpura suggestive of vasculitis; lower extremity evaluation may reveal livedo reticularis and evidence of emboli, suggesting atheroembolic disease. It is beyond the scope of this chapter to detail all the physical findings that can be associated with causes of AKI; however, the presence of neurologic or pulmonary disease, fever, skin lesions, joint abnormalities, or diffuse lymphadenopathy suggests the presence of a systemic disorder associated with AKI.

Laboratory

Blood Urea Nitrogen

Elevations in BUN are dependent on the nitrogen intake, the degree of renal impairment, and the degree of protein catabolism. In the noncatabolic patient with mild renal impairment, daily BUN usually increases <10 to 15 mg per dL per day and SCr <1.5 mg per dL per day. High catabolic states and high-protein diets are associated with greater urea nitrogen production that can exceed 50 mg per dL.

Another condition associated with a BUN increase not proportional to the rise in SCr level and fall in GFR is the presence of decreased intravascular effective volume. Normally, the usual BUN:SCr ratio is about 15:1 and the BUN and SCr increases by 10 to 15 and 1.0 to 1.5 mg/dL/day, respectively, in the absence of GFR. In situations characterized by decreased glomerular perfusion pressure, such as heart failure, BUN can increase independently from SCr. Elevations

in BUN are independent from SCr levels, and these two parameters are a reflection of the severity of renal dysfunction, and actually a consequence of two distinct pathologic processes.[36–38] The activation of RAAS and the sympathetic nervous system are responsible for decreasing the glomerular perfusion pressure and GFR. The increment in vasopressin levels upregulates aquaporin-2 expression and increases water reabsorption. Urea, in contrast to SCr, is not secreted but reabsorbed by the renal tubules. The increased reabsorption of sodium and water, rather than the reduced GFR, enhances reabsorption of urea and increases BUN levels. Thus, BUN levels and BUN/creatinine ratio could be a more effective way to assess circulatory volume than GFR, which is regulated by the pressure difference between glomerular afferent and efferent arterioles.[39] In heart failure, and possibly in other settings where underfilling is part of the pathophysiologic process, the rise in BUN greater than any fall in GFR is a marker of the neurohumoral axis activation.[40]

In selected circumstances it may not be clear if an elevated BUN:SCr ratio is due to an acute or chronic process. In these settings, a review of previous records is helpful. Although the test is not widely avilable, measurement of carbamylated hemoglobin can be helpful. Hemoglobin potentially undergoes nonenzymatic carbamylation of its terminal valine. Thus, similar to the hemoglobin A1C value as an index of blood sugar control, the level of carbamylated hemoglobin is an indicator of the degree and duration of elevated BUN.[103,104] A carbamylated hemoglobin level greater than 80 to 100 μg carbamyl valine per gram hemoglobin suggests the diagnosis of chronic rather than ARF.[105,106]

Serum Creatinine

Clinicians usually follow daily SCr concentrations to assess whether GFR is increasing, decreasing, or constant in patients with AKI. The SCr concentration, however, is dependent on creatinine production, volume of distribution, and renal elimination. However, in patients with AKI, changes in GFR often correlate poorly with changes in SCr concentration. In AKI, three main factors influence the estimation of kidney function: the actual GFR, fluctuations in creatinine production, and fluid balance.[33] Moran and Myers[107] demonstrated this in several patterns of AKI (abrupt and large, slow and progressive, and stepwise). They developed a simple, computerized model of creatinine kinetics in patients with postischemic AKI to calculate GFR based on SCr concentration corrected for changes in creatinine volume of distribution. They suggested that changes in GFR are difficult to evaluate using SCr concentration alone in the setting of AKI.[33]

Jelliffe developed an equation to estimate GFR in patients with unstable (nonsteady state) kidney function that considered fluctuations in kidney function and creatinine production without requiring timed urine collection.[108] The Jelliffe equation is based on the concept that daily changes in SCr depend on the difference between creatinine production and excretion. Bouchard et al. demonstrated that the GFR by Jelliffe correlated best with urinary creatinine clearances.[109] In an ICU cohort the eGFR by Cockcroft–Gault, Modification of Diet in Renal Disease, and Jelliffe overestimated urinary creatinine clearance in 80%, 33%, and 10%, respectively. The relative overestimation of GFR in AKI with both Cockcroft–Gault and MDRD was more prominent when baseline GFR was higher.

Fluid administration is a common and required component of the management of critically ill patients and has recently focused on goal-directed resuscitation with early volume expansion in the ICU course. These strategies frequently result in a relative increase in body weight of 10% to 15% or more, sometimes doubling the total body weight in a short period of time.[110] The fluid accumulation increases the extracellular fluid (ECF), altering the volume of distribution of SCr, and resulting in potential overestimation of the level of kidney function.[34] The masking of AKI severity by volume expansion may be especially problematic in settings where the SCr is rising relatively slowly owing either to lower creatinine generation (e.g., as might be expected in the elderly or patients with less muscle bulk) or to more modest overall injury.

Urine Microscopy

Urinary microscopy (UM) is an integral part of the clinical evaluation of patients with kidney disorders and is frequently utilized to differentiate some clinical conditions (e.g., nephrotic syndrome, urinary tract infection, nephritic syndrome). Drug toxicity has also been assessed by UM, as in the case of indinavir, acyclovir, and amoxicillin, associated with variable degrees of leukocyturia, crystalluria, and cellular casts.[96,111–113]

In the case of AKI, UM has traditionally been used as a tool to differentiate prerenal AKI and ATN. Sediment containing few formed elements or only hyaline casts strongly suggests prerenal azotemia or obstructive uropathy. With ATN, brownish-pigmented cellular casts and many renal tubular epithelial cells are observed in more than 75% of patients. Sufficient red blood cells to cause microscopic hematuria are traditionally thought to be incompatible with a diagnosis of ATN and usually result from glomerulonephritis or structural renal disorders (stones, tumor, infection, or trauma). Red blood cell casts suggest the presence of glomerular or vascular inflammatory diseases of the kidney and rarely, if ever, occur with ATN. Red blood cell casts, however, can be seen rarely in acute interstitial nephritis. The presence of large numbers of polymorphonuclear leukocytes, singly or in clumps, suggests acute diffuse pyelonephritis or papillary necrosis. In allergic tubular-interstitial nephritis eosinophilic casts on Hansel's stain of urine sediment may be diagnostically helpful.[114–116] Eosinophiluria may be also present in some forms of glomerulonephritis and in atheroembolic renal disease but is rarely encountered in ATN.[117] The combination of brownish-pigmented granular casts and positive occult blood tests on urine in the absence of hematuria indicates either hemoglobinuria or myoglobinuria. In AKI, the finding of large numbers of "football-shaped" uric acid

crystals in fresh, warm urine may suggest a diagnosis of acute uric acid nephropathy, whereas the finding of large numbers of "back-of-envelope–shaped" oxalic acid suggests ethylene glycol toxicity.[118] Other agents (e.g., indinavir, sulfadiazine, acyclovir, and methotrexate) also can induce AKI with characteristic crystal appearance on urinalysis.[112,118] The presence of broad casts (defined as more than three white blood cells in diameter) suggests chronic renal disease.

Recently, two different groups have revised the clinical value of performing UM.[119,120] In a pilot study, Chawla et al. developed an AKI cast-scoring index to standardize urine-sediment. The score precision was evaluated in 30 patients with a clinical syndrome compatible with ATN with an interobserver index of 99.8 ± 0.29%, and a coefficient of variation of 1.24%. Urine-sediment was further correlated with outcomes in 18 patients with ATN. The authors found that renal recovery was worse in those patients with a higher cast scoring index (2.55 ± 0.9 vs. 1.7 ± 0.79; P = .04), and area under the ROC curve of the cast scoring system to predict nonrenal recovery was 0.79. In another study, Perazella et al. proposed a different scoring system for differentiating ATN from prerenal AKI (Table 28.8).[120] Using the final AKI diagnosis at discharge as a gold standard, UM at the day of nephrology consultation was highly predictive of ATN. The odds ratio for ATN incrementally increased with a higher score. In patients with initial diagnosis of ATN, any granular casts (GCs) or renal epithelial tubular cells (RETCs) (score 2) resulted in a PPV of 100% and a NPV of 44%. The lack of RTECs or GCs in patients with initial diagnosis of prerenal AKI had a sensitivity of 0.73 and specificity of 0.75 for the final diagnosis of

28.8 Recent Studies Evaluating Urinary Microscopy in Acute Kidney Injury

Reference	Standardized Method of Sample Preparation	Score System Used[a]
Bagshaw et al.[b]	None	Description of common findings in urine sediment of patients with sepsis of 7 studies included (common presence of muddy brown or ECCs, RTECs, and variable trace hematuria and pyuria)
Chawla et al.[c]	**Volume collected:** 10 mL **Centrifugation process:** 5 minutes at 2,000 rpm **Supernatant:** decanted (9.5 mL) **Residual sample for analysis:** 0.5 mL **Resuspend:** by hand Pipette use to dispense 1 drop of sediment to a glass slide and 24 × 30 mm coverslip gently applied	**Grade 1**: None (no evidence of GCs or ECCs) **Grade 2** : Rare (rare GCs or ECCs; at least 1 GC or ECC seen on the entire slide, but 10% of LPFs) **Grade 3**: Moderate (many GCs or ECCs, but not seen on every LPF; casts seen on >10% but <90% of LPFs) **Grade 4**: Sheets (sheets of muddy brown cast; GCs or ECCs seen on >90% of LPFs)
Perazella et al.[d]	**Volume collected:** 10 mL **Centrifugation process:** 5 minutes at 2,000 rpm **Supernatant:** removal by suction (9.5 mL) **Residual sample for analysis:** 0.5 mL **Resuspend:** by hand Pipette use to dispense 1 drop of sediment to a glass slide and coverslip gently applied	**Score 1**: RTECs 0 and GCs 0 **Score 2** : RTECs 0 and GCs 1–5 or RTECs 1–5 and GCs 0 **Score 3**: RTECs 1–5 and GCs 1–5 or RTECs 0 and GCs 6–10 or RTECs 6–20 and GCs 0
Perazella et al.[e]	**Volume collected:** 10 mL **Centrifugation process:** 5 minutes at 2,000 rpm **Supernatant:** removal by suction (9.5 mL) **Residual sample for analysis:** 0.5 mL **Resuspend:** by hand Pipette use to dispense 1 drop of sediment to a glass slide and coverslip gently applied	**RTECs (per HPF)** 0 (0 points) 1–5 (1 point) ≥6 (2 points) **GCs (per LPF)** 0 (0 points) 1–5 (1 point) ≥6 (2 points)

28.8 Recent Studies Evaluating Urinary Microscopy in Acute Kidney Injury (continued)

Reference	Differential Diagnosis Pre-renal vs. ATN	Prediction of Outcomes	Comments
Bagshaw et al.[b]	Not assessed	Not assessed	Only 7 studies (26%) from the 27 included in the systematic review reported urinary microscopy or sediment findings.
Chawla et al.[c]	Not assessed	Nonrenal recovery (need of RRT or death while SCr trend was still rising) **Nonrecovery:** CSI score 2.55 ± 0.93 **Recovery:** CSI score 1.57±0.79 **ROC** area under the curve for CSI was 0.79	Standardized urine sediment processing method Score system for predicting outcomes
Perazella et al.[d]	**Score 1:** OR 9.7 (95% CI, 5.3–18.6) **Score ≥2:** OR 74 (95% CI 16.6–329.1)	Not assessed	Standardized urine sediment processing method Score system for differential diagnosis
Perazella et al.[e]	Score not employed for differential diagnosis	Worsening AKI (increase in AKIN stage, need of RRT, or in-hospital death) adjusted RR compare to 0 points. **1 points** → 3.4 (95% CI 1.3–6.5) **2 points** → 6.6 (95% CI 3.4–9.1) **≥3 points** → 7.3 (95% CI 3.8–9.6)	Standardized urine sediment processing method Score system for predicting outcomes

[a]Reference test use as a gold standard for diagnosis of acute kidney injury.
[b]Bagshaw et al. (2006): No gold standard used for assessing urinary sediment performance, study only resumes common findings in urine sediment of patients with sepsis.
[c]Chawla et al. (2008): Clinical syndrome consistent with ATN determined by the renal consult service.
[d]Perazella et al. (2008): Final diagnosis of the patient type of AKI at discharge (ATN, Pre-renal AKI, or other) as determined by renal consult service.
[e]Perazella et al. (2010): The same as in Perazella et al. (2008).
AKI, acute kidney injury; AKIN, acute kidney injury network; ATN, acute tubular necrosis; SCr, serum creatinine; RRT, renal replacement therapy; ECCs, epithelial cellular casts; GCs, granular casts; RTECs, renal tubular epithelial cells; LPH; low power field; HPF, high power field; RR, risk ratio; OR, odds ratio; CI, confidence interval; CSI, cast scoring index.

From Claure-Del Granado R, Macedo E, Mehta RL. Urine microscopy in acute kidney injury: time for a change. Am J Kidney Dis. 2011;57(5):657–660.

ATN. A scoring point system of UM findings was used to predict adverse outcomes.[121] Correlation of the urinary-sediment score and AKIN stage at nephrology consultation was demonstrated, and the score was associated with a higher risk of worsening AKI in a dose-dependent manner.

Urinary Chemical Indices

Since the 1940, the concentrations of sodium (U_{Na}) and chloride (U_{Cl}) in the urine have been known to be high during established phases of ATN. Although the accuracy of U_{Na} alone in determining the cause of AKI was limited, the renal failure index ($U_{Na} \div$ U/P creatinine) or the fractional excretion of sodium (FE_{Na} or $U/P_{Na} \div$ U/P creatinine $\times$ 100) was found to have a high degree of accuracy in differentiating between reversible prerenal azotemia and ATN. However, there are several caveats when using spot urine chemistries as a diagnostic tool to evaluate the cause of AKI. Despite widespread, routine use, no study has demonstrated that knowledge of these indices either changes management or improves outcome of AKI.

Nearly all studies of spot chemistries have been performed at a single time point often relatively late in the course of AKI. The lack of serial data is important because AKI is a dynamic process. During the early phases of AKI, renal tubular function is intact. Later, cell injury may result in loss of tubular cell polarity. The resulting urine chemistries, therefore, are dependent on the phase of the course in which they were obtained. This may limit the sensitivity and specificity of urine chemical indices. For example, the early course of AKI occurring in the setting of sepsis,

radiocontrast exposure, rhabdomyolysis, and NSAID use is often associated with renal vasoconstriction, hypoperfusion of the kidney, and low FE_{Na}.[122] Later in the course, the FE_{Na} often increases, if tubular necrosis occurs.

Two other points deserve emphasis with regard to use of urine chemistries as an AKI diagnostic tool. Early in the course of urinary tract obstruction, in some patients with nonoliguric ATN, and in some vascular/glomerular disorders (acute glomerulonephritis, vasculitis, thrombotic thrombocytopenic purpura) urinary chemical indices can be indistinguishable from those seen with prerenal AKI. Conversely, several acute renal parenchymal disorders (e.g., interstitial nephritis, severe ischemic nephropathy, and exacerbations of chronic renal failure) can be associated with urine chemical parameters indistinguishable from ATN, suggesting a lack of specificity. Finally, it is important to acknowledge that potentially reversible prerenal AKI with an $FE_{Na} > 1\%$ occurs in selected settings such as recent diuretic use, bicarbonaturia, salt-wasting nephropathy, glycosuria, and mineralocorticoid deficiency. In the setting of prerenal AKI associated with bicarbonaturia, the urinary chloride concentration is low, confirming a prerenal state.[123] In the setting of prerenal AKI associated with diuretic use, the fractional excretion of trace lithium, urea nitrogen, or uric acid continues to be low.[124] Although urine chemical indices are most often used as diagnostic adjuncts in patients with AKI, they may also provide prognostic information. Some studies suggested that in oliguric patients with AKI lower values for FE_{Na} and higher values for U/P osmolality can predict a high likelihood of a response to diuretics.[125]

Miscellaneous Tests

In some circumstances the cause of AKI may not be evident after chart review, history, physical examination, and urinalysis. In some cases a review of the hemogram may be helpful. A peripheral blood smear that reveals rouleaux formation may suggest the presence of a plasma cell dyscrasia. Eosinophilia is compatible with allergic interstitial nephritis, atheroembolic disease, and polyarteritis nodosa. A microangiopathic picture with thrombocytopenia suggests vasculitis, malignant hypertension, the HELLP syndrome, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. The presence of coagulopathy can suggest either disseminated intravascular coagulation or an antiphospholipid antibody syndrome as the cause of AKI. If glomerulonephritis is a diagnostic possibility, then the presence of antineutrophilic cytoplasmic antibodies may suggest a diagnosis of either Wegener granulomatosis (primarily a cytoplasmic pattern) or pauci-immune glomerulonephritis (primarily a perinuclear pattern). Antibodies to glomerular basement membrane are strongly suggestive of Goodpasture syndrome, whereas antinuclear antibodies and antibodies against DNA suggest the presence of systemic lupus erythematosus. The presence of cryoglobulins may point to the presence of circulating immune complexes, a plasma cell disorder, or primary cryoglobulinemia.

Imaging

Ultrasonographic evaluation of the kidney can also help in the diagnosis of AKI. Ultrasound is an excellent modality for structural imaging as it is possible to detect renal parenchyma size, scarring, fibrosis, and polycystic kidneys. The presence of small kidney size strongly supports a diagnosis of chronic renal disease and may also help to differentiate acute from chronic renal failure. The echogenicity of the cortex can be assessed with a hyperechoic cortex (normal cortex is hypoechoic to liver), present in most cases of chronic renal failure, adult polycystic kidney disease being the notable exception. Noncontrast computed tomographic (CT) and magnetic resonance imaging (MRI) scans analyze renal structure and renal artery calcification. Other functional studies, such as mercaptoacetyltriglycine (MAG3) and diethylene triamine pentaacetic acid (DTPA), evaluate renal perfusion, uptake, and excretion of a tracer.

A further role of imaging is to determine the number of present and functioning kidneys. For ARF to occur in previously normal kidneys, the underlying cause must be a bilateral process, or a single functioning kidney must be compromised.

Kidney ultrasonography is most commonly ordered in the setting of AKI to rule out urinary tract obstruction. Although renal ultrasonography is a safe and noninvasive test, because obstruction is a relatively uncommon cause of hospital-acquired AKI, the majority of ultrasonography results obtained are negative. In fact, hydronephrosis (HN), the evidence of obstruction on imaging, is only identified on renal ultrasound in 1% to 10% of patients with AKI.[10,126] Licurse et al.[127] sought to create a stratification system that would help clinicians ascertain the risk of renal obstruction among those with AKI. The idea was to improve the probability of a positive finding on renal ultrasound. The authors identified multiple risk factors for hydronephrosis: history of hydronephrosis, history of abdominal or pelvic cancer, prior pelvic surgery, or a single functioning kidney. Patients with a history of heart failure, granular casts on urinalysis, elevated leukocyte count, documented hypotension, or exposure to aspirin, diuretics, or vancomycin during hospitalization were less likely to have hydronephrosis.[127] Patients in the ICU also have a lower incidence of obstruction.

Obstructed kidneys are typically normal sized with dilated ureters, renal pelvis, and calyceal systems. The urine-filled structures appear as anechoic areas with posterior acoustic enhancement. Ureter and renal pelvis can be dilated without being obstructed, mainly after previous obstruction that leaves a residual dilated collecting system, or as an anatomic variant (enlarged extrarenal pelvis). False negatives can occur in the hyperacute setting if the renal collecting system has not had time to dilate, or if associated with retroperitoneal fibrosis. Noncontrast CT scan is the gold standard for detecting ureteric calculi. The ureters can usually be traced between the kidney and bladder, and a hyperdense stone can be seen at the distal site of hydroureter. More than

99% of renal calculi are radiopaque on CT scan; however, xanthine calculi may be radiolucent and stones associated with indinavir are radiolucent. The obstructed kidney is typically edematous (i.e., swollen) with perirenal stranding. A noncontrast study can usually detect many extrinsic compressing masses, such as retroperitoneal tumors or cervical or colon carcinomas, that may produce bilateral obstruction. Scintigraphic imaging with either Tc-99m-MAG3 or Tc-99m-DTPA can detect ureteric obstruction and the negative predictive value of nuclear medicine scanning is extremely high.

In ATN, ultrasounds will usually show enlarged kidneys with a smooth contour caused by interstitial edema. The cortex can present normal echogenicity with either a normal or hypoechoic medulla. The renal arteries can also be evaluated for the renal index (RI), which is an objective measure of the resistance to renal perfusion. RI is defined as (systolic velocity minus diastolic velocity) divided by systolic velocity, and has been heavily investigated to determine whether elevation in RI can differentiate ATN from renal hypoperfusion not yet complicated by ATN. Unfortunately, RI has inadequate specificity for routine clinical use. The examination of choice in suspected ATN is a MAG3 nuclear medicine study. Scintigraphic imaging examinations in ATN using Tc-99m-MAG3 demonstrate relatively well-preserved on-time renal perfusion and delayed tracer uptake, often with a continuing activity accumulation curve. Excretion of tracer into the collecting system is delayed and reduced, but there is no obstruction to drainage of the collecting systems.

In suspected glomerulonephritis or acute interstitial nephritis, the "gold standard" diagnostic test is a renal biopsy. The main role of imaging is to detect structural signs of chronic renal disease and to exclude other causes of ARF. MAG3 studies will show poorly functioning kidneys, but will not show accumulation pattern or obstruction to drainage. Edema can sometimes be demonstrated with ultrasound, manifesting as hypoechoic large kidneys.

Renal Biopsy

A renal biopsy is rarely undertaken but should be considered in the setting of AKI in the presence of: (1) no obvious cause of AKI, (2) either extrarenal clinical evidence or a history of systemic disease, (3) heavy proteinuria and persistent hematuria, (4) marked hypertension in the absence of volume expansion, (5) prolonged (>2 to 3 weeks) oliguria, and (6) anuria in the absence of obstructive uropathy. In clinical practice, most nephrologists choose to biopsy when they are not confident of the cause of the AKI or when the renal injury has an obscure etiology. In a significant proportion of patients diagnosed with AKI, the clinical context suggests the etiology with a reasonable degree of certainty. In other less clear cases the lack of efficient therapeutic options coupled with the risks of a biopsy decreases the likelihood that the clinician will perform the procedure. However, the development of AKI is often multifactorial, and some other causes of AKI may be misclassified as ATN.

Several studies have examined the clinical utility of renal biopsy in the setting of AKI.[128,129] Rivera et al. obtained data from 9,378 cases with native biopsy-proven renal diseases between 1994 and 2001, investigating clinicopathologic correlations. Acute renal failure was an important cause for performing a kidney biopsy: 12% in that cohort. The majority of the biopsies were in adults and elderly patients, predominantly with the suspicion of vasculitis and crescentic glomerulonephritis (GN).[129] In an Italian survey similar results were found: 34.1% frequency of vasculitis and crescentic GN in 1,059 renal biopsies of patients with AKI.[130] In Baltimore, 259 renal biopsies of adults older than 60 years with ARF showed similar results: 35.2% of the diagnoses were crescentic GN.[131] However, these studies included mostly patients with active urinary sediment, a selection bias resulting from clinical practice of biopsy indication in AKI. In most AKI patients, clinical evaluation eliminates prerenal and postrenal causes of AKI, and the results of the biopsies show what we would find when performing biopsy in AKI patients who are thought not to have ATN.[132] Another issue is determining the number of patients clinically diagnosed with ATN who actually have another disease other than AKI. Most importantly, it is necessary to determine the number of patients with treatable forms of AKI that are not being identified.

Several studies have suggested significant discordance between prebiopsy and postbiopsy diagnoses in the setting of AKI. Haas et al. studied the elderly and found the clinical diagnosis to be incorrect in 34% of cases biopsied, many of them involving potentially treatable entities.[131] Among elderly patients with rapidly progressive renal injury, Uezono et al. found 71% of the patients with crescentic GN and 17% with interstitial nephritis. Prebiopsy and histopathologic diagnoses differed in 15% of patients, and both groups benefited from therapeutic intervention.[133]

These data emphasize the value of renal biopsy in the management of AKI of uncertain origin, irrespective of the age of the patient. Accurate diagnosis is important to direct the appropriate treatment, especially in vasculitis and crescentic GN, in which the delay in diagnosis may affect outcome. Given the safety of the ultrasound tomographic-guided renal biopsy, unclear causes of AKI deserve renal biopsy consideration.

Primary Prevention

The development of AKI contributes to dysfunction of other organs, such as heart, lung, brain, and liver.[134–136] Even small changes in GFR are associated with increased mortality.[21] Consequently, primary prevention and early diagnosis of AKI is of central clinical importance. As shown in the conceptual model of AKI illustrated in Figure 28.5, the first step in preventing AKI is an adequate risk assessment. The prevention of AKI should start with assessment of the risk to develop AKI; identification of comorbidities, nephrotoxic medications in use, and early recognition of acute reversible risk factors

associated with AKI. A surveillance approach, applying close monitoring in patients at risk to develop AKI, is a fundamental key to AKI prevention.[137] General preventive strategies are outlined in Table 28.9. A contemporary study has illustrated the potential value of a computerized surveillance system with electronic notification of clinicians to attenuate nephrotoxin-induced AKI.[138] In this study e-mail messages were sent to clinicians to notify them whenever mild increases in SCr occurred in their patients receiving a nephrotoxic drug.[138] This notification led to earlier discontinuation of the offending agent compared to when clinicians were not notified. Earlier notification and cessation of the offending agent decreased the frequency of development of severe AKI from 7.5% to 3.4%. This study confirms that earlier identification of patients with higher risk allows the physician to apply preventive measurements, adjust or suspend nephrotoxic drugs, and, when possible, delay or avoid progression of renal injury. However, recommendations to prevent AKI are not uniformly followed. Weisbord and associates reviewed the medical records of "at risk" patients who underwent radiologic investigations using radiocontrast.[139] They found that of 144 patients eligible for intravenous volume expansion, 16% failed to receive any intravenous fluids. NSAIDs and COX-2 inhibitors were prescribed for 8% of patients.[139] These results validate the effort to bring to light the importance of surveillance, the continuation of the search for earlier markers of AKI, along with providing education to the medical community to valorize small changes in renal function.

28.9 Prevention of Acute Kidney Injury

Avoidance of Nephrotoxicity
- Recognition of agents with nephrotoxic potential
- Recognition of high-risk populations
- Avoidance of concomitant use of more than one nephrotoxin
- Consideration of alternative therapies
- Use of smallest dose and briefest duration
- Formulation/dosing modification
- Monitoring of blood levels if available
- Frequent measurement of renal function
- Surveillance systems to alert clinicians to changes in renal function
- Hydration

Minimization of Nosocomial Infection
- Meticulous handwashing
- Conservative use and rapid removal of intravascular and intravesicular catheters
- Cautious use of antibiotics based on culture data with automatic stop orders to ensure periodic reassessment
- Aspiration pneumonia precautions (elevate head of bed, attention to gastric residual volume, conservative use of sedatives/hypnotics)

Selected Application of Pharmacologic Intervention
- Extracellular fluid expansion
- Maintenance of high urine flow
- Maintenance of cardiac index and mean arterial pressure
- Renal vasodilators
- Intravenous albumin
- Growth factors
- Calcium channel blockers
- Miscellaneous agents

Selected Application of Nonpharmacologic Interventions
- Preoperative optimization
- Maintenance of high oxygen delivery
- Minimization of artificial ventilation
- Supranormal optimization of cardiovascular hemodynamics
- Prophylactic hemofiltration

Risk Assessment

Although AKI associated with one specific cause is common outside the ICU, most critically ill patients have several etiologic factors associated with the development of AKI. AKI acquired in the hospital is often due to a combination of insults. The most common associated causes are failure of renal autoregulation, direct nephrotoxicity, ischemia reperfusion, and inflammatory states. As multiple factors directly influence renal function, the nature and timing of the inciting event is commonly unknown. Accurate identification of AKI risk factors is a fundamental first step in achieving early diagnosis and implementing preventive strategies.

In the ICU population two large prospective observational studies have provided a better understanding of the risk factors associated with AKI in this setting: BEST and PICARD.[10,98] Both BEST and PICARD found sepsis to be the most common contributing factor to ICU-related AKI. A significant percentage of patients developing severe AKI had baseline CKD. In the BEST study in 47.5% of patients ARF was associated with septic shock. Thirty-four percent of ARF was associated with major surgery, 27% was related to cardiogenic shock, 26% was related to hypovolemia, and 19% of ARF was potentially drug-related.[10]

Volume depletion is one of the most common and important risk factors for AKI. In addition to hypovolemia, renal hypoperfusion may be caused by decreased cardiac output, decreased plasma oncotic pressure, hypotension, and decreased renal prostaglandin synthesis. Preexisting renal disease and advanced age, which is often associated with some degree of decreased renal function, are also common risk factors associated with AKI. Administration of a potentially nephrotoxic agent, or drugs that may enhance

nephrotoxicity, obviously increases the risk of AKI. For example, the concurrent use of furosemide and intravenous contrast agents may increase the risk of AKI.[140] Sepsis, congestive heart failure, nephrotic syndrome, and hepatic disease are common conditions associated with AKI.[10]

Although several individual risk factors are associated with the development of AKI, the combination of risk factors and the development of risk stratification scores could provide better tools to predict AKI in specific patient populations (e.g., after cardiac surgery, contrast exposure, hospital-acquired, general surgery, and high-risk surgery).[141–143] Few models have examined the clinical risk factors for the development of AKI among the ICU population.[144,145] Risk profiling can also be used to establish appropriate criteria for surveillance for AKI in hospitalized patients.[146] The use of models to predict the risk of AKI can help clinicians to identify patients with high risk of developing AKI, improve care, and provide better patient counseling.[50]

Volume Expansion

Regardless of the nature of the insult, hemodynamic stabilization with optimization of the cardiac output and blood pressure are key factors in preventing the initiation or worsening of AKI. The general aim is to optimize volume status based on physiologic measurements, maintain adequate hemodynamic status and cardiac output to ensure renal perfusion, and avoid further insults (e.g., hypotension and hypovolemia). Therefore, fluid management is an important intervention for patients in the initiation or extension phase of AKI. However, once the injury is initiated and the extension phase starts, the impact of volume expansion with intravenous fluids on clinical outcomes has not been well described and needs to be balanced with the unwanted consequence of fluid accumulation and overload.

Although there are no specific guidelines for optimizing hemodynamic and fluid status for renal function preservation, extrapolation of data from clinical settings associated with AKI can be instructive. To improve the evaluation of volume status, international guidelines for management of sepsis from the Surviving Sepsis Campaign recommends invasive monitoring with measurements of central venous pressure and venous oxygen saturation (superior vena cava or mixed) based on the early goal-directed therapy approach.[147] The Rivers study randomized patients with severe sepsis or septic shock to receive 6 hours of standard therapy, or 6 hours of early goal-directed therapy, before admission to the intensive care unit. The protocol ensured that all patients had a central venous pressure of between 8 and 12 mm Hg, a mean arterial pressure of >65 mm Hg, and a urine output of >0.5 mL/kg/min, by the administration of 500-mL boluses of crystalloid or colloid and vasopressor agents as necessary. Early goal-directed therapy patients received a central venous catheter capable of measuring $ScvO_2$, and they had to achieve a $ScvO_2$ of >70%, pursued by red blood cell (RBC) transfusion for anemic patients (hematocrit, <30%) and dobutamine therapy for patients above that threshold. This initial approach, applied for 6 hours, reduced the mortality rate, need of mechanical ventilation and vasopressors, and length of hospital stay.[147]

A number of studies have since established the benefits of adequate fluid expansion and earlier vasopressor administration for rapid shock reversal.[148] However, data from recent studies have shown that fluid expansion should be stopped when patients are no longer fluid responsive.[149] Late and prolonged aggressive resuscitation in critically ill patients is associated with fluid overload and worse outcomes. Data from the Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network indicate that after initial resuscitation, a conservative approach to fluid administration was associated with faster weaning from mechanical ventilation and decreased length of ICU stay, without any deterioration of kidney function or worse kidney outcomes.[149] A liberal fluid approach as part of early goal-directed therapy appears to be beneficial during the first 6 hours of shock, and a conservative approach should be followed after shock resolution. In AKI patients, in the absence of shock, it is still unknown if these same principles apply. The potential risks of fluid accumulation and volume overload in the setting of AKI need to be considered.[110,150]

Another issue in critically ill patients is determining the optimal fluid to use for resuscitation. The recent Saline versus Albumin Fluid Evaluation (SAFE) trial in 6,997 patients found that fluid resuscitation with saline or albumin resulted in similar relative risks of death in critically ill patients.[151] There were also no significant differences in the proportion of patients with new single-organ and multiple-organ failure, length of ICU stay, length of hospital stay, days of mechanical ventilation, or days of renal replacement therapy.[151] In patients with cirrhosis and spontaneous bacterial peritonitis, intravenous albumin (1.5 g per kg at diagnosis followed by 1 g per kg on day 3) decreased the frequency of AKI (defined as a 50% greater increase in pretreatment BUN or serum creatinine to levels >30 and 1.5 mg per dL, respectively) from 33% to 10% ($P = .002$).[152]

Hydroxyethylstarches (HESs) are the most used nonprotein intravascular volume expanders. In addition to their efficiency in fluid management they have anti-inflammatory properties and reduced cost compared with albumin. However, the physicochemical characteristics and the electrolyte composition of the solvent make these coumponds a potential risk to alter coagulation and platelet function. Another concern with the use of HES is the development of AKI. There is a risk of urine hyperviscosity and consequent tubular lumen obstruction, leading to a tubular lesion called "osmotic nephrosis." The HES solutions have different nephrotoxicty potential depending on the degree of substitution at carbons 2 and 6 in the glucose ring in combination with the molecular weight and molar substitution. They are identified by three numbers which indicate the concentration of the solution; the mean MW (kDa); and, most significantly, the molar substitution (e.g., 10% HES 200/0.5). More recent data using a third generation of HES, 130/0.4, have reported no adverse

effects on renal function in patients who are considered to be at higher risk—for example, those with mild to severe renal dysfunction, advanced age, or on high-dose therapy. In patients at risk for AKI, renal function should be closely monitored when these agents are utilized, and the newer generation of isooncotic HES (130/0.4, 6%) should be preferred.

Prevention of Contrast Medium Nephropathy

Common among the various protocols is the need to establish and maintain an adequate hydration status. To prevent contrast medium nephropathy (CIN) low-risk patients should increase their oral fluid intake and high-risk patients should receive intravenous hydration. Hydration with isotonic saline starting the morning of the procedure, or immediately before in cases of emergency interventions, is superior to half-isotonic (0.45%) saline.[153] A randomized, controlled trial (RCT) compared isotonic saline with isotonic sodium bicarbonate (154 mmol per L $NaHCO_3$ in 5% dextrose) at 3 mL/kg/hr starting 1 hour preprocedure followed by 1 mL/kg/hr for the 6 hours after the procedure. CIN was significantly lower in the bicarbonate group, 2% versus 14% in the saline solution group.[154] The mechanism for the superiority of isotonic bicarbonate over isotonic saline is unclear. Animal studies have shown that bicarbonate is capable of scavenging reactive oxygen species, and the increased pH in the proximal tubule and the renal medulla associated with bicarbonate administration could reduce generation of superoxide. In addition, isotonic saline contains high amounts of chloride with a potential vasoconstrictor effect on renal vasculature. Considering that most hydration studies using isotonic bicarbonate use shorter infusion protocols (only 1 hour) than those using isotonic saline (usually 12 to 24 hours), hydration with bicarbonate is also an attractive alternative in the setting of emergency procedures.

Joannidis et al.[155]conducted a meta-analysis to address discordant results of trials evaluating the efficacy of bicarbonate. Although they confirmed that bicarbonate therapy is more effective in preventing contrast media-induced nephropathy, the study's heterogeneity and publication bias were substantial, preventing clear and definitive conclusions.

Iodinated contrast medium (CM) can be categorized according to osmolality into high-osmolal CM (HOCM; ~2,000 mOsm/kg), low-osmolal CM (LOCM; 600–800 mOsm/kg), and iso-osmolal CM (IOCM; 290 mOsm/kg). Evidence to date suggests that the iso-osmolal, nonionic CM are the least nephrotoxic and should therefore be used in patients at high risk for CIN.

The volume of contrast administered is also a crucial risk factor and an independent predictor of CIN. Based on the volume of contrast given [V] and the creatinine clearance [CrCl], a V/CrCl ratio >3.7 was a significant and independent predictor of CIN in the general population. Administration of contrast more than once in a short period of time is another risk factor, and contrast studies should be postponed at least 48 hours after the last infusion of contrast if possible.

Acetylcysteine

N-acetylcysteine (NAC) is a tripeptide analogous to glutathione able to cross cellular membranes. NAC may reduce vasoconstriction and oxygen free radical generation following contrast administration. Because an increased production of free radicals by the kidneys is partly responsible for their cellular damage in postischemic and nephrotoxic AKI, several clinical studies have attempted to use NAC to prevent or attenuate AKI.

In the first study NAC, a dose of 600 mg orally twice daily the day before and the day of the procedure prevented AKI following radiocontrast administration. Since then there has been ongoing debate as to whether NAC is effective for preventing CIN. Marenzi et al. confirmed the preventive and the dose-dependent effect of NAC in CIN prevention in a large single-center RCT.[156] However, in a large randomized study assessing the efficacy of NAC in preventing CIN (487 patients), intravenous NAC 500 mg did not provide renal protection in patients with impaired renal function compared with placebo.[157] Recent meta-analyses concluded that NAC, compared to periprocedural hydration alone, could lower the risk of CIN in high-risk patients. Therefore, NAC use is recommended based on its potential benefit, low cost, and excellent side effect profile. However, NAC should never replace IV fluids which have a more substantial benefit. In practice, we combine both hydration and NAC in patients at risk for CIN.

Prevention of Drug- and Nephrotoxin-Induced Acute Kidney Injury

Amphotericin B

Amphotericin B (AmB) associated nephrotoxicity can occur in as many as one third of treated patients and the risk of AKI increases with higher cumulative doses. Lipid formulations seem to cause less nephrotoxicity compared with the standard formulation, AmB deoxycholate. Amphotericin lipid complex, liposomal AmB, and AmB colloidal dispersion are significantly less nephrotoxic than amphotericin B deoxycholate; however, there are no conclusions in the comparison of AmB colloidal dispersion nephrotoxicity to other lipid formulations. The use of these formulations can help to preserve renal function in patients with systemic fungal infections; still, they are significantly more expensive. Recently, alternative antifungal agents such as itraconazole, voriconazole, and caspofungin have been more commonly used in patients at high-risk for AKI.

Angiotensin-Converting Enzyme Inhibitors, Angiotensin Receptor Blockers, and Nonsteriodal Anti-inflammatory Drugs

ACE inhibitors and angiotensin receptor blockers (ARBs) cause vasodilatation of the efferent glomerular arteriole, further reducing intraglomerular pressure already compromised by the blood pressure lowering effect of these agents.

In patients with renal dysfunction they can contribute to reducing the GFR. In patients with an increase in SCr higher than 30% after the initiation of ACE inhibitor and ARB treatment, bilateral renal artery stenosis, stenosis of the renal artery in a solitary kidney, or diffuse intrarenal small vessel disease should be suspected and these drugs should be discontinued. Although there is very limited information it is generaly advisable to discontinue ACE/ARB during an AKI episode. However, these decisions need to be individualized and ACE/ARB may be restarted when there is recovery of renal function in order to support other organ function (e.g., heart failure).

NSAIDs or COX-2 inhibitors should be used with caution in patients with atherosclerotic cardiovascular diseases, CKD, liver disease, and intravascular volume depletion. As NSAIDs cause acute inhibition of cyclo-oxygenase (COX, type I or II), they can reduce GFR and renal blood flow. In critically ill patients, renal hypoperfusion due to decreased effective arterial volume is relatively common, and inhibition of prostaglandin-induced vasodilation by these agents may further compromise renal blood flow and exacerbate ischemic injury.

Aminoglycosides

Clinical evidence of AKI due to aminoglycoside nephrotoxicity usually occurs 5 to 10 days after initiation of the treatment, is typically nonoliguric, and is associated with decreased urine concentrating ability and urinary magnesium wasting. With multiple daily dosing schedules, elevated aminoglycoside peak levels appear to correlate with nephrotoxicity. Because aminoglycoside uptake by proximal tubular cells is a saturable process, once-daily dosing can decrease tubular cell toxicity by reducing the amount of drug taken up by proximal tubular cells. In the general population extended intervals between doses maintains the target dose while decreasing the risk of nephrotoxicity compared with multiple daily dosing. However, intensive care patients have different volumes of distribution and variable clearance, thereby making it difficult to maintain correct serum levels with longer intervals. As these drugs are entirely excreted by glomerular filtration, patients with compromised renal function are at increased risk for nephrotoxicity. In these patients the administration of a large single dose can be associated with a decreased uptake and lower antimicrobial effect.[158] Therefore, to treat serious infections in critically ill patients, dosing with maximum concentration (C_{max}), monitoring, and minimal inhibitory concentration (MIC) evaluation of the pathogen are necessary.[158]

Uric Acid Nephropathy and Tumor Lysis Syndrome

Acute uric acid nephropathy is caused by deposition of uric acid crystals in the interstitium and tubules associated with tumor lysis syndrome (TLS). The early recognition of patients at high risk for TLS is the first step to prevent the development of AKI. In patients with high-grade hematologic malignancies, risk factors for TLS are large tumor burden, lactate dehydrogenase levels above 1,500 IU, extensive bone marrow involvement, and high tumor sensitivity to chemotherapeutic agents. In patients with low or intermediate risk of TLS, allopurinol can be used as a hypouricemic agent and should be started 2 days before chemotherapy. Aggressive hydration with isotonic saline is initiated 2 days before the chemotherapy to maintain a high urinary output, allowing the elimination of uric acid and phosphate. If urinary output decreases despite adequate fluid intakes, a loop diuretic should be added, but renal replacement therapy will be required if oliguria persists.[159] The use of urine alkalinization to promote elimination of urates is not recommended as it can induce calcium phosphate deposition and therefore aggravate TLS. In addition to the hydration, recombinant urate oxidase can reduce uric acid levels and the risk of uric acid deposition nephropathy.[160] Recombinant urate oxidase should be initiated in high-risk patients or for established TLS.

MANAGEMENT OF ACUTE KIDNEY INJURY

After the kidney insult has occurred measures should be directed to avoid further injury, facilitate repair and recovery, and prevent AKI complications (Fig. 28.10). The timing of interventions is crucial to their effectiveness. Various approaches have been applied but are best appreciated in the context of specific scenarios. Initial management includes careful assessment of the etiology of kidney dysfunction and patient volume status. The main goals are maintenance of adequate hemodynamic status to ensure renal perfusion and avoidance of further kidney injury. Appropriate therapeutic interventions to reduce kidney function loss, prevention, and treatment of the associated complications of AKI need to be instituted concurrently. Any potentially nephrotoxic agents should be avoided, including intravascular radiocontrast. Antimicrobial agents such as aminoglycosides, amphotericin, acyclovir, and pentamidine should be avoided or their doses adjusted to avoid further insult.

Fluid and Electrolyte Management

Although early and vigorous resuscitation with crystalloid solutions and aggressive infection control can reduce the incidence of AKI (see previous), the role of fluid resuscitation in established AKI is less clear. Volume status is one of the most difficult parameters to assess and fluid resuscitation should target a predefined preload, stroke volume, or cardiac output rather than a set mean arterial pressure. Many clinical studies have demonstrated the poor value of right atrial pressure and pulmonary artery occlusion pressure in predicting volume expansion efficacy. Other bedside indicators of preload, such as the right ventricular end-diastolic volume (evaluated by thermodilution) and the left ventricular end-diastolic area (measured by echocardiography), have also been shown to be ineffective in differentiating volume

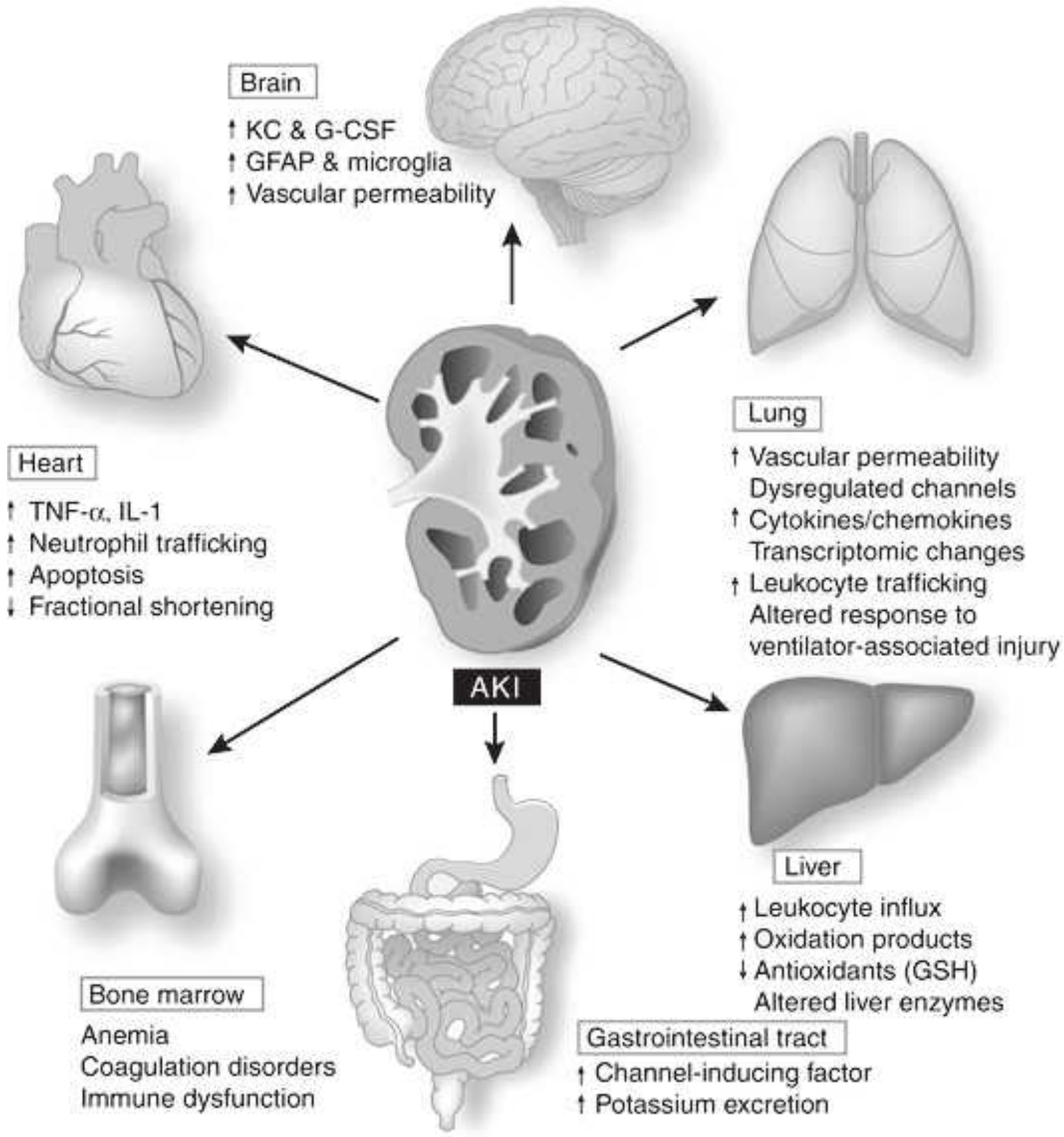


FIGURE 28.10 Acute kidney injury (AKI)-induced distant organ effects. AKI leads to changes in distant organs, including brain, lungs, heart, liver, gastrointestinal tract, and bone marrow. Changes have been described in organ function, microvascular inflammation and coagulation, cell apoptosis, transporter activity, oxidative stress, and transcriptional responses. *AKI*, acute kidney injury; *G-CSF*, granular colony-stimulating factor; *GFAP*, glial fibrillary acidic protein; *GSH*, glutathione; *IL-1*, interleukin-1; *KC*, keratinocyte-derived chemokine; *TNF-α*, tumor necrosis factor-α. (From Scheel PJ, Liu M, Rabb H. Uremic lung: new insights into a forgotten condition. *Kidney Int*. 2008;74(7):849–851.)

responder from nonresponder patients.[161] In critically ill patients receiving mechanical ventilation, respiratory changes in left ventricular stroke volume can predict fluid responsiveness. In hypovolemic patients, positive-pressure ventilation may induce a fall in the venous return and, consequently, in cardiac output. Based on the positive relationship between ventricular end-diastolic volume and stroke volume, the expected hemodynamic response to volume expansion is an increase in right ventricular end-diastolic volume, left ventricular end-diastolic volume, stroke volume, and cardiac output. Because a decrease in ventricular contractility decreases the slope of the relationship between end-diastolic volume and stroke volume, the increase in stroke volume as a result of end-diastolic volume increase depends on ventricular function.

Volume expansion in critically ill patients can frequently result in a relative increase in body weight of 10% to 15% or more, sometimes doubling the total body water in a short period of time. Some recent studies have demonstrated the role of fluid accumulation on adverse outcomes showing the association between fluid accumulation and mortality and the benefits of restrictive fluid management strategies in acute respiratory distress syndrome (ARDS). A prospective multicenter observational study (PICARD) found that patients with fluid overload, defined as increase in body weight relative to baseline >10%, had significantly higher mortality at 60 days (46% vs. 32%).[110] In addition, increases in the total body water alter the volume of distribution of creatinine resulting in underestimation of serum values.[34] The resulting underestimation of the severity of renal dysfunction may delay recognition and adequate treatment of AKI. In AKI patients presenting with fluid overload the evaluation of kidney function should consider the effect of fluid balance in order to prevent underestimation of AKI severity, correctly modify drug dosing, and avoid use of nephrotoxic agents.

In patients with positive fluid balance, large fluid intakes, and inadequate urine output, loop diuretic therapy can be initiated in conjunction with measures to optimize systemic and kidney perfusion. Although administration of furosemide facilitates fluid management, concerns of possible harm from loop diuretics in AKI surfaced after studies showed that associated diuretic use had an increased adjusted risk of death and nonrecovery of renal function. In urgent situations, morphine and nitrates can be used to alleviate the respiratory symptoms. Morphine reduces patient anxiety and decreases the work of breathing; it can be administered intravenously at an initial dose of 2 to 4 mg over a 3-minute period and can be repeated if necessary at 5- to 15-minute intervals. Nitrates are the most commonly used vasodilators in pulmonary edema. Nitroglycerin reduces left ventricular filling pressure via venodilation and an initial dose of 5 μg per minute of intravenous nitroglycerin is commonly used

in addition to diuretic therapy. When fluid overload cannot be quickly treated with medical management, positive ventilation pressure may need to be initiated with or without endotracheal intubation, and dialysis depends on the clinical situation.

Loop Diuretics and Natriuretics

Although loop diuretics are often prescribed in established AKI,[162] a recent meta-analysis confirmed that their use is not associated with reduced mortality or better kidney recovery.[163] Two prospective cohort studies evaluating diuretic use in AKI and mortality yielded controversial results with one study showing an increase[164] and the other study showing no effect.[165] However, an association between diuretic use and a shorter duration of dialysis was found in this meta-analysis.[163] Still, two other meta-analyses have shown that loop diuretics do not affect mortality, need for dialysis, or number of dialysis sessions required.[166] In regard to morbidity, diuretics are associated with an increased risk of ototoxicity.[166] Concomitant prescription of aminoglycosides and diuretics should be avoided due to an increased risk of ototoxicity. Well-designed trials of diuretics are required to assess their benefits and potential side effects in AKI. In the meantime, we suggest that a trial of diuretics may be utilized to enhance urine output; however, if this approach is not successful, escalating doses of diuretics should be avoided.

Atrial natriuretic peptide (ANP) has been studied as a treatment for AKI in four RCTs.[45,167–169] ANP was shown to reduce need for dialysis but did not reduce mortality.[45] In the largest study published so far, ANP improved overall dialysis-free survival in the subgroup of oliguric patients.[167] A subsequent trial, including 222 oliguric patients, did not confirm that ANP reduces mortality or dialysis-free survival.[169] The most recent study evaluated the use of ANP treatment for a mean of 5.3 ± 0.8 days in 61 patients who underwent cardiac surgery. The use of ANP decreased the probability of dialysis and improved dialysis-free survival.[168] Larger studies are required to confirm the benefits of ANP in AKI.

Nesiritide, a B-type natriuretic peptide, is currently approved by the FDA for the treatment of heart failure. Nesiritide induces vasodilation and indirectly increases cardiac output, having no inotropic or heart rate effect. In some individuals, a resultant decrease in the neurohormonal activation can result in natriuresis and diuresis. In adults with acute decompensated heart failure, nesiritide reduces pulmonary capillary wedge pressure, reduces right atrial pressure and systemic vascular resistance, decreases symptoms of heart failure, and enhances clinical status. However, questions regarding the risks of nesiritide therapy have recently been raised. The most frequently reported adverse effect is dose-related hypotension and an acute increase in SCr concentration. This effect in kidney function has not been shown to negatively affect mortality and reviews of large, observational, registry databases do not suggest an adverse inpatient mortality effect compared with other vasodilator therapies.[170]

Vasoactive Agents

"Renal-dose" dopamine (0.5 to 3 μg/kg/min), given as a specific vasodilatator to increase renal blood flow and prevent AKI, does increase urine output but does not affect AKI outcome or mortality.[171] Dopexamine, a synthetic dopamine analogue, is a dopamine 1 and less potent dopamine 2 receptor agonist. Small studies performed in patients undergoing liver transplant surgery have not found a beneficial effect of dopexamine in preventing AKI.

No RCTs have assessed the effect of norepinephrine on prevention of AKI. In a meta-analysis, fenoldopam, a dopamine receptor-1 agonist increasing blood flow to the renal cortex and outer medulla, was shown to reduce the risk of AKI in postoperative or critically ill patients (odds ratio, 0.43).[172] A large RCT will need to be performed to confirm these findings. Intrarenal administration of fenoldopam allows the use of a substantial dose of fenoldopam mesylate while avoiding systemic adverse effects such as hypotension. In a registry of 268 patients treated with intrarenal fenoldopam, infused for at least 1 hour, the incidence of CIN was less than 1%, compared to 27% based on historic rates in that population. Although we are still waiting for additional studies to confirm these results, it may be a promising preventive measurement for patients at high risk of CIN.

Vasopressors are often considered to be detrimental for organ perfusion. However, experimental and clinical data suggest a beneficial effect of norepinephrine on the urine output in sepsis. A small prospective study including 14 patients in septic shock showed that norepinephrine improved SCr and creatinine clearance when mean arterial pressure was raised above 70 mm Hg.[173] However, in a small RCT including 28 patients, increasing mean arterial pressure from 65 to 85 mm Hg with norepinephrine did not improve renal function.[174]

Fenoldopam has shown improved outcomes in some studies. In a recent meta-analysis, fenoldopam decreased the need for dialysis (7% vs. 10%) and in-hospital mortality (15 vs. 19%) in ICU patients.[172] However, this meta-analysis had limitations, such as no standardized criteria for initiation of dialysis, heterogeneity of populations and AKI definitions, dosage and duration of treatments, and the absence of independent measure of GFR. An adverse effect of fenoldopam is hypotensive episodes, and it may be more frequent and deleterious outside RCTs.[172,175] These results show that, although widely promoted, the use of fenoldopam needs to be confirmed with an adequately powered trial.

Avoidance of Hyperglycemia

Effective management of hyperglycemia in critically ill patients has been a major topic of discussion since a landmark study demonstrated a significant reduction in mortality and morbidity in surgical patients who were treated with an intensive regimen to control blood glucose. Subsequent studies have highlighted the importance of hyperglycemia for adverse outcomes in various populations and proposed algorithms

for glycemic control. A systematic review of intensive insulin therapy in critically ill patients found a 38% reduction in the incidence of AKI, whereas other negative trials showed no benefit and an increased risk of hypoglycemia.[8]

In critical illness membrane expression of GLUT-1, GLUT-2, and GLUT-3 proteins is upregulated and allows glucose to enter cells more in proportion to extracellular glucose levels. This contributes to glucose overload in several tissues, including brain neurons, hepatocytes, endothelial cells, and renal tubules. These events are associated with various cytokines (tumor necrosis factor α [TNF-α] and interleukin 6 [IL-6]), hormones (cortisol, catecholamines, and growth hormone), and other molecules (vascular endothelial growth factor and transforming growth factor [TGF]) that are also upregulated in renal failure.

The kidney plays an important role in glucose homeostasis. In humans renal glucose production contributes approximately 25% to systemic glucose production, whereas renal glucose uptake accounts for 20% of systemic glucose removal. Because glucose homeostasis in the kidney is regulated by insulin, loss of kidney metabolic function could account for a component of insulin resistance as a result of loss of a major target organ for insulin action. Uremia is also associated with decreased hepatic and peripheral glucose uptake and a reduction in peripheral tissue glucose transporters. The kidney also metabolizes insulin and reduced renal function prolongs the half-life of insulin and can contribute to hypoglycemic events. One of the major risk factors for development of hypoglycemia in the ICU is the presence of preexisting renal dysfunction and the need for kidney replacement therapy.

Some studies have shown that maintaining blood glucose levels around 110 mg per dL reduced the onset of acute kidney injury from 12.3% to 9% ($P = .04$) and need for dialysis by 41%. Whereas the lowered blood glucose level was related to reduced mortality and other complications, the insulin dosage was an independent determinant for prevention of AKI.[176] Two large intervention studies in medical and surgical ICU patients confirmed a similar association and found that the development of newly acquired AKI decreased by 75% and 45%, respectively.[177] In a large observational study, patients who did and did not have diabetes and required glycemic control had more infections, anemia, and AKI (11% and 7% versus 4%; $P < 0.001$) compared with control subjects.[178] Additional observational studies from different populations suggest a linkage of hyperglycemia and the metabolic syndrome with the development of AKI. Most of these studies used a doubling of creatinine or a creatinine level >2.5 mg per dL as a criterion for AKI; however, a more sensitive criterion (0.5 mg per dL creatinine change) would likely increase the incidence of AKI. Whether these associations are simply a consequence of the deranged metabolic milieu that accompanies critical illness or there is a direct effect of hyperglycemia and insulin resistance on the kidney still needs more evaluation.

Although several pieces of the puzzle linking hyperglycemia and kidney function are still missing, there is enough evidence now to suggest that the kidneys are active in the process and a target for new injury. On the basis of these conclusions hyperglycemia should be considered a major risk factor for AKI in the ICU and should prompt specific measures. Clinicians should seek out a history of hyperglycemia as part of the evaluation of critically ill patients who are at risk or develop AKI and institute preventive and therapeutic measures.

However, achieving glycemic control is not easy and hypoglycemic events are commom in AKI patients. In a recent large, international, randomized trial in critically ill patients (NICE sugar study), intensive glucose control increased the absolute risk of death to 90 days compared to conventional glucose control. Severe hypoglycemia was significantly more common with intensive glucose control. In a meta-analysis including 26 trials, a total of 13,567 patients, and data from the NICE sugar study, the relative risk (RR) of death with intensive insulin therapy compared with conventional therapy was 0.93. Patients in surgical ICUs presented a benefit from intensive insulin therapy (RR 0.63), whereas patients in medical ICU did not (RR 1.0).

Based on these recent studies it appears that intensive insulin therapy significantly increased the risk of hypoglycemia and is not associated with a benefit in mortality among critically ill patients. Whether there is a benefit in preventing or ameliorating AKI is still unclear. We would recommend maintaining appropriate control of blood glucose in the 120 to 140 mg per dL range.

Protective Mechanical Ventilation

Mechanical ventilation is associated with the disruption of pulmonary epithelium and endothelium, lung inflammation, atelectasis, hypoxemia, and the release of inflammatory mediators.[179,180] These inflammatory mediators can cause injury to lungs and other organs.[181] Traditional approaches to mechanical ventilation use tidal volumes of 10 to 15 mL per kg of body weight and may cause stretch-induced lung injury in patients with acute lung injury.[182,183] Treatment with a ventilation approach designed to protect the lungs from excessive stretch resulted in improvements in several important clinical outcomes in patients with acute lung injury and ARDS.[183] Thus, in mechanically ventilated patients an important preventive measure is to avoid excessive lung stretch during adjustments to mechanical ventilation, and a lower tidal volume protocol should be used in patients with acute lung injury and ARDS.

Mechanical ventilation has been shown to be an important independent factor for mortality in AKI patients, and the time on mechanical ventilation is also associated with increased mortality.[10] In addition, AKI is a risk factor for prolonged mechanical ventilation.[184] Patients with prolonged mechanical ventilation are predisposed to pulmonary infections, and infection is the leading cause of death in patients with AKI.

In patients in need for renal support, removal of fluid with ultrafiltration can improve pulmonary edema resulting in better oxygenation.[122] However, in these patients, remov-

ing fluid faster than interstitial fluid can be mobilized into the circulation can induce hypotension and contribute to prolonged AKI. A judicious rate of fluid removal should be individualized to the clinical status of the patient in order to avoid this vicious cycle.

Pharmacologic Approaches

A variety of drugs are effective in altering the course of experimental models of ATN. However, only a few have consistently shown benefits in preventing or attenuating established AKI (Table 28.10).

Statins

Statins induce downregulation of angiotensin receptors, decrease endothelin synthesis, decrease inflammation and improve endothelial function by inhibiting nuclear factor κB, decrease expression of endothelial adhesion molecules, increase nitric oxide bioavailability, attenuate production of reactive oxygen species, and protect against complement-mediated injury. All of these mechanisms may be involved in the protective effect against CIN. A number of publications support the potential for kidney protection with statin administration.[185] A recent prospective study evaluated the effect of statins to decrease the incidence of CIN during percutaneous coronary intervention. Patients receiving statins prior to the procedure had a significant decrease of CIN (3% vs. 27%).[186] However, in a retrospective cohort study evaluating patients undergoing major vascular procedures,[187] perioperative statin administration did not improve renal function, reduce length of stay, or reduce mortality. No benefit was observed in patients with a preexisting creatinine clearance <40 mL per min. Currently, there is no basis to recommend the initiation of statin therapy specifically for the pericontrast period to prevent CIN. Patients who are already on statin therapy, or need it for other indications, should be maintained on statins through contrast procedures.

Calcium Channel Blockers

Calcium antagonists have been shown to reverse the afferent arteriolar vasoconstriction induced by a variety of stimuli and also have an independent natriuretic effect.[188] These drugs were exhaustively evaluated in the prevention of AKI, especially in the context of transplant-associated nephropathy. If administered prophylactically calcium blockers protected against posttransplantation delayed graft failure in some studies. However, a large multicenter RCT evalu-

28.10 Drugs Tested in Clinical Studies for Prevention or Treatment of Acute Kidney Injury

Drugs	Results
Prevention	
Dopamine	No effect on kidney function
Fenoldopam	Controversy: no effect on kidney function or beneficial effect on kidney function
Loop diuretics	No effect on kidney function
N-acetylcysteine	Variable beneficial effect in contrast-induced nephropathy
Statins	Beneficial effect on kidney function
Calcium channel blockers	No effect on kidney function
Adenosine antagonists	Controversial effect on kidney function
Multipotent stem cells	Beneficial effect on kidney function in Phase 1 studies
Erythropoietin	Uncertain based on a large Phase 2/3 trial in intensive care unit patients
Small interfering ribonucleic acid	Beneficial effect on kidney function, undergoing Phase 2/3 trials
Treatment	
Loop diuretics	No effect
Atrial natriuretic peptide	Possible beneficial effect on survival and kidney function
Dopamine	No effect on mortality or kidney function
Norepinephrine	Possible beneficial effect on kidney function
Fenoldopam	Controversy: No effect on mortality or kidney function Beneficial effect on mortality and need for dialysis
Insulin	Controversial effect
Mesenchymal stem cells	Beneficial effect on kidney function
Erythropoietin	Beneficial effect on kidney function
Alkaline phosphatase	Beneficial effect on kidney function

ating the effect of isradipine on renal function, incidence and severity of delayed graft function, and acute rejection after kidney transplantation did not find any benefit.[189] A systematic review evaluated the benefits and harms of using calcium channel blockers in the peritransplant period in patients at risk of ATN after cadaveric kidney transplantation.[190] The authors suggested calcium channel blockers given in the perioperative period reduced the incidence of ATN posttransplantation. However, the heterogeneity of the trials makes the comparison of studies difficult. The use of calcium channel blockers during renal transplant surgery may be of benefit in extended donor criteria transplants (e.g., donors older than 60 years, predonation SCr level higher than 1.5 mg per dL, cerebrovascular disease as the cause of death), or those with prolonged ischemia times.

Adenosine Antagonists

Small clinical studies evaluating the role of theophylline, an adenosine antagonist, in the prevention of contrast nephropathies have shown discordant results. A recent study, including seven RCTs, concluded that the prophylactic administration of theophylline or aminophylline appeared to protect against CIN.[191] However, this meta-analysis included studies that did not control for hydration status. A recent RCT adding theophylline to NAC showed a reduced incidence of CIN. Additional selective blocker agents, such as rolofylline, have maintained renal function in patients with decompensated heart failure, although they have not been assessed for prevention of AKI. At the moment it remains unclear if theophylline as a solo agent might be useful in preventing contrast nephropathy. Further studies are necessary prior to its routine use.

Disorders of Electrolyte and Uric Acid Metabolism

Hyperkalemia, hyponatremia, metabolic acidosis, and hyperuricemia often occur in AKI.

Potassium

A rise in plasma potassium concentrations to >5.5 mEq per L is a frequent complication seen in 50% of patients with AKI. Hyperkalemia is due to continued potassium release from cells, or dietary potassium, in the face of impaired renal potassium elimination. The potassium concentration of intracellular water is about 155 mEq per L in skeletal muscle. Thus, in conditions such as tumor lysis syndrome and rhabdomyolysis, dangerous levels of hyperkalemia can occur quickly. In patients with rhabdomyolysis induced by extensive traumatic muscle crush injury, plasma potassium concentrations can rapidly increase from normal to life-threatening levels. Other factors including a cellular shift of potassium due to academia; hyperosmolality or potassium loads from exogenous sources such as blood, dietary intake, potassium salts (e.g., salt substitutes); or large doses of penicillin G can also contribute to hyperkalemia. AKI induced by NSAIDs can also be associated with marked hyperkalemia. The effect of these agents in suppressing renin and aldosterone secretion may be responsible in part.

The primary risk of hyperkalemia is on cardiac conduction where it may cause bradycardia or asystole. If echocardiogram (ECG) changes are present, the administration of intravenous calcium is urgent. Concomitantly, sources of oral or intravenous potassium should be identified and removed, including drugs with effect on potassium handling such as beta-adrenergic antagonists, potassium-sparing diuretics, ACE inhibitors, ARBs, and other drugs that inhibit renal potassium excretion.

The next step is to enhance the shift of potassium to the intracellular space using parenteral glucose and insulin infusions. The onset of action is within 20 to 30 minutes, and the effect lasts for 2 to 6 hours. Continuous infusions of insulin and glucose-containing intravenous fluids can be used to prolong their effect. Sodium bicarbonate also promotes shift of K^+ into the intracellular space, the effect occurs in less than 15 minutes, and has 1 to 2 hours' duration. This therapy can be started if there is no concern of fluid overload (44.6 mEq intravenously over 5 minutes); however, the potassium-lowering effect of sodium bicarbonate is most prominent in patients with metabolic acidosis. Beta-adrenergic agonists given as aerosols are also effective but more likely to produce side effects and so are not often prescribed to treat hyperkalemia.

Potassium excretion should be increased by the administration of loop diuretics and cation exchange resins, such as Kayexalate or calcium resonium. The resins can be administered orally or rectally, as a retention enema. In case of hyperkalemic emergencies, rectal administration is preferred, as the colon is the major site of action of this drug. If hyperkalemia is unresponsive to conservative measures, or occurs in patients with ESRD, emergency hemodialysis is the treatment of choice. As it may take some time to initiate RRT, medical management should always be used while waiting for dialysis to be started. Monitoring for potassium levels should continue following conservative or dialytic management to prevent and treat rebound hyperkalemia from the underlying process.

Acid-Base Disorders

In AKI, metabolic acidosis is the most common acid-base abnormality. The metabolic acidosis results from continued production of nonvolatile acid and decreased renal ability to excrete acid. In severe catabolic states, the usual daily production of 1 mEq per L of nonvolatile acid can be markedly increased. Accumulation of phosphate and unexcreted unmeasured anions—such as sulfate, urate, hippurate, hydroxypropionate, furanpropionate, and oxalate—are contributory. Hypoalbuminemia can attenuate this acidification process, and it is exacerbated by lactic acidosis. Despite retention of unmeasured anions, the anion gap remains within

normal limits in 50% of patients. Although metabolic acidosis is frequent, triple acid-base disturbances can also occur.

The approach to acid-base disturbances in AKI needs to be adjusted to the underlying causes. There is controversy surrounding the optimal treatment of acute metabolic acidosis. When metabolic acidosis is simply a complication of AKI, sodium bicarbonate can be administered if the serum bicarbonate concentrations fall below 15 to 18 mmol per L. Bicarbonate administration in lactic acidosis due to an underlying shock is controversial given the possibility of an increase in CO_2 generation, worsening of the intracellular acidosis, and volume overload. Rapid improvement in the metabolic status may also enhance hypocalcemia, which may lower cardiac output. Therefore, since the benefit of bicarbonate in patients with lactic acidosis due to an underlying shock seems limited, most physicians would restrict the administration of sodium bicarbonate to patients with severe metabolic acidosis (arterial pH below 7.10 to 7.15) to maintain the pH above 7.15 to 7.20 until the primary process can be reversed. Alternative forms of base treatment have not been studied extensively in patients with AKI. Tris(hydroxymethyl)aminomethane (THAM) is excreted in the urine and its clinical efficacy compared to sodium bicarbonate remains unproven.[192] We do not recommend its use in patients with AKI, especially in patients with hyperkalemia, because THAM does not decrease potassium levels in contrast to bicarbonate and can even cause hyperkalemia. Restriction of protein intake has also been suggested as a method of acidosis control since protein breakdown has been associated with worsening acidosis.

Sodium

Hyponatremia is a common complication of AKI and is caused by an absolute or relative increase in solute-free water intake. Rare associations with hyponatremia and AKI include toxin ingestion,[193,194] rhabdomyolysis, infection,[195] and hypothyroidism.[19] The treatment consists of water restriction to below the level of output. Salt restriction is usually necessary to treat fluid overload and/or edema. In cases of true volume depletion with associated prerenal AKI, isotonic saline will need to be administered to correct both disorders.

Intensive care patients with hypernatremia are more prone to AKI. In most cases, treatment of the underlying cause will be necessary and water deficit will need to be estimated. Water should be administered orally or intravenously as dextrose in water to correct serum sodium at a maximum rate of 10 mmol/L/day. Dialysis and continuous RRT, in particular, may be required to optimally correct sodium disorders in AKI.

Renal Replacement Therapy

The issues regarding RRT in AKI are currently the source of much debate and investigation. The areas of debate include when to initiate, what modality to use, and the dose of therapy to deliver.

Dialysis Initiation

Whether or not to provide dialytic support, and when to initiate, are two of the fundamental questions facing nephrologists and other intensivists in most cases of severe AKI. The optimal timing of dialysis for AKI is not defined. The association of early initiation of dialysis with survival benefit was first suggested by case series with historical controls conducted in the 1960s and 1970s.[197–199] However, the relevance of these studies to current practice is questionable, given that BUN concentrations at the start of dialysis in the "early" treatment groups in these previous studies are considered high by modern standards. In the modern dialysis era, few studies have examined the association of the timing of initiation of dialysis in AKI with mortality. Moreover, changes in illness severity, especially in later years, make comparisons of studies extremely difficult. Single-center studies that were restricted to AKI after trauma[200] and coronary artery bypass surgery[201,202] suggested a benefit to dialysis initiation at lower BUN concentrations. In a broader population, Bouman et al.[203] randomized 106 critically ill patients with AKI to early versus late initiation of dialysis. The early initiation group started dialysis within 12 hours of low urine output, less than 30 mL per hour for 6 hours, not responding to diuretics or hemodynamic optimization, or creatinine clearance less than 20 mL per minute. The late initiation group started dialysis when classic indications were met. The study did not find differences in ICU or hospital mortality between the groups of early and late initiation, or in renal recovery among survivors. A prospective multicenter observational cohort study[204] performed by the Program to Improve Care in Acute Renal Disease (PICARD) analyzed dialysis initiation—as inferred by BUN concentration in 243 patients from five geographically and ethnically diverse clinical sites. Survival rates were slightly lower for patients who started dialysis at higher BUN concentrations, despite a lesser burden of organ system failure. Adjusting for age, hepatic failure, sepsis, thrombocytopenia, and serum creatinine and stratified by site and initial dialysis modality, initiation of dialysis at higher BUN was associated with an increased relative risk for death (95% CI, 1.16 to 2.96).

Although the maintenance of BUN concentrations below arbitrarily set levels is usually a reference for starting dialysis treatment, BUN reflects factors not directly associated with kidney function such as catabolic rate and volume status. SCr is influenced by age, race, muscle mass, catabolic rate, and its volume of distribution varies on fluid overload patients. Thus, neither creatinine nor BUN should be used to absolutely determine when to initiate dialysis. In a prospective multicenter observational study conducted at 54 intensive care units (ICUs) in 23 countries,[205] timing of RRT was stratified into "early" or "late" by median urea at the time RRT started and also categorized temporally from ICU admission into early (less than 2 days), delayed (between 2

and 5 days), or late (more than 5 days). Timing by serum urea showed no significant difference in mortality (63.4% for urea ≤24.2 mmol per L vs. 61.4% for urea >24.2 mmol per L). However, when timing was analyzed in relation to ICU admission, late RRT was associated with greater crude mortality (72.8% late vs. 62.3% delayed vs. 59% early, P = .001) and covariate-adjusted mortality (OR, 1.95; 95% CI, 1.30–2.92; P = .001). Overall, late RRT was associated with a longer duration of RRT and stay in hospital and higher rate of dialysis dependence.

There are potential safety concerns regarding earlier initiation of dialysis, including increased risk for infection from an indwelling dialysis catheter, hypotension, delayed renal recovery, and leukocyte activation from contact with dialysis membranes.[206,207] The concept that dialysis initiation would prolong the course of AKI was supported by experimental data showing renal lesions consistent with fresh ischemia in dialyzed animals without systemic hypotension, long after their initial renal injury. In the presence of ischemia, the vasculature of normal kidneys responds with vasodilation as part of the autoregulatory response to maintain renal blood flow and GFR. In ATN, autoregulation is impaired; as a result, recurrent ischemic tubular injury is more likely to occur, thereby delaying the restoration of function. However, it is difficult to document that earlier initiation of dialysis is harmful because patients with more severe forms of renal injury may develop indications for dialysis earlier in their ICU course and may be more likely to develop irreversible disease independent of therapy. Several factors can influence the survival and recovery of renal function in dialytic AKI patients. Whether these risks outweigh the potential benefits of earlier initiation of dialysis is still unclear.[207]

In current practice the decision to dialyze is based most often on clinical features of volume overload and biochemical features of solute imbalance (e.g., azotemia, hyperkalemia). Data from an RCT comparing IHD to CRRT suggest that the indication for dialysis is an important determinant of outcome.[208] In that study patients dialyzed predominantly for solute control experienced better outcomes than those dialyzed predominantly for volume overload. Patients dialyzed for control of both azotemia and volume overload experienced the worst outcome. Volume resuscitation is a common strategy used in the treatment of multiorgan failure, particularly when accompanied by sepsis syndrome and hypotension. It is often applied indiscriminately in the setting of oliguric AKI, where it is assumed that providing additional volume will improve renal perfusion, prompting correction of renal dysfunction. Although this may be of great benefit to patients with prerenal azotemia, excessive volume administration can lead to pulmonary edema, compromising oxygenation and ventilation, and hastening the need for dialysis. In critically ill patients, especially in the postoperative period and in septic patients after volume expansion, the increase in total body water can reach more than 10 L within 7 days.[149,209] Mukau et al.[210] found that 95% of their patients with postoperative AKI had fluid excess of more than 10 L at the time of dialysis.

Despite recent evidence suggesting positive fluid balance as possibly harmful for ICU patients, the association between fluid balance and outcomes in AKI patients is not completely defined. These patients are expected to present higher positive fluid balance; however, the impact in the prognosis is poorly understood. Payen et al.[211] analyzed data from the Sepsis Occurrence in Acutely Ill patients (SOAP) study, a multicenter observational cohort study including 198 ICUs. In AKI patients, mean daily fluid balance was significantly more positive among nonsurvivors than survivors (0.98 ± 1.5 versus 0.15 ± 1.06 L per 24 hours, P <0.001). Bouchard et al.[150] found that fluid overload, defined as a >10% increase in body weight relative to baseline, was associated with significantly higher mortality rates at 60 days (46% vs. 32%; P = .006). The adjusted odds ratio for death associated with fluid overload at dialysis initiation was 2.07 (95% CI, 1.27–3.37). In that study, among the dialyzed patients, survivors had lower fluid accumulation at dialysis initiation compared with nonsurvivors (8.8% vs. 14.2% of baseline body weight) (Fig. 28.11).

These factors collectively suggest the need to develop evidence-based, patient-specific, and nonbiased indications for the initiation of dialysis in AKI (Table 28.11). Timing of RRT, a potentially modifiable factor, might exert an important influence on patient survival. However, it largely depended on its definition. We favor utilizing an approach that recognizes that the strategy in treating AKI is to minimize and avoid uremic and volume overload complications. Thus, it is not necessary (and arguably harmful) to wait for progressive uremia to initiate dialytic support. The indications for dialysis should include a consideration of the need for renal support (as well as renal replacement), and the timing of dialysis should be based on the goals to be achieved.

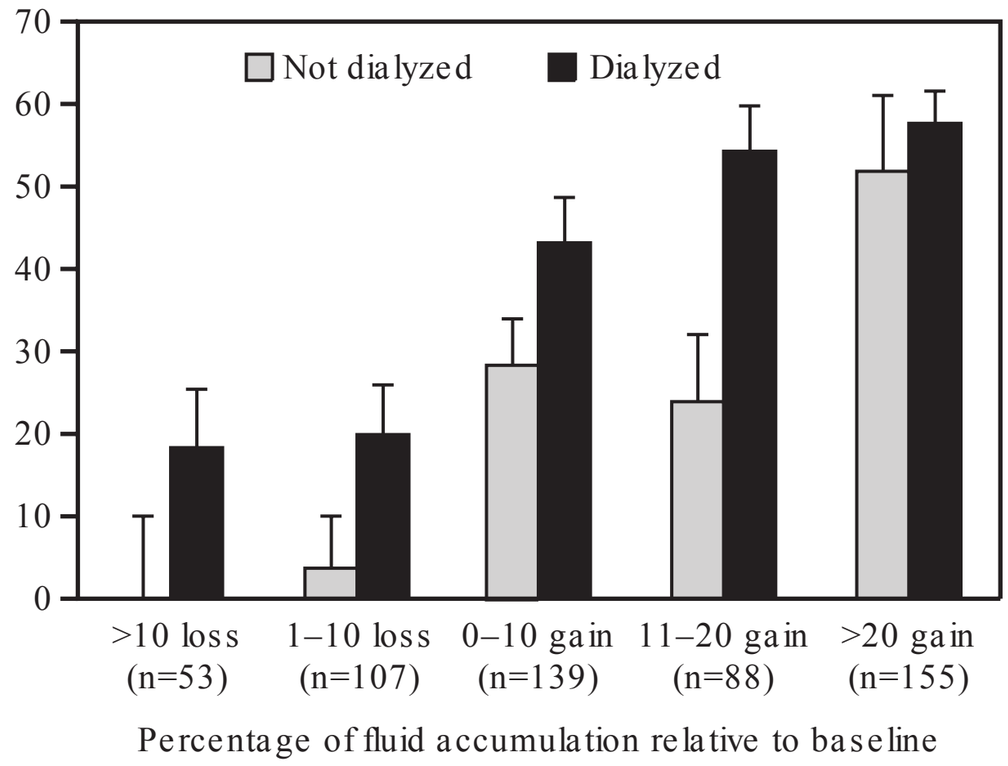


FIGURE 28.11 Mortality rate by final fluid accumulation relative to baseline weight and stratified by dialysis status. (From Bouchard J, Mehta RL. Fluid accumulation and acute kidney injury: consequence or cause. *Curr Opin Crit Care*. 2009;15(6):509–513.)

28.11 Prognostic Factors in Acute Kidney Injury

Severity of Renal Dysfunction
- Magnitude of rise in serum creatinine concentration
- Urinalysis
- Fractional excretion of sodium
- Presence of oliguria or anuria
- Requirement for renal replacement therapy
- Duration of renal dysfunction

Underlying Health of the Patient
- Age
- Presence of chronic kidney disease
- Presence, severity, and reversibility of underlying disease

Clinical Circumstances
- Cause of the renal failure
- Severity and reversibility of acute process(es)
- Number and type of other organ systems failed
- Development of sepsis and other complications

Intermittent Versus Continuous

The choice of intermittent or continuous therapy is currently based on the experience of the nephrology team and the availability of therapies. When both therapies are available, the indication of CRRT or IHD is based on the patient's neurologic, hemodynamic, and catabolic status. Ideally, the therapy should be tailored to the patient's demands, which changes daily in the critically ill. It is now accepted that more than one therapy will be utilized for managing patients during the course of AKI. Transitions from CRRT to IHD are common and reflect the changing needs of patients during their AKI course. For instance, patients in the ICU may initially start on CRRT when they are hemodynamically unstable, transition to SLED-EDD when they improve, and leave the ICU on IHD. In the recent ATN trial, 57% of the patients had more than one therapy whereas 23% and 20% had IHD and CRRT alone.[212] We recommend that all therapies should be utilized as indicated to best support patient needs through their course.

The comparison of the operating characteristics of the two therapies will help to recognize the strengths and weaknesses of each modality. Fluid removal is a desirable component of any renal replacement therapy and is a major goal of renal replacement for AKI.[213] Fluid removal and, hence, fluid balance, is limited to the period of dialysis. If the patient is hemodynamically unstable during this period, it may be difficult to remove any fluid. Fluid removal is slower and hypotension is uncommon with peritoneal dialysis and CRRT. It has been suggested that the latter modality may be associated with an improved outcome, due perhaps to more stable hemodynamics; however, this has not been rigorously demonstrated.[207] The high efficacy of these therapies in continuous fluid removal allows for use in situations other than renal failure, such as heart failure.[214] Pediatric patients are better suited for PD and CRRT, and these modalities have been used successfully in the management of AKI in neonates.[215,216]

The continuous removal of fluid permits the delivery of optimal nutrition as fluid load becomes a nonlimiting factor. Two other factors influence the overall nutritional balance of the patient in dialysis: the composition of the dialysate and hemofiltrate solutions. Although lactate-based dialysate and hemofiltrate solutions can rarely result in hyperlactatemia and worsening of acid-base status, they can cause higher urea generation rates compared to bicarbonate solutions.[217,218] The content of glucose in dialysate solutions results in glucose absorption during the dialysis procedure, which contributes to the caloric load. This glucose content is also associated with an increase in endogenous insulin secretion in most patients, and some patients may require exogenous insulin.[219] The use of a lower dextrose concentration-based dialysate in CRRT usually prevents this complication. Another nutritional factor is the dialysance of amino acids, vitamins, and trace elements across the filter. Losses appear to depend more on the serum levels than on the underlying clinical status of the patient.[220] To avoid potential harm, vitamin supplementation should be provided for all patients on CRRT regardless of dialysis dose, and pharmacists should be consulted to optimize drug dose adjustments.[221] With the massive expansion of therapeutic alternatives in critical care (especially antibiotics), much more research is required to understand optimal drug during CRRT.

The effect of the dialysis modality on outcome is still a major question to be answered. In four prospective cohort studies,[222–225] none suggest differences in mortality between modalities. A recent systematic review[226] identified nine RCTs that compared CRRT versus intermittent methods.[227–234] The relative risk of death associated with CRRT was not significantly different than with intermittent hemodialysis (RR 1.10; 95% CI, 0.99–1.23). The last Cochrane Review comparing dialysis modalities concluded that, in hemodynamically stable patients, modality does not appear to influence outcomes. In hemodynamically unstable patients, CRRT may be preferable as patients on CRRT maintain higher mean arterial pressure and show a trend toward lesser need for escalation of vasopressor therapy and arrhythmias.[235]

Dose of Dialysis

Until recently, dialysis dose was considered to play a pivotal role in improving outcomes in critically ill patients requiring CRRT. The relationship between treatment dose and patient outcome in AKI was first investigated prospectively in a single-center study where 425 subjects were randomized to 45, 35, and 20 mL/kg/h of postdilution continuous venous-venous hemofiltration (CVVH). Subjects receiving doses of

45 and 35 mL/kg/h experienced lower mortality rates compared to subjects receiving 20 mL/kg/h, 42% and 43% versus 59%, respectively ($P < 0.005$). After this study was published, three other RCTs showed contradictory results. Bouman et al.[203] found no difference in mortality among subjects who received higher hemofiltration volumes—48.2 mL/kg/h versus 19.5 mL/kg/h. Tolwani et al.[236] randomized 200 patients for CVVHDF using two different ultrafiltration volumes. The intensive group received 29 mL/kg/h against 17 mL/kg/h for those in the standard group. There was no significant difference in the mortality rate between groups: 64% versus 60% ($P = .56$). Adding a diffusive component (18 mL/kg/h of dialysate) in 206 patients submitted to hemofiltration (25 mL/kg/h of replacement fluid), Saudan et al.[237] showed a significant decrease in mortality—46% versus 61% ($P = .0005$). In this study, subjects in the hemodiafiltration group received substantially more overall solute clearance than subjects in the hemofiltration group, making it difficult to determine if the reduction in mortality was attributable to the higher dose or the addition of diffusive clearance.

Schiffl et al.[238] conducted an RCT comparing conventional alternate day dialysis to daily dialysis among 160 patients with AKI, assessing 14-day survival. The groups were similar with respect to baseline characteristics and illness severity and were analyzed by intention to treat. In the daily group, the weekly delivered Kt/V was 5.8 ± 0.4, and in the conventional group it was 3.0 ± 0.6. The duration of therapy was 3.3 hours per session in the daily group and 3.4 hours per session in the conventional group. The daily HD group had improved survival (28% vs. 46%, $P = .01$) and recovered kidney function more quickly (9 ± 2 days vs. 16 ± 6 days, $P = .001$). Factors significantly associated with an increased odds of death included alternate day HD (vs. DHD) (OR 3.92, 95% CI, 1.68–9.18, $P = .002$), higher APACHE III scores (OR 1.06, 95% CI, 1.01–1.12 per point increase, $P = .02$), oliguria (OR 3.02, 95% CI, 1.35–6.77, $P = .007$), and sepsis (OR 3.27, 95% CI, 1.43–7.50, $P = .005$).[238] The Schiffl study was the first randomized trial suggesting that patients with AKI benefited from more frequent HD and, consequently, a higher weekly Kt/V.[238]

Two recent large multicenter RCTs did not find benefit of intensive dose of dialysis over a standard dose.[212,239] The ATN trial was a randomized multicenter study including 1,124 critically ill AKI patients with sepsis or at least one nonrenal organ dysfunction. This trial aimed to provide a definitive conclusion on the benefits of intensive versus less-intensive dialysis dosage.[212] Intensive dosage was defined as CRRT with an effluent rate of 35 mL/kg/h, IHD or SLED six times per week, and less-intensive dosage as CRRT with an effluent rate of 20 mL/kg/h, and IHD or SLED three times per week. Each IHD or SLED treatment was aimed to achieve a single-pool Kt/V_{urea} of 1.2 to 1.4. The mean delivered dosages (5.4 treatments per week vs. 3 treatments per week at Kt/V of 1.3 or effluent rate of 35.8 vs. 22.0 mL/kg/h) were almost identical to the prescribed dosages. Subjects were switched from one modality to another according to their Sequential Organ Failure Assessment (SOFA) cardiovascular score (IHD when the score was 0 to 2 and CRRT or SLED when the score was 3 or 4). Baseline characteristics were similar between the groups. There were no differences in the primary endpoint, mortality at 60 days, in the duration of renal replacement therapy, or rate of recovery of kidney function or nonrenal organ failure between the groups. In contrast to the Bouman and Tolwani studies,[203,236] the sample size of the ATN study was sufficiently large so that there was adequate power to detect modest differences in mortality.

In the RENAL study 1,508 critically ill adults meeting predetermined criteria for the initiation of RRT were randomly assigned to postdilution CVVHDF with an effluent flow of 40 mL/kg/h or 25 mL/kg/h.[239] All patients received CRRT as their first mode of RRT and only 7% of patients received IHD later in their ICU stay. Thus, the RENAL study constitutes a more direct measure of the relationship between intensity of CRRT and survival.

The design of the ATN or the RENAL studies did not include predetermined strategies for some parameters that may have influenced the results, such as the timing of initiation of therapy, fluid balance, and site of delivery of replacement fluids (pre- vs. postdilution). It is important to note that subjects in the less intensive group received more renal replacement therapy than most patients in routine clinical practice. Therefore, practitioners should not conclude that dose is unimportant. In AKI there is a marked discrepancy between prescribed and delivered dose of dialysis. The delivered Kt/V in AKI patients have been shown to be 30% lower than prescribed,[238,240] resulting from hypotension, dialyzer clotting, and vascular access recirculation.[241]

The ideal dialysis prescription for AKI should incorporate an assessment of the dose of dialysis delivered. Unfortunately, there are no standard methods for assessing the dose of dialysis in AKI. In ESRD, the dose of dialysis prescribed and delivered is usually based on an assessment of the amount of urea removed, using urea kinetic modeling either via direct dialysis quantification or by using regression formulas incorporating fractional urea reduction.[242] A key feature of these methods is the assumption that patients with ESRD are in steady state with respect to urea generation, volume status, and renal and extrarenal clearance. However, dialysis dosing in AKI needs to account for highly variable body water volumes and varying urea generation rates, as well as different methods of dialysis and changes in renal and extrarenal clearance. Unfortunately, these issues have not been accurately quantified or adequately studied in prospective cohort studies or clinical trials conducted to date.

In general, the dose of dialysis is based on modality-specific criteria (e.g., membrane choice, operational characteristics, and the duration of each dialysis session). For patients treated with IHD, the frequency of dialysis is another determinant of the overall dose of dialysis delivered. Table 28.3 shows a comparison of the factors affecting dose of dialysis for IHD and CRRT. Several investigators have attempted to quantify the dose of dialysis delivered in AKI

using methods used for patients with ESRD. Clark et al.[243] compared IHD to CRRT techniques using a computer model to derive the required IHD frequency (per week) or required CRRT for a given patient weight for desired BUN values of 60, 80, and 100 mg per dL. For the attainment of intensive IHD metabolic control (BUN = 60 mg per dL) at steady state, a required treatment frequency of 4.4 dialyses per week was predicted for a 50-kg patient. However, the model predicted that the same degree of metabolic control could not be achieved even with daily IHD therapy in patients 90 kg or more. On the other hand, for the attainment of intensive CRRT metabolic control (BUN = 60 mg per dL), required urea clearance rates of approximately 900 mL per hour and 1,900 mL per hour were predicted for 50 and 100 kg patients, respectively. These data suggest that, for many patients, rigorous control of azotemia equivalent to that readily attainable with most CRRT programs can be achieved with intensive (nearly daily) IHD regimens only. In practice, the frequency of dialysis usually depends on the patient's clinical and biochemical status. It is noteworthy that reimbursement policies in the United States currently do not support the practice of daily IHD.

Other promising concepts should also be prospectively tested to improve our current understanding of the pathophysiology of AKI and help to better define dialysis dosage requirements. To improve the definition of dialysis dosage, other dialysis parameters, such as fluid balance, need to be assessed. In CRRT the effluent volume per se may not accurately reflect clearance as clotting of filter is associated with declining efficacy in effluent saturation. Although current RRT substitute small solute and volume clearances, the later parameter has never been included in randomized studies on dialysis dosage in AKI.[211,212,239] More importantly, fluid excess has been shown to be independently associated with increased mortality in one adult and several pediatric observational studies in AKI.[101,211,215] Fluid excess was usually defined as a proportion of initial hospital admission weight. In the largest pediatric study, the percentage fluid excess at dialysis initiation was significantly lower in survivors versus nonsurvivors (14.2 ± 15.9% vs. 25.4 ± 32.9%; $P < .03$) even after adjustment for severity of illness.[101] Therefore, fluid excess may contribute to imbalances between groups and should be better characterized in future studies. Results from the ongoing RENAL trial, a multicenter trial comparing an augmented versus a normal CRRT regimen, may add additional insight into the question of dialysis dose and outcome (Fig. 28.12).

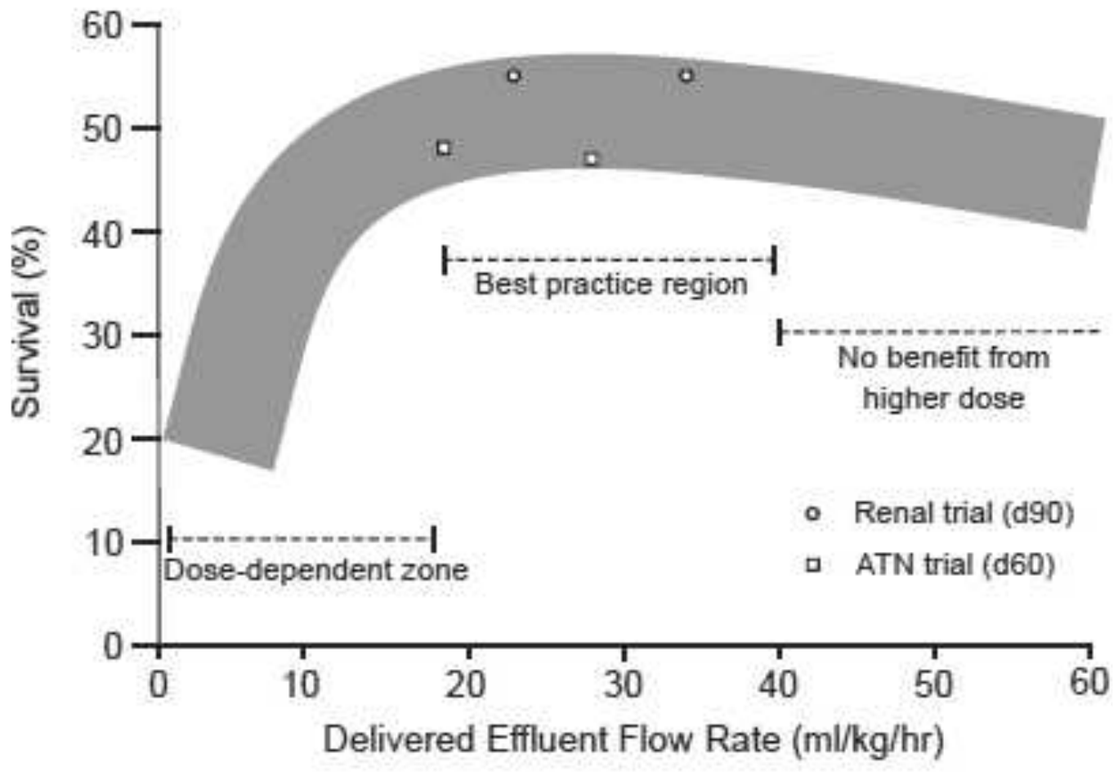


FIGURE 28.12 Possible relationship between delivered dose of continuous renal replacement therapy and survival, with results from the ATN and RENAL. These studies indicate a plateau response at the dose ranges examined. Acute tubular necrosis (ATN) doses are corrected for predilution. To reproduce these results, clinicians will need to prescribe continuous renal replacement therapy doses above the lower target dose in the trial protocols (20 or 25 mL/kg/min) as larger periods of filter downtime can be expected outside a clinical trial environment. Below this best-practice region, survival is likely to be dose-dependent; however, the exact nature of this relationship has not been formally determined. Doses above the best-practice region are unlikely to be beneficial to unselected patients and could potentially be harmful. *ATN*, Veterans Affairs/National Institutes of Health Acute Renal Failure Trial Network; *RENAL*, Randomized Evaluation of Normal versus Augmented Level. (Adapted from Prowle JR, Schneider A, Bellomo R. Clinical review: Optimal dose of continuous renal replacement therapy in acute kidney injury. *Crit Care*. 2011;15(2):207.)

Nutritional Considerations

AKI patients present an increased risk of protein–energy malnutrition due to poor nutrient intakes and high catabolic rates. Nutritional support should be directed to ensure adequate nutrition, prevent protein–energy wasting with its concomitant metabolic complications, promote wound healing and tissue repair, support immune system function, accelerate recovery, and reduce mortality.

In critically ill patients, the metabolic response to stress causes increased production of some cytokines (IL-1, IL-6, TNF-α), counterregulatory hormones (catecholamines, cortisol, glucagon), and immune mediators (thromboxane A2, prostaglandin F2a, prostaglandin E2).[244] The activation of stress-mediated response causes skeletal muscle breakdown, impairs amino acid transport into skeletal muscles, suppresses insulin-mediated protein synthesis, depletes body energy reserves and constitutive proteins, and increases urea production and peripheral insulin resistance.[245] As a result, critically ill patients have loss of body energy reserves (glycogen, protein, and fat stores), hypertriglyceridemia, hyperglycemia, and negative nitrogen balance.

Severe malnutrition occurs in up to 42% of patients with AKI. Severely malnourished patients have a significantly increased in-hospital length of stay, increased risk for comorbidities (sepsis, septic shock, hemorrhage, intestinal occlusion, cardiac dysrhythmia, cardiogenic shock, acute respiratory failure), and increased in-hospital mortality.[246] Nutritional assessment is difficult, especially in AKI patients

presenting higher metabolic demands. Subjective Global Assessment (SGA) accesses nutritional status, requires no additional laboratory testing, and is highly predictive of outcome.[247]

Patients with AKI should receive a basic intake of at least 1.5 g/kg/day of protein and an energy intake of no more than 30 kcal nonprotein calories or 1.3 × BEE (Basal Energy Expenditure) calculated by the Harris–Benedict equation. Thirty to 35% of calories should come from lipid, as lipid emulsions. Monitoring of nitrogen balance to assess the effectiveness of supplemental nutritional therapy is determined by measuring protein intake over 12 or 24 hours and urinary excretion of urea nitrogen over the same time interval. A positive or negative protein balance is used to determine the adequacy of protein intake of the patient. It is calculated as follows:

$$\text{Nitrogen balance} = (\text{protein intake}/6.25) - (\text{UUN} + 4),$$

Protein intake and urinary urea nitrogen (UUN) are each expressed in grams.

The enteral route should be the first choice for nutritional support if the gastrointestinal tract is functioning, whereas parenteral nutrition should be reserved when the gastrointestinal tract cannot be used, or when the enteral route appears inadequate to reach nutrient intake goals.[248] AKI itself and other factors commonly present in critically ill patients, such as medications, hyperglycemia, and electrolyte disorders, can impair gastrointestinal motility.

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CHAPTER

29 Pathophysiology of Ischemic Acute Kidney Injury

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INTRODUCTION

Acute kidney injury (AKI) is defined as a sudden decrease in the glomerular filtration rate (GFR) occurring over a period of hours to days. The Acute Dialysis Quality Initiative (ADQI) has developed the RIFLE classification of AKI that divides AKI into the following stages: (1) risk, (2) injury, (3) failure, (4) loss of function, and (5) end-stage kidney disease.[1–3] The RIFLE criteria have been validated in multiple studies (i.e., as the RIFLE class increases so does mortality).[1–3] The term AKI, in general, replaces the term acute renal failure (ARF) and ARF is restricted to patients that have AKI and need renal replacement therapy. AKI has also replaced the term acute tubular necrosis (ATN). The term ATN emerged from the early observation on renal biopsy that necrosis of some renal tubular epithelial cells may occur in humans with acute renal injury.[4] Tubular epithelial casts (muddy brown casts) are excreted in the urine of these patients. However, it is now known that the tubular necrosis is quite patchy and alone could not account for GFR less than 10 mL/min/1.73m^2, the functional hallmark of clinically significant AKI.[4] Moreover, a percentage, ranging from 30% to 70%, of the urinary tubular epithelial cells has been shown to be viable by culture and the exclusion of vital dyes.[5,6] This observation is somewhat surprising because, generally, cells, which are separated from their extracellular matrix, undergo apoptosis.[7–10] Emerging results suggest that adhesion molecules (e.g., cadherins and integrins) may allow a cell-to-cell or a cell-to-matrix adhesion that not only avoids apoptosis, but also may contribute to intratubular obstruction.[11–14] Intraluminal tubular casts on a renal biopsy are a hallmark of clinical AKI, and earlier nephron dissection studies by Jean Oliver demonstrated a preferential location of these casts in the medullary collecting duct.[15] This location is of particular relevance to the overall low GFR in AKI because thousands of nephrons drain into a single medullary collecting duct.

There is debate about whether the pathophysiology of clinical AKI is primarily tubular or vascular. In fact, initial rat micropuncture studies were unable to consistently detect an elevation in tubular pressures in experimental ARF and thus the term vasomotor nephropathy replaced the ATN term for the clinical syndrome.[16–18] Experimental results have emerged that both vascular and tubular factors are involved in the pathogenesis of clinical AKI. Most recently, the role of endothelial injury and dysfunction[19] in promoting an inflammatory response in AKI[20] has received prominence. Thus, this chapter will discuss the potential tubular and vascular factors, as well as inflammatory processes, involved in the pathogenesis of ischemic AKI (Fig. 29.1). Toxic AKI will be discussed in another chapter. However, it must be emphasized that AKI in humans is frequently multifactorial. Both ischemic and toxic insults combine in a synergistic fashion to cause clinical AKI.

The understanding of the pathogenesis of ischemic AKI is of considerable importance for several reasons. First, this clinical syndrome is quite frequent, occurring in 5% to 10% of hospitalized patients and in 30% to 40% of intensive care unit (ICU) patients.[21–23] The incidence is likely to increase in the future because of the use of newer nephrotoxic drugs and the performance of more complex procedures in older patients.[24] Second, ischemic AKI has a very high mortality particularly when requiring dialysis to treat the resultant uremic syndrome. Overall mortality averages from 40% to 50%; however, the patient in the ICU with ischemic AKI may have a mortality in excess of 80%, particularly if the patient has multiorgan failure.[25–30] It is widely quoted that the mortality of AKI has only improved slightly in the last 40 years.[31] However, a study[32] suggests there has been improvement in AKI mortality between the late 1970s and the early 1990s. Third, there is considerable evidence that a functional component of the renal failure exists. Specifically, a histologic examination of the kidney from patients with clinical AKI exhibits normal glomeruli, occasional tubular necrosis, some intraluminal casts, and modest interstitial edema.[4] There is virtually no evidence for irreversible tissue damage and the morphologic changes alone fail to support the presence of a GFR less than 10 mL/min/1.73m^2.

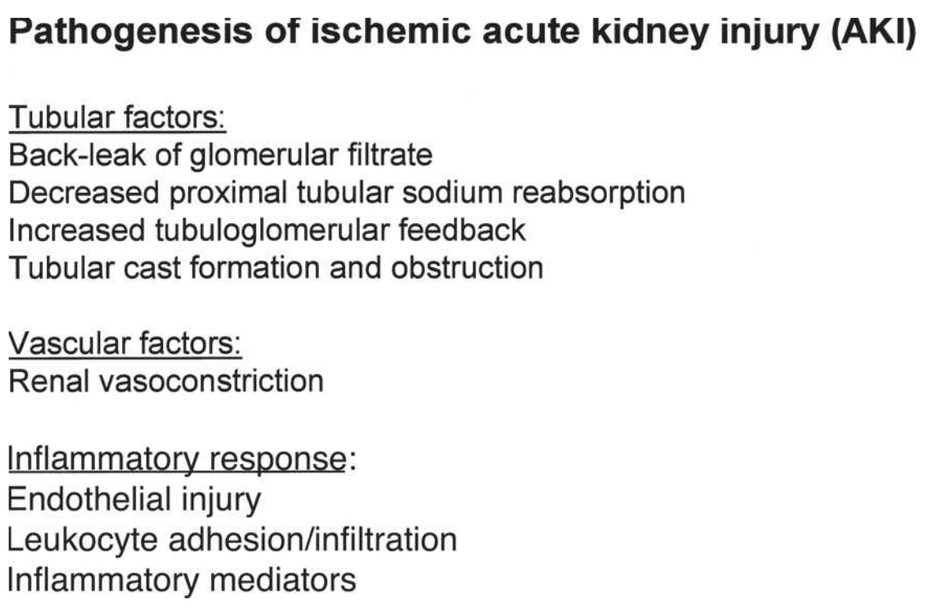

FIGURE 29.1 Vascular and tubular factors and inflammatory processes are involved in the pathophysiology of ischemic acute kidney injury.

EXPERIMENTAL MODELS OF ACUTE KIDNEY INJURY

Available models to study the pathophysiology of renal cell ischemia are listed in Table 29.1.[33] An understanding of these models will allow a better interpretation of the multiple studies discussed in this chapter.

Proximal and distal tubular cells in culture have been widely used to study tubular injury. These cells change from their normal dependence on oxidative mitochondrial metabolism to glycolysis under culture conditions.[34] As a result, these cultured tubules become less susceptible to oxygen deprivation. Thus, exposure to drugs like antimycin-A, ionomycin, or a combination to induce "chemical" ATP depletion and subsequent necrosis or apoptosis is used. Cultured cells also undergo considerable structural change, which includes simplification of both their apical and basolateral compartments.[35] The presence of necrosis rather than apoptosis in these cells may be related to the level of ATP depletion. In cultured mouse proximal tubules subjected to ATP depletion below 15% of control values, the cells died of necrosis; whereas in ATP depletion to 25% to 70% of control values all the cells died of apoptosis.[36] Therefore, although cultured tubule cells are the least complex model and allow for an understanding of the mechanisms involved, the therapeutic implications for in vivo AKI are limited.

Freshly isolated rat or rabbit proximal tubules in suspension are also widely used to study proximal tubular injury.[37–45] The method of isolation of tubules is by collagenase digestion and Percoll centrifugation. Hypoxia is achieved by gassing the suspension with 95%N_2/5%CO_2 for up to 15 minutes, thereby reducing the pO_2 to approximately 30 mm Hg. Lactic dehydrogenase (LDH) release into the suspension medium is measured as an index of lethal membrane injury.[46,47] The tubules are preincubated with cytoprotective agents and enzyme inhibitors before the induction of hypoxia, and the effect of these agents on cell membrane injury can be determined. The presence of necrosis rather than apoptosis during short periods of hypoxia (15 to 30 minutes) in this model was demonstrated using DNA-specific dyes, such as Hoechst 33342 and propidium iodide.[48] Another study has also demonstrated that during hypoxia there is endonuclease activation without morphologic features of apoptosis in the same model of rat proximal tubules.[49] Freshly isolated tubules are valuable for both structural and metabolic investigations because they retain the biochemical properties of the in vivo state and a high degree of structural integrity, and are highly polarized and fully differentiated.[33] However, the tubules are highly sensitive to ATP depletion, and severe hypoxia or anoxia results in necrosis of more than 50% of the cells after 30 minutes. Isolation methods also expose the tubules to repeated 4°C exposure and collagenase.

In whole animal studies—usually rats, rabbits, or mice—a clamp model of ischemic AKI is used.[50–52] Ischemic AKI is generally induced by (1) clamping of both the right and left renal pedicles or renal arteries or (2) unilateral renal pedicle or artery clamp preceded by contralateral nephrectomy. The renal vessels are clamped for varying periods of time, generally from 30 to 60 minutes, followed by varying periods of reperfusion. This results in a reversible model of AKI in which the blood urea nitrogen (BUN) and serum creatinine reach a peak at 24 to 48 hours reperfusion and

29.1 Available Models to Study the Pathophysiology of Renal Cell Ischemia (Increasing Order of Complexity)

Model	Origin
Cultured tubular cells	Primary culture of human, rat, and mouse; Madin-Darby canine kidney (MDCK) cells (distal); porcine renal epithelial (LLC-PK1) cells (proximal); opossum kidney (OK) cells; human kidney (HK) cells
Freshly isolated proximal tubules in suspension	Rabbits, rats, mice
Isolated perfused kidney	Rats
Whole animals	Rabbits, rats, mice, dogs (not much used anymore)
Human patients	Renal biopsy studies; urine and serum biomarkers of acute kidney injury

Adapted from Reference 33.

then gradually normalize over the next 7 days.[50–53] However, renal vessel clamping in rats results in extensive necrosis of proximal tubules. This necrosis is much more extensive than is seen in humans with ischemic AKI. Nevertheless, although animal models of ischemic AKI are complex with many experimental limitations, they provide important leads for future therapeutic clinical interventions.

In the isolated perfused kidney model, the kidney is removed from the animal. The perfusate usually consists of a Krebs-Henseleit buffer with albumin. Urine is collected by cannulation of the ureter. This model allows for the study of factors independent of changes in systemic hemodynamics and neural activity. Further advantages include the study of specific circulatory factors or pharmacologic agents that are added to the perfusate. These agents are thus delivered directly to the kidney. The disadvantages of the model are (1) the absence of red blood cells in the perfusate impairs oxygen delivery to the medullary thick ascending limb (mTAL) and (2) perfusate flow greatly exceeds normal in vivo values. The isolated perfused kidney is regarded as a model of selective hypoxia to the medullary thick ascending limb.[54]

Studies in patients with AKI, although having important experimental limitations, have the most direct therapeutic value. An analysis of urine cytology represents a noninvasive method for potentially defining the cause of AKI.[4] Meyers and colleagues[55–57] have examined patients with ischemic AKI postrenal transplantation by obtaining biopsies of these allografts at the time of transplantation. Biomarkers of kidney injury would greatly facilitate the early detection and the precise diagnosis of AKI.

BIOMARKERS OF ACUTE KIDNEY INJURY

AKI is usually diagnosed by recording increases in serum creatinine and decreased urine output over several days. However, serum creatinine is not a good marker of renal function in AKI because its concentration can be affected by factors not related to renal function such as the volume of distribution, muscle mass, and creatinine secretion.[28] When the kidney is injured and the true GFR suddenly drops, but there is a slow increase in serum creatinine over days. This new steady state, which may take up to 7 days, is reached when creatinine generation equals creatinine excretion. In contrast to serum troponin in myocardial infarction, an increase in serum creatinine lags and may not be directly related to tubular injury in AKI. Recent studies have examined urine and serum biomarkers of kidney injury that have the potential to facilitate the diagnosis of AKI.

Recently described molecules such as the cytokine interleukin (IL)-18,[58] kidney injury molecule-1 (KIM-1),[59] cysteine-rich protein 61 (Cry61),[60] neutrophil gelatinase–associated lipocalin (NGAL),[61] and sodium/hydrogen exchanger isoform 3 (NHE3)[62] have demonstrated compelling results as markers of AKI at the preclinical level. Studies have been initiated to explore these molecules in human AKI.

Because of the crucial importance of early therapy in the management of AKI, markers are being explored for early diagnosis. NGAL was investigated as an early biomarker for AKI following cardiopulmonary bypass in 45 patients.[63] Urine and serum were collected at baseline and at frequent intervals for 5 days following a cardiopulmonary bypass. All patients who developed AKI (defined as a 50% increase in serum creatinine) displayed a significant increase in serum and urine NGAL very early after the cardiopulmonary bypass compared to patients without AKI. These results show that NGAL may be a sensitive, early urinary, and serum biomarker for AKI.

In a nested case-control study within the adult respiratory distress syndrome (ARDS) network trial, urinary IL-18 was investigated as an early marker of AKI.[64] Median urine IL-18 levels were significantly higher in AKI cases (defined as a 50% increase in serum creatinine) as compared to controls. On multivariable analysis, urine IL-18 values predicted the development of AKI 24 and 48 hours later after adjusting for demographics, sepsis, acute physiology and chronic health evaluation (APACHE) III score, serum creatinine, and urine output. After controlling for other parameters, a rise in urine IL-18 by 25 pg per milliliter was associated with an increased odds ratio of AKI by 19% for the next 24 hours. Urine IL-18 performed as a diagnostic test with an area under the receiver operator characteristic curve of 73%. The conclusion of this study is that urinary IL-18 levels can be used for the early diagnosis of AKI.

A recent study demonstrated that serum cystatin C appears to increase 24 to 48 hours before serum creatinine in patients with AKI.[65] However, cystatin C is a marker of GFR, or a functional marker, and is not a structural or biochemical marker of renal tubular injury.

There are multiple promising serum and urinary biomarkers (eg, IL-18, neutrophil-gelatinase-associated lipocalin [NGAL], kidney injury molecule-1 [KIM-1], cystatin C, liver fatty acid-binding protein [L-FABP]), which detect AKI before the rise in serum creatinine and predict outcomes in patients with AKI.[66] Prospective studies to determine the use of these biomarkers in larger populations have been initiated. In this regard, a National Institutes of Health (NIH)-funded clinical consortium consisting of investigators from nine academic centers called TRIBE-AKI (Translational Research Investigating Biomarkers in Early Acute Kidney Injury) has been established. Currently, the consortium is performing a prospective multicenter observational cohort study of 1,800 patients receiving cardiac surgery to determine whether urine IL-18, urine NGAL, and serum cystatin C are biomarkers for the early diagnosis and long-term outcomes of AKI. Ultimately, disease control studies to determine the impact of a biomarker screening on AKI morbidity and mortality are desirable. In this regard, a prospective study in over 500 ICU patients in New Zealand determined whether erythropoietin therapy decreases the incidence of AKI, as determined by serum creatinine and serum cystatin C, and lowers levels of urinary IL-18, NGAL, and KIM-1.[67] In this

study, early intervention with erythropoietin based on urine biomarker levels did not affect the outcome of AKI.[67]

BACK-LEAK OF GLOMERULAR FILTRATE POSTRENAL ISCHEMIA

It has been proposed that GFR in ischemic AKI is really not as low as measured because glomerular filtrate leaks across the damaged epithelial monolayer and/or the tubular basement membranes. In some toxic experimental models of ischemic AKI, diffuse tubular necrosis and basement membrane damage has been associated with evidence for back-leak of glomerular filtrate. The term "back-leak" of glomerular filtrate refers to the unregulated passage of salt and water from the tubular lumen into the interstitium and later back into the renal venous capillaries and renal veins.[68] However, the level of epithelial and basement membrane damage with these experimental toxic models (e.g., cisplatin, mercuric chloride) is virtually never observed in human AKI. Myers et al.[69] have performed human studies using solute sieving curves in search of tubular back-leak of glomerular filtrate. They found dextran sieving curves could sometimes exceed inulin sieving curves, thus providing evidence in support of the back-leak of solutes (i.e., dextran), which are normally unable to cross intact tubular epithelial basement membranes. However, even when accepting the validity of this method for documenting tubular back-leak of filtrate, the calculated amount would only account for a decrease in renal function of 8% to 10%. Thus, although tubular back-leak of glomerular filtrate might occasionally occur in patients with severe ischemic AKI, it is unlikely to be a dominant pathogenic factor.

The tight junction of polarized tubular epithelial cells is the most apical component of the junctional complex and serves as an important permeability barrier.[70] Tight junctions also control cell polarity.[71] However, the tight junction in the proximal tubule is relatively "leaky" with as much as one-third of proximal sodium reabsorption occurring via the paracellular route. The tight junctional complex is a dynamic and regulated structure. Some of its protein components have been identified and include the transmembrane protein occludin. Nontransmembrane proteins on the cytosolic leaflet include zona occludens (ZO)-1, ZO-2, cingulin, 7H6, and several unidentified phosphoproteins. Interactions of some of these proteins with the actin cytoskeleton is a major determinant of the tight junction structure and may also play a role in the regulation of tight junction assembly.[70] The integrity of the tight junction is disrupted during ischemic injury and must be reestablished for recovery.[72] There is in vitro experimental evidence in cell culture studies for an impaired tight junction between tubular epithelial cells undergoing chemical anoxia.[73] Ruthenium red, which normally is impermeable to tight junctions, has been shown to enter the ZO after chemical hypoxia and renal ischemia.[73,74] Energy depletion abolishes the gate function of the tight junction, as determined by the dramatic decrease in transepithelial resistance, but it leaves the fence function intact, as determined by the maintenance of lipid polarity.[75] In an ATP depletion–repletion model in Madin-Darby canine kidney cells, tight junction proteins such as ZO-1 reversibly form large complexes and associate with cytoskeletal proteins.[76] A model has been proposed in which a key, potentially regulated step in the generation of the ischemic epithelial cell phenotype is the interaction between tight junction proteins and fodrin and/or other cytoskeletal proteins.[76] Intracellular calcium plays a role in tight junction reassembly after ATP depletion.[72] In this study, the role of intracellular calcium in tight junction reassembly after ATP depletion–repletion was studied using the cell-permeable calcium chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid-AM (BAPTA-AM). Lowering intracellular calcium during ATP depletion was associated with a significant inhibition of the reestablishment of the permeability barrier following ATP repletion as measured by transepithelial electrical resistance and mannitol flux, marked alterations in the subcellular localization of occludin by immunofluorescent analysis, and decreased solubility of ZO-1, and other tight junction proteins by the Triton X-100 extraction assay. This suggested that lowering intracellular calcium potentiates the interaction of tight junction proteins with the cytoskeleton.

Studies have also shown the importance of small GTPases (Rho, Rac, Cdc-42) in the integrity of the ZO.[77] ATP depletion with chemical hypoxia has been demonstrated to inactivate these GTPases and thereby contribute to increased paracellular back-leak of filtrate.[71,78] Expression of constitutively active ras homolog gene family, member A (RhoA) GTPase in ATP-depleted Madin Darby canine kidney (MDCK) cells prevents tight junction disassembly.[71] Cyanide-induced chemical hypoxia increases the kinase activity of c-Src and causes its translocation to cell–cell junctions where it binds to and phosphorylates β-catenin and p120, suggesting that this may contribute to the loss of epithelial barrier function.[79] It is likely that the effects on tight junction integrity seen in ATP depletion are due, at least in part, to the inhibition of Na-K-ATPase.[80] The relevance of these cell culture observations to clinical ischemic AKI in patients remains to be proven.

DECREASED TUBULAR SODIUM REABSORPTION

Proximal tubular injury, whether it be sublethal reversible dysfunction, necrosis, or apoptosis, has been extensively studied. Mechanisms of proximal tubular injury that will be discussed in this section are outlined in Table 29.2. The study of proximal tubular injury is of special relevance in order to explain the decreased tubular sodium reabsorption that occurs with a postrenal ischemia.

In the normal kidney, Na^+ is vectorially transported from the proximal tubule lumen across the apical membrane microvilli into the tubular epithelial cells and then across the basolateral membrane into the interstitium and the peritubular circulation.[81] Na^+ influx into the polarized proximal tubular epithelial cells across the apical membrane is passive

29.2 Mechanisms of Hypoxic/Ischemic Proximal Tubular Injury

Sublethal Reversible Injury
- Cytoskeletal disruption and loss of polarity
- Loss of tight junction function
- Loss of cell-matrix adhesion
- Abnormal gene expression

Necrosis
- Severe ATP depletion (15% of normal)
- Calcium influx
- Calcium-dependent phospholipase A_2 ($cPLA_2$)
- Calcium-dependent cysteine proteases (e.g., calpain)
- Calcium-independent PLA_2
- Caspase-1
- Interleukin-18 (IL-18)
- Metalloproteases
- Oxygen radicals
- Lipid peroxidation
- Deficiency of glycine
- Nitric oxide (generated by iNOS)
- Endonuclease activation
- Deficient heat stress response
- Potassium efflux
- Klotho deficiency

Apoptosis
- Mild ATP depletion (25%–50% of normal)
- Caspase-3
- Caspase-1
- Caspase-6
- Endonuclease activation
- Serine proteases
- Insulinlike growth factor I receptor deficiency
- Deficient heat stress response
- Erythropoietin
- NGAL
- Ghrelin
- Bcl-2 proteins
- Mitochondrial fragmentation

iNOS, inducible nitric oxide; NGAL, neutrophil gelatinase–associated lipocalin.

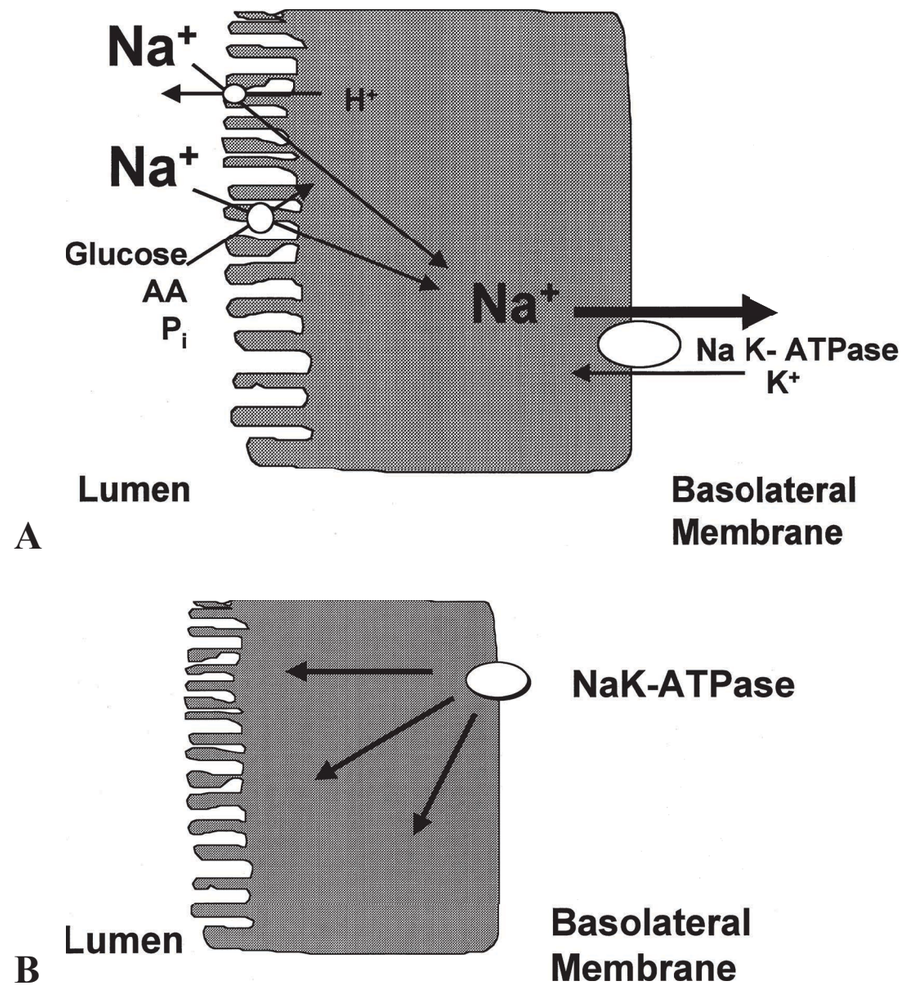

FIGURE 29.2 **A:** Normal reabsorption of sodium in the proximal tubule. The NaK-ATPase pump is located on the basolateral surface of the proximal tubule. **B:** Translocation of the Na-K-ATPase pump away from the basolateral proximal tubule membrane during hypoxia/ischemia. The loss of polarity of proximal tubule cells during chemical anoxia and ischemia results in a translocation of the Na-K-ATPase. During ATP depletion in cultured cells, the Na-K-ATPase translocates to the apical membrane. In human kidney allografts with delayed function, the Na-K-ATPase translocates to the cytoplasm. The translocated Na-K-ATPase remains functional.

down the Na^+ gradient via the H^+/Na^+ exchanger and various Na^+ cotransporters. The Na^+ gradient is maintained by an active transport via the Na^+/K^+-ATPase at the basolateral membrane of the proximal tubular cells (Fig. 29.2A).

The earliest signs of clinical AKI are urinary muddy brown casts and an increased fractional excretion of sodium (FE_{Na}).[82,83] The proximal tubule is the most frequent morphologic site of injury in ischemic AKI in both humans and animals.[84] The S_3 segment of the proximal tubule is particularly prone to ischemic injury, perhaps because of its location in the outer medulla, which is relatively hypoxic compared to the renal cortex.[84] The proximal tubule nephron site is also associated with impaired vectorial sodium transport. The earliest morphologic changes with ischemic injury include invagination and sloughing of the brush border membrane into the lumen, an abnormality that is compatible with impairment and a loss of apical sodium antiporters and cotransporters responsible for sodium entry into the proximal tubular epithelium.[85–87] The tubules lose their polarity.[83] In vitro studies have shown that ATP depletion leads to dephosphorylation and inactivation of the actin binding protein, ezrin, and activation of the actin depolarizing protein in the proximal tubule membrane.[88,89] This leads to a disruption of the microvillar actin and a loss of the brush border membrane.[90] A loss of polarity of the proximal tubule cells during chemical anoxia and ischemia has also been shown with the translocation of the Na-K-ATPase to the apical membrane (Fig. 29.2B).[91–93] The translocated Na-K-ATPase remains functional.[94] In MDCK cells exposed to ATP depletion, there is a loss of polarity of Na-K-ATPase and a dissociation of the membrane–cytoskeleton complex at the spectrin–ankyrin interface.[95] Thus, sodium transport back

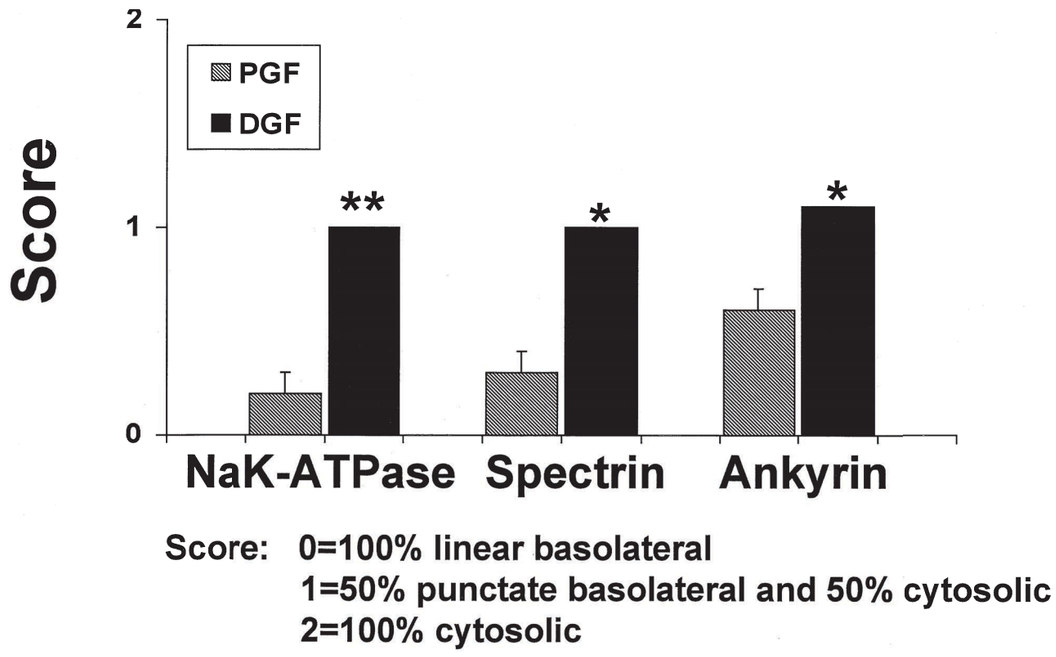

FIGURE 29.3 The cellular location on immunohistochemistry of the actin binding proteins, ankyrin and spectrin, and Na-K-ATPase in cadaveric transplanted kidneys with prompt graft function (PGF) and delayed graft function (DGF) was compared. In those kidneys with DGF, approximately 50% of the ankyrin, spectrin, and Na-K-ATPase was translocated from the basolateral membrane to the cytoplasm. Whereas those kidneys with a PGF had only minimal translocation of these proteins from the basolateral membrane. * $P < .01$ versus PGF; ** $P < .05$ versus PGF. Adapted from Alejandro et al.[55]

across the apical membrane and into the proximal lumen, as well as decreased proximal tubular sodium reabsorption, has been proposed in response to hypoxia and ischemia.

Studies in cadaveric transplanted kidneys with both prompt and delayed graft functions have been compared relative to the cellular location of the actin binding proteins, ankyrin and spectrin, and Na-K-ATPase using selective antibodies. In kidneys with delayed graft function, approximately 50% of the ankyrin, spectrin, and Na-K-ATPase were translocated from the basolateral membrane to the cytoplasm (Fig. 29.3). Those kidneys with prompt graft function had only minimal translocation of these proteins from the basolateral membrane (Fig. 29.3).[55] These observations therefore have contributed to our understanding of reversible and sublethal tubular dysfunction in ischemic kidneys in vivo (Fig. 29.3).

Because proximal tubular cells in a culture convert from primarily oxidative to glycolytic metabolism and alter their phenotype,[34,35] confirmation of experimental results in other systems is advisable. The use of freshly isolated proximal tubules to study the response to hypoxia has also been enlightening. These tubules, in general, maintain their phenotype and oxidative metabolism but are more sensitive to hypoxia, as assessed by LDH release, than in vivo tubules.[96–99] Hypoxia for 15 to 30 minutes causes a reproducible release of LDH, and the cells die by necrosis.[48,49] The central role of intracellular calcium and various protective maneuvers against hypoxic injury have been demonstrated in these isolated proximal tubules. However, before discussing the role of intracellular calcium in proximal tubular injury, we shall briefly consider adenine nucleotides. It is unquestioned that the first effect of ischemia, hypoxia, or mitochondrial inhibition in most in vitro and in vivo models is to compromise adenine nucleotide metabolism. A decreased production of ATP precedes the increase in intracellular calcium.

Adenine Nucleotides

The removal of oxygen from renal cells or whole kidneys results in prompt decreases in the cellular ATP pool. Initially, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) concentrations increase,[52] and further catabolism of AMP to adenosine and then to hypoxanthine and, in some species to xanthine, occurs as the ischemic period is prolonged.[100,101] The provision of exogenous ATP-$MgCl_2$ to ischemic rat kidneys protects against ischemic injury.[102] Mechanisms whereby a loss of ATP results in cellular injury include the loss of purine nucleosides themselves (in some species, the generation of oxygen free radicals during reperfusion), and the loss of many metabolic functions (e.g., phosphorylation of important enzymes, ion channels, and the functions of ion transporters that are dependent on adequate ATP levels).

Ischemic preconditioning protects the heart, and in some studies the kidneys, from subsequent ischemia–reperfusion injury. Ischemic preconditioning appears to be mediated via the activation of adenosine receptors, specifically the A_1 adenosine receptors. In support of this are studies that the exogenous administration of adenosine or A_1 adenosine agonists mimic ischemic preconditioning in cardiac muscle.[103] It was recently demonstrated that rat kidneys can be preconditioned to attenuate ischemic–reperfusion injury. In this study, adenosine infusion before the ischemic insult protects renal function via A_1 adenosine receptor activation, and adenosine A_1 antagonism blocks adenosine-induced protection. In a more recent study, acute and delayed protection against renal ischemia was seen with an A_1 adenosine receptor agonist.[104] In addition, adenosine A_3 receptor activation before the ischemia worsens the renal ischemia–reperfusion injury and A_3 receptor antagonism protects renal function.[103] A_2A adenosine receptors mediate the inhibition of ischemic AKI in rats due to an inhibitory effect on neutrophil adhesion.[105,106] A combined infusion of an A_{2A} adenosine receptor agonist and a type IV phosphodiesterase (PDE 4) inhibitor leads to enhanced protection against ischemia–reperfusion injury in mice.[107] Protection against renal ischemia–reperfusion injury by A_2A receptor agonists or endogenous adenosine requires the activation of receptors expressed on bone marrow–derived cells.[108] The A_2A adenosine receptor may be a novel therapeutic target in renal ischemia–reperfusion injury.[109,110]

Intracellular Calcium

The normal regulation of epithelial cell calcium is demonstrated in Figure 29.4A. Calcium exists in the cell as cytosolic free calcium, which is the smallest pool, but the most critical for regulation of intracellular events. Calcium is also bound to proteins and anions in the cytosol and to membrane

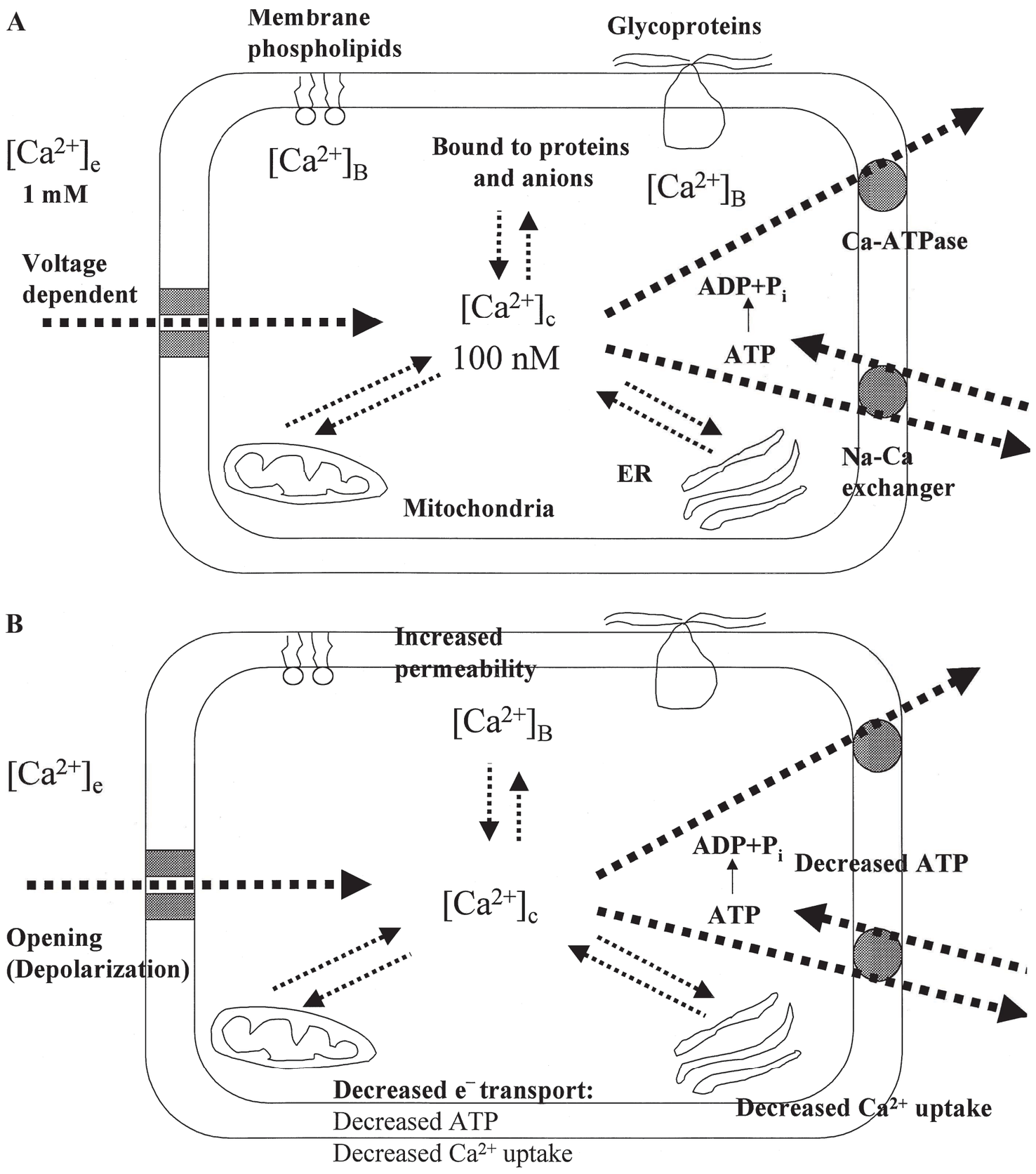

FIGURE 29.4 **A:** The normal regulation of epithelial cell calcium. The cytosolic free calcium $[Ca^{2+}]_c$ is the smallest amount, but the most critical for the regulation of intracellular events. The concentration of calcium in the cytosol is about 100 nM, which is 1/10,000 of the extracellular calcium, which is in a micromolar concentration of $[Ca^{2+}]_e$. Calcium is also bound to proteins and anions in the cytosol and to membrane phospholipids and glycoproteins $[Ca^{2+}]_b$. The largest pool of intracellular calcium is in the mitochondria and the endoplasmic reticulum. The large electrochemical gradient is maintained by binding calcium to intracellular components and by apical and basolateral transport systems. Transport systems, which maintain the large electrochemical gradient between intracellular and extracellular Ca^{2+}, may be voltage dependent or ATP dependent like the $Ca2^{+}$ ATPase pump and the $Na^{+}/Ca2^{+}$ exchanger. During cell injury, active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic calcium. **B:** During epithelial cell injury, several factors favor increases in cytosolic free calcium. There is (1) depolarization or an opening of voltage-dependent channels; (2) increased membrane permeability; and (3) decreased mitochondrial electron transport leading to decreased ATP levels. The decreased ATP leads to decreased calcium uptake by mitochondria and the endoplasmic reticulum, and a decreased ability to pump calcium out of the cell.

phospholipids and glycoproteins. The largest pools of intracellular calcium are in mitochondria and the endoplasmic reticulum.[111,112] The concentration of calcium in the cytosol is about 100 nM, which is 1/10,000 of extracellular calcium concentrations.[111] The large electrochemical gradient between intracellular and extracellular calcium is maintained by the binding of calcium to intracellular components and by apical and basolateral transport systems. Transport systems may be voltage dependent or ATP dependent. Calcium efflux is mediated in basolateral membranes by both calcium ATPase, which is ATP dependent, and by a Na^{+}/Ca^{2+} exchanger on the basolateral membrane, which is ATP independent.[113] Normally, the cell membrane is impermeable to calcium and maintains the steep calcium gradient between cytosolic free calcium and the extracellular space.[112] However, when cytosolic calcium increases in response to either increased cellular membrane permeability or decreased calcium efflux, or both, the mitochondria and the endoplasmic reticulum (ER)

actively increase their calcium uptake. Mitochondrial uptake and retention of calcium becomes substantial only when cytosolic levels exceed 400 to 500 nM, as occurs with cell injury.[111] Mitochondrial uptake is regulated by a calcium uniporter in the mitochondrial inner membrane. Thus, during cell injury, active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic calcium.

During epithelial cell injury, several factors favor increases in cytosolic free calcium (Fig. 29.4B). This includes (1) decreased mitochondrial electron transport leading to decreased ATP levels, (2) increased membrane permeability, and (3) depolarization or opening of voltage-dependent channels. The decreased ATP leads to a decreased calcium uptake by mitochondria and ER and a decreased ability to pump calcium out of the cell.

With this background on the normal regulation of cell calcium, we shall now consider the role of intracellular calcium in tubular injury. In 1981, it was proposed that calcium ions were important participants in the functional, biochemical, and morphologic disturbances that characterize AKI.[114,115] Numerous studies over the past 15 years in different injury models and cell types have demonstrated an increase in cytosolic calcium in renal epithelial cell injury. These studies are summarized in Table 29.3.

The crucial questions to implicate calcium as a primary factor in cell injury are (1) whether the increase in cytosolic calcium precedes the injury and (2) whether preventing the rise in cytosolic calcium attenuates the injury.[116,117] To investigate whether hypoxia is associated with an increase in free cytosolic calcium in proximal tubular cells, which precedes any evidence of membrane damage, a video imaging technique was developed in which free intracellular calcium could be measured simultaneously with staining of nuclei with the membrane impermeable indicator, propidium iodide, as an index of hypoxia-induced membrane damage.[118] Propidium iodide enters the cell through the damaged plasma membrane and stains the cell nucleus. The percent of nuclei that stain with propidium iodide is quantitated and is an index of plasma membrane damage. Hypoxia in rat proximal tubules is associated with a significant rise in cytosolic calcium, which antecedes evidence of membrane damage as assessed by propidium iodide staining.[99] Cytosolic calcium increased from 170 to 390 nM during 5 minutes of hypoxia. The increase in cytosolic calcium preceded propidium iodide–detectable cell injury (Fig. 29.5). The increase in cytosolic calcium that preceded the hypoxic membrane damage was promptly reversible with reoxygenation after 8 minutes of hypoxia. This is important because if cytosolic calcium is increased only after lethal cell membrane damage, reoxygenation should not have

29.3 Increases in Cytosolic Calcium in Renal Epithelial Cell Injury

Injury Model	Cell Type	Reference
Calcium ionophore	Rabbit proximal tubules	Mandel and Murphy, 1984[467]
Anoxia	LLCMK2 cells	Snowdowne et al., 1985[468]
Chemical ATP depletion	MDCK cells	McCoy et al., 1988[469]
Calcium ionophore, chemical ATP depletion	Cultured rabbit proximal tubules	Phelps et al., 1989[470]
Chemical anoxia	Rabbit proximal tubules	Weinberg et al., 1991[471]
Hypoxia	Rabbit proximal tubules	Jacobs et al., 1991[472]
Hydrogen peroxide	LLCPK1 cells	Ueda and Shah, 1992[473]
Anoxia and hypoxia	Rat proximal tubules	Almeida et al., 1992[474]
Chemical anoxia	Opossum kidney cells	Li et al., 1993[475]
Hypoxia-reoxygenation	Primary culture rat proximal tubules	Greene and Paller, 1994[476]
Hypoxia	Rat proximal tubules	Kribben et al., 1994[99]
Anoxia	Rabbit proximal tubules	Rose et al., 1994[477]

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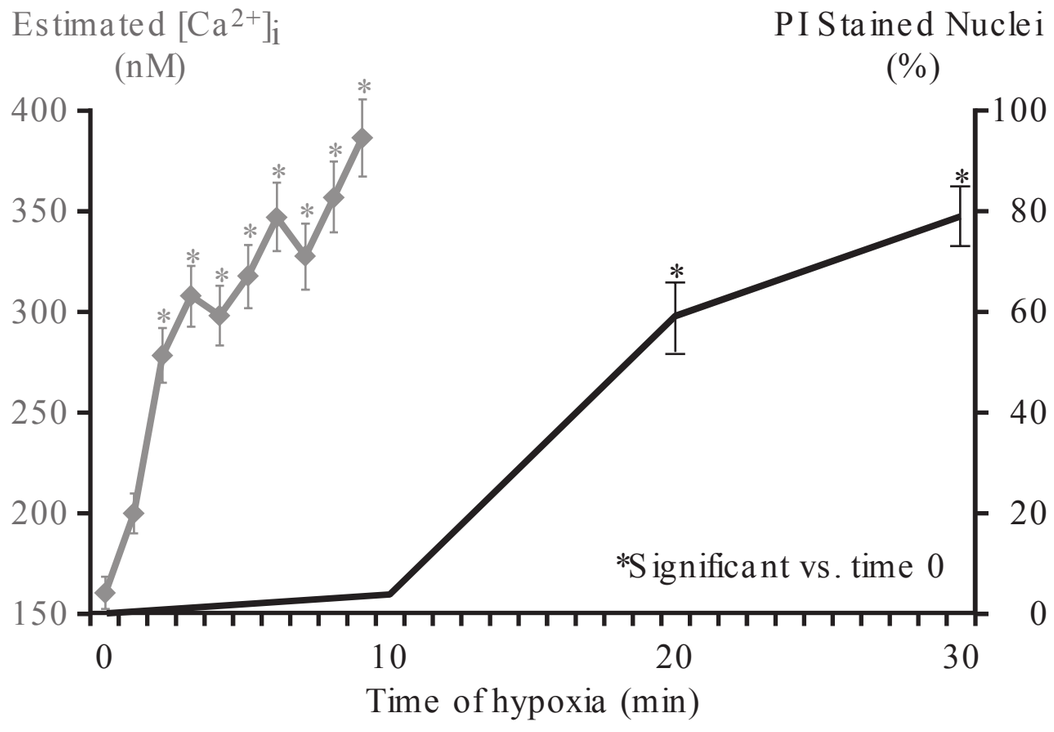


FIGURE 29.5 In isolated proximal tubules, the increase in free cytosolic calcium as measured by Fura-2 precedes the cell membrane damage as assessed by propidium iodide (PI) staining. Adapted from Kribben et al.[99]

normalized cytosolic calcium. The 10 minutes of cytosolic calcium rise correlated significantly with subsequent cell damage observed at the 20 minute mark. The pivotal role of the rise in cytosolic calcium during hypoxia was further demonstrated by using the intracellular Ca^{2+} chelator BAPTA to prevent the rise in cytosolic calcium; this approach resulted in marked cytoprotection against hypoxic tubular injury.

What are the mechanisms whereby increases in cytosolic free calcium could lead to cell membrane injury? Potential calcium-dependent mechanisms include changes in the actin cytoskeleton of proximal tubule microvilli,[119] activation of calcium-dependent PLA_2,[40] and activation of the calcium-dependent cysteine protease, calpain.[116,117,120,121]

Calcium-Dependent Changes in the Actin Cytoskeleton

The role of calcium in pathophysiologic alterations of the proximal tubule microvillus actin cytoskeleton was studied in freshly isolated tubules.[119] Precisely defined medium calcium levels were defined using a combination of the metabolic inhibitor, antimycin, and the ionophore, ionomycin, in the presence of glycine to prevent lethal membrane damage. Increases of intracellular calcium to 10 μM were sufficient to initiate concurrent actin depolymerization, fragmentation of F-actin into forms requiring high-speed centrifugation for recovery, redistribution of villin to sedimentable fractions, and structural microvillar damage consisting of severe swelling and fragmentation of actin cores. However, during ATP depletion induced by antimycin alone or hypoxia alone, initial microvillar damage was calcium independent. This study suggests that both ATP depletion-dependent but Ca^{2+}-independent, as well as Ca^{2+}-mediated processes, can disrupt the actin cytoskeleton during acute proximal tubule cell injury; that both types of change occur, despite protection afforded by glycine and reduced pH against lethal membrane damage; and that Ca^{2+}-independent processes primarily account for prelethal actin cytoskeletal alterations during simple ATP depletion of proximal tubule cells.

In normal proximal tubule cells, actin is concentrated in apical brush border microvilli, along with the actin-binding protein, villin. Villin plays an important role in actin bundling and in microvillar assembly but can also act as an actin-fragmenting protein at higher calcium concentrations. The effects of ischemic injury and reperfusion on the distribution of villin and actin in the proximal tubule cells of rat kidneys were examined.[122] This study demonstrated that villin may be involved in the initial disruption of the actin cytoskeleton during reperfusion injury and that its migration back to the apical domain of these cells accompanies the reestablishment of a normal actin distribution in the brush border.

ATP depletion results in the conversion of monomeric G-actin to polymeric F-actin during tissue ischemia.[123] This conversion results from altering the ratio of ATP-G actin and ADP-G actin, causing a net decrease in the concentration of thymosin actin complexes as a cosequence of the differential affinity of thymosin beta 4 for ATP and ADP-G actin.[124] Recent studies suggest that the actin-binding protein tropomyosin binds to and stabilizes the apical actin microvilli under physiologic conditions in proximal tubules.[125]

Activation of Phospholipase A_2

Phospholipase A_2 (PLA_2) enzymes are important regulators of prostaglandin and leukotriene synthesis and can directly modify the composition of cellular membranes.[126] PLA_2 enzymes are also potent regulators of inflammation. The cytosolic form, $cPLA_2$, preferentially releases arachidonic acid from phospholipids and is regulated by changes in intracellular calcium concentration.[127]

PLA_2 enzymatic activity was measured in cell-free extracts prepared from rat renal proximal tubules.[40] Both soluble and membrane-associated PLA_2 activity were detected. All PLA_2 activity detected during normoxia was calcium dependent. The fractionation of cytosolic extracts by gel filtration revealed three peaks of PLA_2 activity. Exposure of tubules to hypoxia resulted in stable activation of soluble PLA_2 activity, which correlated with the disappearance of the highest molecular mass form (>100 kDa) and the appearance of a low–molecular-mass form (approximately 15 kDa) of PLA_2. Hypoxia also resulted in the release of a low–molecular-mass form of PLA_2 into the extracellular medium. Pretreatment of tubules with glycine before hypoxia blocked this release of PLA_2 but not the activation of soluble PLA_2 activity. This study provides direct evidence for calcium-dependent PLA_2 activation during hypoxia. However, calcium-independent forms of PLA_2 have also been found to play a role in hypoxic proximal tubular injury.[128]

The mechanism of PLA_2-induced cell membrane damage is interesting. Membrane phospholipid breakdown has been observed to occur in a number of tissues during ischemia.[129] In proximal tubules, hypoxia has been shown to

cause an increase in free fatty acids, which was initially believed to contribute to cell injury.[130] However, a study from our laboratory has shown that unsaturated free fatty acids protect against hypoxic injury in proximal tubules and that this protection may be mediated by a negative feedback inhibition of PLA_2 activity.[41] This protective effect of unsaturated free fatty acids has been confirmed by Zager et al.[131] The injurious effect of PLA_2 could be related to a direct disruption of cell membrane integrity by attacking the phospholipid component of cell membranes or through the accumulation of lysophospholipids, which have been shown to disrupt cell membranes and cause cytotoxicity.[132]

Activation of Calpain

The cysteine proteases are a group of intracellular proteases that have a cysteine residue at their active site. The cysteine proteases consist of three major groups: cathepsins, calpains, and the newly discovered caspases. The major groups of cysteine proteases are shown in Table 29.4. The cathepsins are non–calcium-dependent lysosomal proteases that do not appear to play a role in lethal cell injury.[133–135] Calpain is a calcium-activated neutral protease (CANP).[136] It has absolute dependence on calcium. There are two major ubiquitous or conventional isoforms of calpain, the low calcium sensitive μ-calpain and the high calcium sensitive m-calpain.[137,138] The isoenzymes have the same substrate specificity but differ in affinity for Ca^{2+}. μ-Calpain is activated by micromolar concentrations of Ca^{2+}, and m-calpain is activated by millimolar concentrations of Ca^{2+}. The millimolar concentrations of intracellular calcium needed for the activation of m-calpain are not seen in normal cells, and phosphatidylinositol is thought to lower the calcium concentration required for half the maximal autolysis of m-calpain.[139] Procalpain exists in the cytoplasm as an inactive proenzyme and becomes active proteolytically only after it has become autolysed at the cell membrane. Activity of the autolysed calpain is subject to a final regulation by calpastatin.[140,141] Calpastatin is a specific endogenous inhibitor of calpain. It is as widely distributed in nature as the enzyme itself. Calcium is required for calpastatin to bind to calpain and thus for the inhibitory effect of calpastatin on calpain.

Postulated functions of calpain include platelet activation and aggregation, cytoskeleton and cell-membrane organization,[142] and the regulation of cell growth.[143–146]

The calcium-dependent calpains have been shown to be mediators of hypoxic/ischemic injury to the brain, liver, and the heart.[147–150] The role of the calcium-dependent cytosolic protease, calpain, in hypoxia-induced renal proximal tubular injury has also been demonstrated.[43] Tubular calpain activity increased significantly by 7.5 minutes of hypoxia, before there was significant LDH release, and further increased during 20 minutes of hypoxia. Chemically dissimilar cysteine protease inhibitors markedly decreased LDH release after 20 minutes of hypoxia and completely prevented the rise in calpain activity during hypoxia. This role of calpain in proximal tubule injury has subsequently been confirmed by other groups.[151,152] This increased calpain activity has subsequently been shown to be associated with a breakdown of the cytoskeletal protein, spectrin, both in vitro[45] and in vivo,[153] as well as increasing Na-K-ATPase into the cytoplasmic fraction of the cell.

Recent studies have demonstrated that calpain mediates progressive plasma membrane permeability and the proteolysis of cytoskeleton-associated paxillin, talin, and vinculin during antimycin A or hypoxia-induced proximal tubular cell death.[154] Novel nonpeptide calpain inhibitors are protective against antimycin A–induced calcium influx and hypoxia/reoxygenation-induced proximal tubular cell death.[155] In novel in vivo studies, calpastatin transgenic mice that had a decreased activation of calpain in the kidney

29.4 The Major Groups of Cysteine Proteases

	Cathepsins	Calpains	Caspases
Family	B,H,L,S (lysosomal)	μ and m Calpain Tissue specific isoforms	1–14
Location	Lysosome	Cytoplasm	Cytoplasm
Activation	Calcium-independent	Calcium dependent	Caspase activated
Optimal pH	5-6	7.4	7.4
Functions	Intracellular protein degradation	Intracellular signaling Cytoskeletal stability Necrosis and apoptosis	Apoptosis/necrosis Cytokine activation

Adapted from Edelstein[121] with permission.

were generated.[156] In an anti-glomerular basement membrane (GBM) model, calpastatin-transgenic mice had less severe glomerular injury and a reduction in nuclear factor kappa-B (NF-κB) activation, suggesting a role for calpain in inflammation.

Caspases

Caspases are another group of intracellular cysteine proteases. Caspases participate in two distinct signaling pathways: (1) the activation of proinflammatory cytokines and (2) the promotion of apoptotic cell death.[9,157–162] Caspases 3 and 7 are the major mediators of apoptosis. The term "caspase" embodies two properties of these cysteine proteases in which "c" refers to "cysteine" and "aspase" refers to their specific ability to cleave substrates after an aspartate residue. The members of the caspase family are divided into subfamilies based on substrate specificity and function.[163] Caspase-1 (previously known as IL-1–converting enzyme [ICE]) plays a major role in the activation of proinflammatory cytokines. For many years it was not known how caspase-1 was activated. It has recently been discovered that procaspase-1 is activated in a complex called the inflammasome.[164,165] The inflammasome is a protein scaffold that contains NALP (NACHT, LRR, and pyrin domain–containing) proteins, an adaptor protein called ASC (apoptosis-associated specklike protein containing a caspase-recruiting domain [CARD]), procaspase-1, and caspase-5. The interaction of the CARD of procaspase-1 is mediated by the CARD of ASC and the CARD present in the C-terminus of NALP-1. Active caspase-1 in the inflammasome is a regulator of the unconventional protein secretion of leaderless proteins like IL-1α and fibroblast growth factor (FGF)-2.[166] In a recent study, the inflammasome components NLRP3 and ASC were highly expressed in the renal tubular epithelium of humans and mice.[167] The absence of Nlrp3, but not ASC, protected against ischemic AKI.[167] Activation of caspases-1, 8, 9, and 3 have been described in hypoxic renal epithelial cells[168–170] and cerebral ischemia.[171] Although cells contain many caspases, the targeted disruption of specific caspase genes in mice has provided much insight into the functions of individual caspases during cell death.[172]

Although caspases play a crucial and extensively studied role in apoptosis, there is now considerable evidence that the caspase pathway may also be involved in necrotic cell death.[173] The inhibition of caspases protects against necrotic cell death induced by the mitochondrial inhibitor, antimycin A, in PC12 cells, Hep G2 cells, and renal tubules in a culture.[174,175] Caspases are also involved in hypoxic and reperfusion injury in cultured endothelial cells.[176] Rat kidneys subjected to ischemia demonstrate an increase in both caspase-1 and caspase-3 mRNA and protein expression.[177] Caspases play a role in hypoxia-induced necrotic injury of isolated rat renal proximal tubules.[48] In this study, caspase activity was increased in association with cell membrane damage as assessed by LDH release. A specific caspase inhibitor attenuated the increase in caspase activity and markedly protected against cell membrane damage. To specifically identify the caspase involved in proximal tubular injury, proximal tubules were isolated from capsase-1 knockout mice and exposed to hypoxia.[178] Proximal tubules from caspase-1 knockout mice were protected against hypoxic injury, demonstrating the role of caspase-1 in directly causing cell membrane damage in proximal tubules.[178]

A study[179] investigated the role of caspase inhibition and apoptosis in ischemic AKI in mice in vivo. A relationship between apoptosis and subsequent inflammation was found. At the time of reperfusion, administration of the antiapoptotic agents insulin-like growth factor 1 (IGF-1) and ZVAD-fmk (a caspase inactivator) prevented the early onset of not only renal apoptosis, but also inflammation and tissue injury. Conversely, when the antiapoptotic agents were administered after the onset of apoptosis, these protective effects were completely abrogated.

There appears to be an interaction between caspases and calpain during hypoxia-induced injury in the proximal tubule, because caspase inhibition was shown to decrease calpain activity during hypoxia.[48,121] Recent in vivo studies[153] suggest that caspase-mediated degradation of the endogenous inhibitor of calpain, calpastatin, is a mechanism whereby the calcium-mediated activity of calpain is increased.

Caspases in Cold Ischemia

Preservation injury, also known as cold ischemia, is an important clinical problem in kidney transplantation. Significant damage to the kidney may occur during harvest, cold storage, and transport. An ongoing area of interest is identifying methods to reduce organ injury during this process. The primary consequence of cold ischemic injury is delayed graft function (DGF) in kidney transplants.[180–182] These consequences have both short-term and long-term effects. In a kidney transplant, for example, DGF increases patient morbidity in the short term because the hospital stay is longer and dialysis may be required. In the long term, DGF independently predicts reduced 1- and 5-year graft survival.[183]

Both human and animal studies suggest that the adverse impact of cold ischemia may be associated with apoptosis. In human kidney transplant biopsies performed after 1 hour of reperfusion, apoptosis of tubular cells correlated significantly with cold ischemic time.[184] Biopsies of human donor kidneys, which subsequently developed postoperative AKI, demonstrated increased renal tubular epithelial cell apoptosis.[185] Prolonged cold ischemia has also been shown to increase apoptotic cell death in rat kidney allografts at 24 weeks posttransplant.[186] Mitochondria undergo significant changes during ischemia and may contribute to preservation injury.[187]

Caspases have been studied in cold ischemic kidneys.[188] Kidneys were stored for 48 hours at 4°C to produce cold ischemia. Caspase-3 activity was massively increased (100-fold) in cold ischemic kidneys compared to controls. On immunoblot analysis, the processed form of caspase-3

was increased in cold ischemic kidneys compared to controls. The increase in caspase-3 was associated with significantly more renal tubular apoptosis and brush border injury. The pan-caspase inhibitor prevented the formation of the processed form of caspase-3 and the increase in caspase activity, and reduced apoptosis and brush border injury. The results of this study suggest that caspase inhibition may prove useful in kidney preservation.

In a pig model of cold ischemia in which the heart was stopped to mimic donation after cardiac death (DCD), there was a massive increase in apoptosis, caspase-3/7 activity, and caspase-3 protein expression.[189] Apoptosis was compared in DCD kidneys subjected to static versus pulsatile perfusion for 24 hours. Pulsatile perfusion significantly reduced proximal tubular apoptosis and was associated with increased Bcl-2 and hypoxia-inducible transcription factor-1α in the kidney.

The relevance of these studies to organ preservation and the subsequent risk of graft dysfunction is substantial. Caspase inhibitors are a particularly attractive approach to reduce the incidence of DGF in kidney transplantation.

Caspase-1 and IL-18

Caspase-1 is a proinflammatory caspase that cleaves precursor IL-1β and precursor IL-18. Caspase-1-/- mice developed less ischemic AKI as judged by renal function and renal histology (Fig. 29.6A).[190] IL-1β receptor knockout mice or mice treated with IL-1β receptor antagonist (IL-1Ra) are not protected against ischemic AKI.[30] Because caspase-1 also activates IL-18, lack of the mature form of IL-18 in these caspase-1-/- mice was investigated as a possible mechanism of this protection against AKI. Kidney IL-18 was more than 100% increased in wild-type AKI as compared to sham-operated controls. On an immunoblot analysis, there was

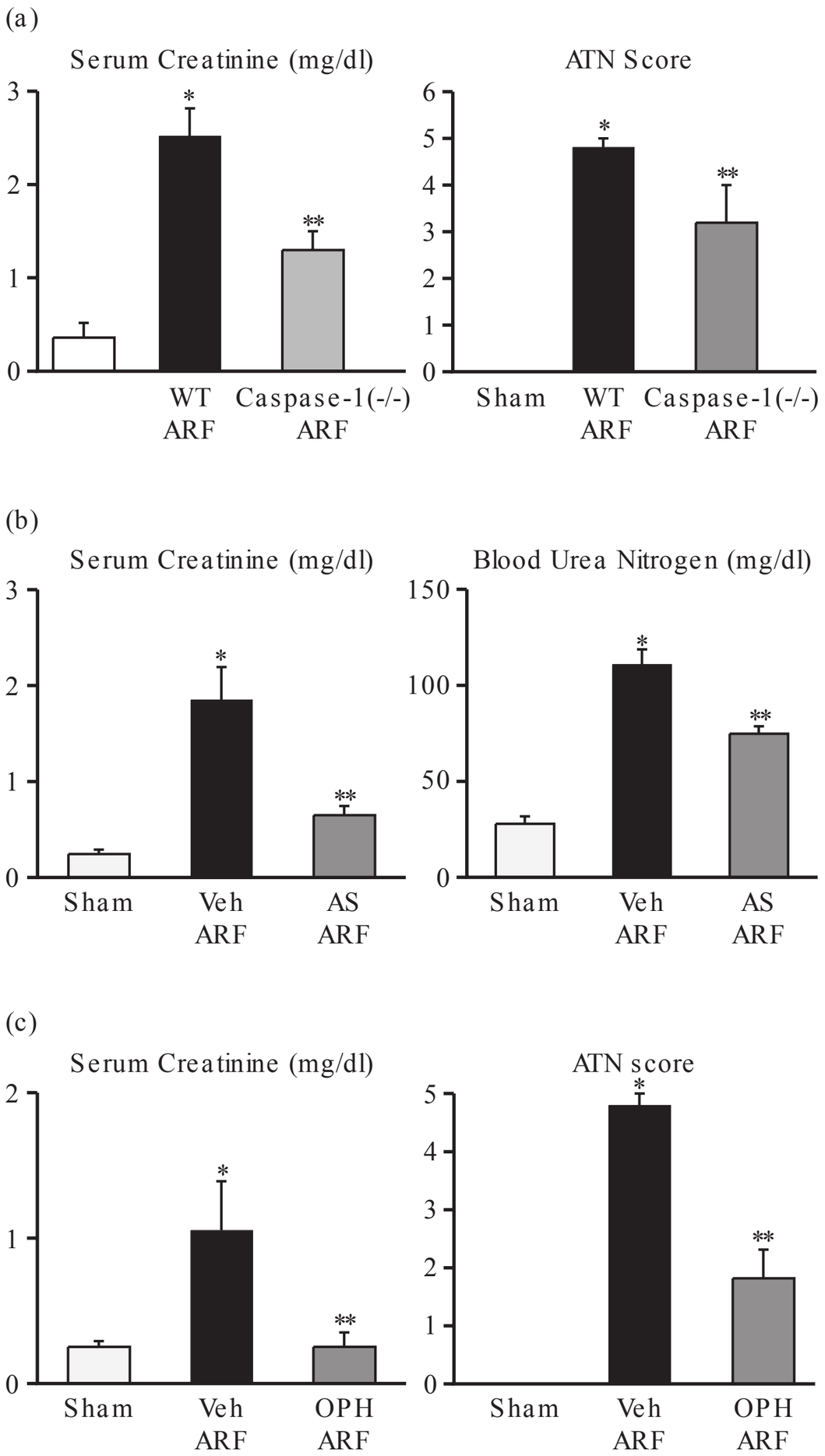


FIGURE 29.6 Caspases and interleukin (IL)-18 in ischemic acute kidney injury (AKI). **A:** Caspase-1-/- mice are protected against ischemic AKI. Caspase-1-/- mice developed less severe AKI, as determined by the serum creatinine and AKI scores compared with wild-type (WT) mice with AKI. * $P < .001$ versus sham; ** $P < .01$ versus WT AKI. **B:** Caspase-1 converts the pro to mature IL-18. Mice treated with IL-18 antiserum (AS) are functionally protected against ischemic AKI. In vehicle-treated mice with ischemic AKI (Veh AKI), serum creatinine and blood urea nitrogen (BUN) levels were significantly increased at 24 hours compared with sham-operated controls. In mice treated with neutralizing IL-18 AS, the serum creatinine and BUN levels were significantly reduced. * $P < .01$ versus sham, ** $P < .01$ versus vehicle-treated mice with Veh AKI. **C:** Mice treated with the pan-caspase inhibitor OPH-001 are protected against ischemic AKI. In vehicle-treated mice with ischemic AKI, the serum creatinine and AKI scores were significantly increased at 24 hours of postischemic reperfusion compared with sham-operated controls. In mice treated with OPH-001 (OPH) before the induction of ischemic AKI, the serum creatinine and AKI scores were significantly decreased compared with sham-operated controls. * $P < .001$ versus sham; ** $P < .01$ versus vehicle-treated mice with Veh AKI; not significant versus sham. ARF, acute renal failure; ATN, acute tubular necrosis. Reproduced from Melnikov et al.,[191] with permission.

a conversion of the precursor to the mature form of IL-18 in AKI wild-type mice, but not in the caspase-1-/- AKI mice and sham-operated controls. To further analyze the role of IL-18, wild-type mice were injected with rabbit antimurine IL-18 neutralizing antiserum prior to the ischemic insult. These mice were protected against AKI to a similar degree as caspase-1-/- mice (Fig. 29.6B).

Caspase-deficient mice have provided extensive information on the role of individual caspases in disease processes. The study of caspase inhibitors is an important step toward the possible therapeutic effect of caspase inhibition in ischemic AKI. Mice with ischemic AKI treated with newly developed caspase inhibitor, Q-VD-(Ome)-OPH (OPH-001) had a marked reduction (100%) in BUN and serum creatinine and a highly significant reduction in the morphologic AKI score compared with vehicle-treated mice (Fig. 29.6C).[191] OPH-001 significantly reduced the increase in caspase-1 activity and IL-18 and prevented neutrophil infiltration in the kidney during ischemic AKI. To further investigate whether this lack of neutrophil infiltration was contributing to the protection against ischemic AKI, a model of neutrophil depletion was developed. Neutrophil-depleted mice had a small (18%) reduction in serum creatinine during ischemic AKI but no reduction in the AKI score despite a lack of neutrophil infiltration in the kidney. Remarkably, caspase-1 activity and IL-18 were still significantly increased in the kidney in neutrophil-depleted mice with AKI. Thus, to investigate the role of IL-18 in ischemic AKI in the absence of neutrophils, neutrophil-depleted mice were treated with an IL-18–neutralizing antiserum. IL-18-antiserum–treated neutrophil-depleted mice with ischemic AKI had a significant reduction (75%) in serum creatinine and a significant reduction in the AKI score compared to vehicle-treated neutrophil-depleted mice. These results suggest a novel neutrophil-independent mechanism of IL-18–mediated ischemic AKI.

In other studies, it was determined whether IL-18–binding protein transgenic (IL-18BP Tg) mice are protected against ischemic AKI.[192] IL-18 function is neutralized in IL-18BP Tg mice. IL-18BP Tg mice with AKI had significantly lower BUN, serum creatinine, and ATN score than wild-type mice. The number of macrophages in the kidney was significantly reduced in IL-18BP Tg compared with wild-type mice. The proinflammatory chemokine, CXCL1 (also known as KC or IL-8), was significantly reduced in the kidneys of IL-18BP Tg mice compared to wild-type mice. This study demonstrates that protection against ischemic AKI in IL-18BP Tg mice is associated with less macrophage infiltration and less production of CXCL1 in the kidney.

The effects of different caspase inhibitors on ischemic AKI in the rat kidney have been studied.[193] A caspase-1 inhibitor significantly reduced functional and histologic evidence of ischemic AKI compared to a caspase-3 inhibitor. Another group of investigators found that caspase-1–deficient mice were not protected against renal ischemia.[194] In this study, the model of renal ischemia was 45 minutes of unilateral renal pedicle clamping with contralateral nephrectomy. This model produces a milder form of functional injury than bilateral clamping. At 24 hours, BUN and creatinine were lower in the caspase-1-/- mice than in the wild-type, but the decrease was not statistically significant.

Matrix Metalloproteinases

Matrix metalloproteinases are a large family of zinc-dependent matrix-degrading enzymes that include interstitial collagenases, stromelysins, gelatinases, elastases, as well as membrane-type matrix metalloproteinases. They play a crucial role in remodeling the extracellular matrix, which is an important physiologic feature of normal growth and development. In the kidney, interstitial sclerosis and glomerulosclerosis have been associated with an imbalance of extracellular matrix synthesis and degradation.[195] Alterations in renal tubular basement membrane matrix proteins, laminin and fibronectin, occur after renal ischemia-reperfusion injury.[196] The role of matrix metalloproteinases in this process has been studied.

In endothelial cells isolated from ischemic kidneys, the proteolytic activity of proMMP-2, proMMP-9, and MMP-9 was increased. Occludin, an in vivo MMP-9 substrate, was partly degraded in the endothelial fractions during ischemia, suggesting that the upregulation of MMP-9 had a functional effect to degrade occluding. These data suggest that AKI leads to the degradation of the vascular basement membrane and to increased permeability related to the increase of MMP-9.[197] In renal cells, in vitro cleavage of cadherins in normal rat kidney (NRK) cells requires active membrane-type (MT)1-MMP (MT1-MMP), also known as MMP-14.[198] The disruption of cadherin/catenin complexes in AKI may be associated with the transtubular back-leak of glomerular filtrate. In contrast to the potential injurious role of some MMPs, MMP9 protects the S3 segment of the proximal tubule and the intercalated cells of the collecting duct from apoptosis in AKI, most likely by releasing soluble stem cell factor (sSCF), an MMP9 substrate.[199]

Meprin A is a zinc-dependent metalloendopeptidase that is present in the brush border membrane of renal proximal tubular epithelial cells. The redistribution of this metalloendopeptidase to the basolateral membrane domain during AKI results in degradation of the extracellular matrix and damage to adjacent peritubular structures. The effect of meprin A, the major matrix degrading metalloproteinase in rat kidney, on the laminin–nidogen complex was examined. Nidogen-1 (entactin) acts as a bridge between the extracellular matrix molecules, laminin-1 and type IV collagen, and thus participates in the assembly of basement membranes. Following ischemic injury, meprin A undergoes redistribution and/or adherence to the tubular basement membrane. Nidogen breakdown products are produced as the result of partial degradation of tubular basement membrane by meprin A following renal tubular ischemia–reperfusion injury.[200]

The susceptibility of inbred strains of mice to ischemic and nephrotoxic acute renal failure was studied in mice with normal and low meprin A activity.[201] The strains of mice with normal meprin A developed more severe renal functional and structural injury following renal ischemia or the injection of hypertonic glycerol compared to the two low meprin A strains. These findings suggest that meprin A plays a role in the pathophysiology of AKI following ischemic and nephrotoxic acute renal failure insults to the kidney.[201] A recent study[202] demonstrated that meprin inhibition protects against ischemic AKI in vivo in rats.

Nitric Oxide

NO is a lipophilic, highly reactive free radical gas with diverse biomessenger functions.[203] NO mediates diverse functions including vasodilatation, platelet aggregation inhibition, neurotransmission, inflammation, antimicrobial and antitumor actions, and apoptosis.[203] Whether the net effects of NO are beneficial or deleterious is determined by the cell type, the concentration of NO, the duration of production, and the composition of the surrounding microenvironment.[203] There are three major nitric oxide synthase (NOS) isoforms in the kidney: neuronal NOS or nNOS (also known as NOS1), inducible NOS (iNOS, also known as NOS2), and endothelial NOS or eNOS (also known as NOS3) (Table 29.5).[204] The macula densa is the principal site of nNOS expression in the kidney.[205,206] In situ hybridization studies in the NRK demonstrate iNOS mRNA in the S3 segment of the proximal tubule, the cortical and medullary thick ascending limb, the distal convoluted tubule, and the cortical collecting duct and the inner medullary collecting duct.[207] eNOS mRNA has been detected in glomeruli, preglomerular vasculature, and proximal and distal tubules.[208] eNOS protein is mainly present in the endothelium of intrarenal, afferent, efferent, and glomerular arterioles and the medullary vas recta.[205] Expression of eNOS protein in tubules has not yet been reported.[204] nNOS and eNOS are continuously present, are activated by calcium, and are also termed constitutive NOS (cNOS).[209,210] In contrast, iNOS is induced when the cells have been stimulated by certain cytokines, microbes, and microbial products, and thus is called iNOS.[211,212] The time course of both calcium-dependent and -independent NOS activity in the rat renal cortex and medulla has been studied.[213] Calcium-dependent NOS activity in the cortex and the medulla decreased in the early phase of AKI and then increased in the recovery phase in the cortex. iNOS activity increased in the early phase of AKI in both the cortex and the medulla and was maintained at higher levels in the medulla. However, in another study, L-arginine improved the deficiency of constitutive NOS activity and improved the recovery phase of ischemic AKI in rats.[214]

Studies in freshly isolated proximal tubules from knockout mice have also revealed the role of NO in hypoxic/ischemic tubular injury. Hypoxia-induced proximal tubule damage, as assessed by LDH release, was no different between wild-type and mice in which eNOS and nNOS had been knocked out. However, proximal tubules from iNOS knockout mice demonstrated resistance to the same degree of hypoxia.[42] The iNOS knockout mice also had less renal failure and better survival than the wild-type mice after renal artery clamping.[215] An induction of heat shock protein was also observed in the iNOS knockout mice as a potential con-

29.5 Nitric Oxide Synthase (NOS) Isoforms

	Tissue Distribution		
Isoform	**Body**	**Renal**	**Phenotype of Knockout Mouse**
nNOS (Type 1)	Neurons, skeletal muscle, penis	Macula densa	Protection against cerebral ischemia[478,479]
iNOS (Type 2)	Constitutive: ileum, uterus, skeletal muscle. Induced: macrophage, VSMC	Constitutive: mTAL, proximal tubule	Less hypotensive response to LPS[480] Increased mortality in polymicrobial sepsis[481] No protection against LPS-induced AKI[375] Protection against ischemic AKI[215]
eNOS (Type 3)	Endothelium	Glomerular vessels, intrarenal arteries	Hypertension[482] Increased susceptibility to stroke[483] and myocardial ischemia[484]

nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; VSMC, vascular smooth muscle cell; mTAL, medullary thick ascending limb; LPS, lipopolysaccharide; AKI, acute kidney injury.

tributor to the protection. Chiao et al.[216] produced further results in a renal artery clamp model in mice in which alpha melanocyte-stimulating hormone (αMSH) was shown to block the induction of iNOS, decrease neutrophil infiltration, and afford functional protection. A subsequent study examined the relative importance of αMSH on the neutrophil pathway by examining the effects of αMSH in ICAM-1 knockout mice and neutrophil-poor isolated perfused kidneys where neutrophil effects are minimal or absent.[217,218] In this study, it was found that αMSH decreases renal injury when neutrophil effects are minimal or absent, indicating that αMSH inhibits neutrophil-independent pathways of renal injury.

Hypoxia was found to increase NO release from freshly isolated proximal tubules and this effect was blocked by L-NAME, a nonspecific NOS inhibitor, but not by the inactive D-NAME compound.[39,219] The NO release during hypoxia was accompanied by LDH release and was reversed by L-NAME administration. Interestingly, however, the administration of L-NAME, the nonspecific NOS inhibitor, to the rat kidney clamp model actually worsened the renal failure.[220] This result was interpreted as an overriding blocking effect of eNOS activity with the nonspecific effects of L-NAME.[68] This would worsen the renal vasoconstriction and the resultant injury, thus obscuring any salutary effect at the level of the proximal tubule.[221] Thus, opposing abnormalities in NO production within the endothelial and tubular compartments of the kidney may contribute to renal injury.[68] Reduced eNOS-derived NO production causes vasoconstriction and worsens ischemia; increased iNOS-derived NO production by tubular cells adds to the injurious effects of ischemia on these cells. Therapeutic interventions to modulate NO production in ischemic AKI may require the selective modulation of different NOS isoforms in the tubular and vascular compartments of the kidney.[222] On this background, Noiri and colleagues[220] performed studies using a specific antisense oligonucleotide to iNOS. The ischemia-induced upregulation of iNOS and nitrite production were both blocked by the antisense oligonucleotide. Most importantly, the BUN and serum creatinine did not rise after the renal ischemic insult in the animals treated with the antisense oligonucleotide against iNOS.

Noiri and colleagues also studied the relationship between NO and osteopontin during ischemic AKI. Osteopontin is a negatively charged glycosylated phosphoprotein that is expressed in many tissues, including the renal epithelial cells. Osteopontin serves both a cell attachment function and a cell signaling function via the alpha-v beta-3 integrin. Effects on gene expression include suppression of the induction of NOS by inflammatory mediators. Osteopontin may play an important role in the pathophysiology of AKI. Osteopontin knockout mice subjected to renal ischemia developed worse renal failure and more structural damage than wild-type controls.[223] This was associated with the augmented expression of inducible NOS and the prevalence of nitrotyrosine residues in kidneys from osteopontin knockout mice versus wild-type counterparts. This study[220] provides strong evidence of the renoprotective action of osteopontin in acute renal ischemia.

The protective effect of 17 β-estradiol against ischemic AKI in rats is due to the activation of the PI3K/Akt pathway followed by increased eNOS phosphorylation.[224]

The microvillar actin and cellular integrins are potential substrates of NO action, which could contribute to the ischemia-mediated sloughing of the brush border membrane and the detachment of the proximal tubule epithelial cells from their extracellular matrix.[11,225–227] Such an effect would not only result in impaired tubular sodium reabsorption, but would also provide intraluminal cellular debris as a component of tubular cast formation.

Heat Shock Proteins

The stress response is a highly conserved homeostatic mechanism that allows cells to survive a variety of different stresses.[228] Stresses that trigger the heat shock response include hyperthermia, hypothermia, the generation of oxygen radicals, hypoxia/ischemia, and toxins.[229] On a molecular level, their function is to protect cells from environmental stress damage by binding to partially denatured proteins, dissociating protein aggregates, regulating the correct folding, and cooperating in transporting newly synthesized polypeptides to the target organelles.

The proteins induced by these stresses belong to a family of proteins called heat shock proteins (HSP). The proteins are identified by their molecular weight. The most important families include proteins of 90, 70, 60, and 27 kDa.[229] HSP 90 is essential for cell viability. It is associated with the steroid hormone receptor and is a general chaperone with ATPaselike activity. In stressed cells, it associates with the cytoskeletal protein, actin. The HSP 70 family includes proteins that are both constitutively expressed and induced by stress. They are the most highly induced proteins by stress and function as chaperones, binding to unfolded or misfolded proteins. The HSP family is restricted to the mitochondrial matrix where it functions as an unfoldase. The HSP 27 family has functions similar to HSP 70. Ubiquitin is a stress protein that binds denatured proteins and targets them for proteolysis by the proteasome.

Renal ischemia results in both a profound fall in cellular ATP and a rapid induction of the 70 kD heat shock protein family, HSP-70.[230,231] The relationship between cellular ATP and the induction of the stress response in the renal cortex during renal ischemia has been studied. Van Why et al.[232] demonstrated that a 50% reduction in cellular ATP in the renal cortex must occur before the stress response is detectable, that a reduction of ATP below 25% of control levels produces a more vigorous response, and that reperfusion is not required for the initiation of a heat shock response in the kidney.[232]

Ischemic AKI also induces differential expression of small HSPs. In sham-operated kidneys, HSP 25 localized to glomeruli, vessels, and collecting ducts, whereas another

stress protein, αB-crystallin, localized primarily in medullary thin limbs and collecting ducts. After ischemia, HSP 25 accumulated in proximal tubules in the cortex and the outer medulla, whereas αB-crystallin labeling became nonhomogeneous in the outer medulla, and increased in the Bowman capsule. This study demonstrates that there is striking differential expression of HSP 25 and αB-crystallin in various renal compartments.[233]

In vitro studies have demonstrated that HSP induction protects cultured renal epithelial cells from injury. It has been determined that prior heat stress protects opossum kidney (OK) cells, a cultured renal epithelial cell line, from injury mediated by ATP depletion.[234] Also, HSP 70 overexpression is sufficient to protect cultured proximal tubule (LLC-PK1) cells from hyperthermia but is not sufficient to protect against hypoxia.[235]

During ischemic AKI, the question of whether prior HSP induction by hyperthermia is protective is controversial. One study[236] found that prior heat shock protected kidneys against warm ischemia. Another study[237] investigated the protective effect of heat shock proteins on ischemic injury to renal cells in two different experimental models: ischemia-reflow in intact rats and medullary hypoxic injury as seen in the isolated perfused rat kidney. The prior induction of HSP by hyperthermia was not protective against the functional and morphologic parameters of ischemic AKI in either of these models.[237] These variable results may be explained by the complexity of the intact animal compared to cultured cells; the degree, duration, and timing of the hyperthermic stimulus; and the differential response of mature and immature kidneys.[24,238]

Pharmacologic agents have been used to increase stress protein expression. In a recent study, the induction of HSP 70, a potent antiapoptotic agent, inhibited ischemic renal injury in mice.[239] Recently, inhibitors of the proteasome have been identified that can block the rapid degradation of abnormal cytosolic and ER-associated proteins. The hypothesis that proteasome inhibitors, by causing the accumulation of abnormal proteins, might stimulate the expression of cytosolic heat shock proteins and/or ER molecular chaperones and thereby induce thermotolerance was tested in Madin-Darby canine kidney cell cultures.[240] The inhibition of proteasome function induced heat shock proteins and ER chaperones and conferred thermotolerance in these cells. Thus, these agents may have applications in protecting against cell injury.[240] Another study[241] determined that proteasome inhibition protects against the morphologic and functional abnormalities in ischemic AKI in rats.[241] However, the effect of proteasome inhibition on HSP induction during ischemic AKI was not determined in this study.

The mechanism of HSP protection against ischemic AKI is interesting. It has been suggested that HSPs participate in the postischemic restructuring of the cytoskeleton of proximal tubules.[242] It was found that HSP 72 complexes with aggregated cellular proteins in an ATP-dependent manner, suggesting that enhancing the HSP 72 function after an ischemic renal injury assists refolding and stabilization of Na(+)-K(+)-ATPase or aggregated elements of the cytoskeleton, allowing reassembly into a more organized state.[243] Another study examined the temporal and spatial patterns of HSP 25 induction in relation to the actin cytoskeleton.[244] This study suggested that there are specific interactions between HSP 25 and actin during the early postischemic reorganization of the cytoskeleton. In another study,[245] the Brown Norway rat was resistant to renal failure and AKI compared to the Sprague-Dawley rat. The Brown Norway rat had no distribution of Na-K-ATPase into detergent soluble cortical extracts, and the immunohistochemistry showed that baseline HSP 72 and 25 expression was increased in proximal tubules of the Brown Norway rats compared to the Sprague-Dawley rats.

Another potential mechanism of HSP protection against proximal tubular injury is the inhibition of apoptosis. OK proximal tubule cells exposed to ATP depletion develop apoptosis by morphologic and biochemical criteria. Prior heat stress reduced the number of apoptotic-appearing cells, significantly decreased DNA fragmentation, and improved cell survival compared with controls.[246] This study demonstrated that novel interactions between HSP 72 and the antiapoptotic protein Bcl2 may be responsible, at least in part, for the protection afforded by prior heat stress against ATP depletion injury.

Altered Gene Expression

During renal ischemia in vivo, the reaction of the renal epithelial cells is heterogeneous.[247] Some cells, especially those of the proximal tubule, undergo necrosis. Other cells undergo apoptosis, and still others survive the ischemic injury intact. In addition, injured tubules are relined with new cells actively engaged in DNA repair and synthesis. Thus, surviving tubular cells can reenter the cell cycle and replicate. These cells may undergo partial dedifferentiation that allows them to undergo mitosis.[248] The complex events that mediate this heterogeneous response of tubular cells are being studied. This response of tubular cells may involve the early immediate gene response.

Immediate early genes and proto-oncogenes are induced during the early reperfusion period after renal ischemia.[249] There is c-Fos and c-Jun activation as well as an increase in DNA synthesis.[250] There is an accumulation of early growth response factor 1 (Egr-1) and c-Fos mRNAs in the mouse kidney after occlusion of the renal artery and reperfusion.[251,252] Transient expression of the genes c-Fos and Egr-1 may code for DNA binding transcription factors and initiate the transcription of other genes necessary for cell division.[253] JE and KC, growth-factor–responsive genes with cytokinelike properties that play a role in inflammation, are also expressed during early renal ischemia.[254] These genes may code for proteins with chemotactic effects that can attract monocytes and neutrophils into areas of injury.[252] Studies demonstrate that c-Fos and c-Jun are expressed following renal ischemia as a typical immediate

early gene response, but they are expressed in cells that do not enter the cell cycle.[248,255] The failure of the cells to enter the cell cycle may depend on the coexpression of other genes.

DNA synthesis occurs in the proximal tubule, whereas the induction of the early gene response is restricted to cells of the thick ascending limb and collecting duct.[252] Thus, the immediate early gene response does not always occur in cells that undergo DNA synthesis, suggesting that the role of the early gene response is not necessarily proliferative in this setting. The role of the stress response during renal ischemia and the fate of the cells undergoing it are unknown. This immediate early gene response may play a role in the protection of tubular cells against injury. Alternatively, it may be important in mounting a response that will later help the regeneration of other tubular cells because the products of some of these genes are localized to cells that do not undergo cell death from apoptosis or necrosis.[256] The immediate early gene response may be the response to sublethal injury, allowing the cell to dedifferentiate.[253]

The pathways that lead to the early gene response are interesting. At least two quite different pathways lead to the activation of c-Jun.[257–259] Growth factors activate c-Jun via the mitogen-activated protein kinases (MAPKs), which include extracellular-regulated kinases (ERKs) 1 and 2. This pathway is proliferative in nature. In contrast, the stress-activated protein kinase (SAPK) pathway is separate from the MAPK pathway. These kinases include c-Jun N-terminal kinase (JNK) 1 and 2. Activation and the effect on cell fate of the SAPK pathway is very different from the MAPK pathway. The SAPK pathway is essentially antiproliferative and can lead to either cell survival or cell death. During renal ischemia, SAPKs are activated, and the inhibition of SAPK after ischemia protects against renal failure.[260,261] Thus, it is possible that manipulation of this pathway could lead to therapies that may ameliorate AKI.

Numerous recent studies have analyzed gene expression during ischemic AKI. Cell communication, apoptosis, and inflammation genes distinguish primary allograft function in human kidney transplantation.[262] In renal ischemia–reperfusion in mice, there was an increase in genes involved in cell structure, extracellular matrix, intracellular calcium binding, and cell division/differentiation.[263] In another study in mice, there were consistent patterns of altered gene expression in the first 24 hours of postischemic reperfusion.[264] These genes included transcription factors, growth factors, signal transduction molecules, and apoptotic factors. In ischemia–reperfusion in the rat, alterations in the expression of 18 genes were identified by microarray analysis.[265] Nine genes were upregulated (ADAM2, HO-1, UCP-2, and thymosin β4 in the early phase, and clusterin, vanin1, fibronectin, heat-responsive protein 12, and FK506-binding protein in the established phase). Nine genes were downregulated (glutamine synthetase, cytochrome p450 IId6, and cyp 2d9 in the early phase, and cyp 4a14, Xist gene, peroxisome proliferator-activated receptor gamma (PPARγ), α-albumin, uromodulin, and ADH B2 in the established phase). Changes in the gene expression of ADAM2, cyp2d6, fibronectin, HO-1, and PPARγ were confirmed by quantitative real-time polymerase chain reaction (PCR). One of the problems with microarray analysis in the whole kidney during ischemic AKI in vivo is identifying which of the numerous cell types in the kidney is the source of the gene alteration. Laser capture microdissection of immunofluorescently defined cells (IF-LCM) can isolate pure populations of targeted cells from a sea of surrounding cells with excellent preservation of mRNA.[266] This technique has been used to label and isolate thick ascending limb cells in the kidney for mRNA analysis.[266]

In ischemic AKI in mice, 24,600 genes were tested by transcriptional analysis.[267] At days 3, 10, and 28 after ischemic AKI, 242, 146, and 46 genes were upregulated, respectively, and 85, 35, and zero genes were downregulated, respectively. Gene expression changes were primarily related to immune and inflammatory pathways both early and late after AKI. The most highly upregulated genes late after AKI were hepatitis A virus cellular receptor 1 (Havcr1) and lipocalin 2 (Lcn2), which code for KIM-1 and NGAL, respectively.

The PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play crucial roles in the regulation of cellular differentiation, development, and metabolism and tumorigenesis. Increased expression of PPARα[268] or PPARβ/δ[269] protects aginst ischemic AKI. Maintenance of free fatty acid oxidation in the proximal tubule may be the mechanism of the protection against ischemic AKI.[270]

Apoptosis

Apoptosis was first described by Kerr et al.[271] The term comes from the ancient Greek word that means "the dropping off as of leaves from a tree." The term stresses the facts that apoptosis is a physiologic form of cell death, occurs in the individual cell (or leaf) in a programmed pattern, and can be triggered according to a program regulated by external stimuli (autumn).[7] Thus, apoptosis is the name given to the process of physiologic or programmed cell death. Apoptotic cells undergo a series of morphologically identifiable changes in their pathway to cell death.[272] The morphologic, biochemical, and molecular characteristics of apoptosis versus necrosis are very different (Table 29.6). Autophagy has been proposed as a third mode of cell death.[273] Autophagy is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules. Autophagy permits a starving cell, or a cell that is deprived of growth factors, to survive. There are differences in the mode of death and different morphologic, biochemical, and molecular attributes between apoptosis, necrosis, and autophagy.[273]

The triggers of apoptosis include (1) cell injury (e.g., ischemia, hypoxia, oxidant injury, nitric oxide, cisplatin); (2) loss of survival factors (e.g., deficiency of renal growth factors, impaired cell-to-cell or cell-to-matrix adhesion);

29.6 Morphologic, Biochemical, and Molecular Differences Between Apoptosis and Necrosis

Apoptosis	Necrosis
Individual cells shrink and detach from other cells	Multiple cells swell but remain attached
Plasma membranes remain intact	Plasma membrane disruption
Cell excludes DNA-specific dye, propidium iodide	Propidium iodide enters cell and stains nucleus
Nuclear condensation, fragmentation, and pyknosis	Nuclear swelling and autolysis
Apoptotic bodies	No apoptotic bodies
Nuclear DNA fragmentation	Nuclear DNA fragmentation
Programmed by gene activation	No gene activation
Phagocytosis of cellular fragments	Cellular lysis
No inflammation	Inflammation

Autophagy has been proposed as a third mode of cell death.[273] Autophagy is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules, permitting a starving cell or a cell that is deprived of growth factors to survive.

and (3) receptor-mediated apoptosis (e.g., Fas [CD 95] and transforming growth factor [TGF]β).[274]

The two major pathways of apoptosis involve Fas and p53.[9,10,275] In the tumor necrosis factor (TNF) receptor superfamily, Fas antigen (CD 295) is the most important factor. Engagement of Fas by its ligand (FasL) results in apoptosis. The tumor suppressor gene, p53, mediates apoptosis in cells in which the DNA has been damaged. The cascades involving Fas and p53, which are centrally important in cell death, are shown in Figure 29.7.

Caspases are the major mediators of the cell death in apoptosis and also play a role in necrotic cell death. The central role of caspases in cell death is supported by caspase-8, 9, and 3 knockout mice that have strong phenotypes based on apoptotic cell death defects, developmental defects, and usually fetal/perinatal mortality.[162] Caspase-7, like caspase-3, is an executioner caspase and is downstream of the initiators, caspase-8 and 9. Both the intrinsic and extrinsic pathways activate caspase-7. Caspase-3 and 7 exhibit very similar substrate specificities in peptide hydrolysis assays in vitro.[276] However, the role of caspase-7 during the execution phase of apoptosis is obscure.[277] Caspase-7 is unable to cleave the well-known caspase-3 substrates including fodrin, gelsolin, DNA fragmentation factor 45 (DFF45), inhibitor of apoptosis proteins (IAP), and signal transducer and activator of transcription 1 (STAT-1).[277] Also, caspase-7 is unable to activate caspases that would normally be activated by caspase-3.[278] It is known that caspase-3 and 7 can act independently as executioners of apoptosis. Both caspase-3 and caspase-7 deficient mice are perinatally lethal due to a lack of apoptosis.[279] Caspase-7 may play a more specialized role in apoptosis than caspase-3.[277] Both caspase-3 and 7 are critical mediators of mitochondrial events of apoptosis.[280] Caspases have been described in detail earlier in this chapter.

Caspase-dependent or independent endogenous endonuclease activation, resulting in DNA fragmentation, is considered a characteristic biochemical marker for apoptosis.[49] However, DNA fragmentation also occurs in cellular necrosis.[49,281,282] The differentiation of apoptosis from necrosis in tubular cells, therefore, is still difficult[283] and requires both the demonstration of DNA fragmentation, usually using a histochemical technique based on terminal deoxynucleotidyl transferase (TdT) reactivity with DNA breaks, as well as morphologic evidence of apoptosis by light and electron microscopy. Pathways usually associated with apoptosis (e.g., endonuclease activation, increased mitochondrial permeability) may also be associated with necrosis, suggesting that apoptotic and necrotic cell death may share the same pathways.[284] Both apoptosis and necrosis can occur in tissues exposed to ischemia–reperfusion or cultured cells exposed to hypoxia.[285]

The number of in vitro and in vivo studies where apoptosis is described in renal tubules is increasing. These studies are summarized in Tables 29.7 and 29.8. A feature of in vitro prolonged ATP depletion leads to necrosis, whereas milder and shorter ATP depletion leads to apoptotic cell death. A similar pattern has emerged from the in vivo studies (Table 29.8) (i.e., the same insult in a mild form can lead to apoptosis and, when severe, can lead to necrosis).

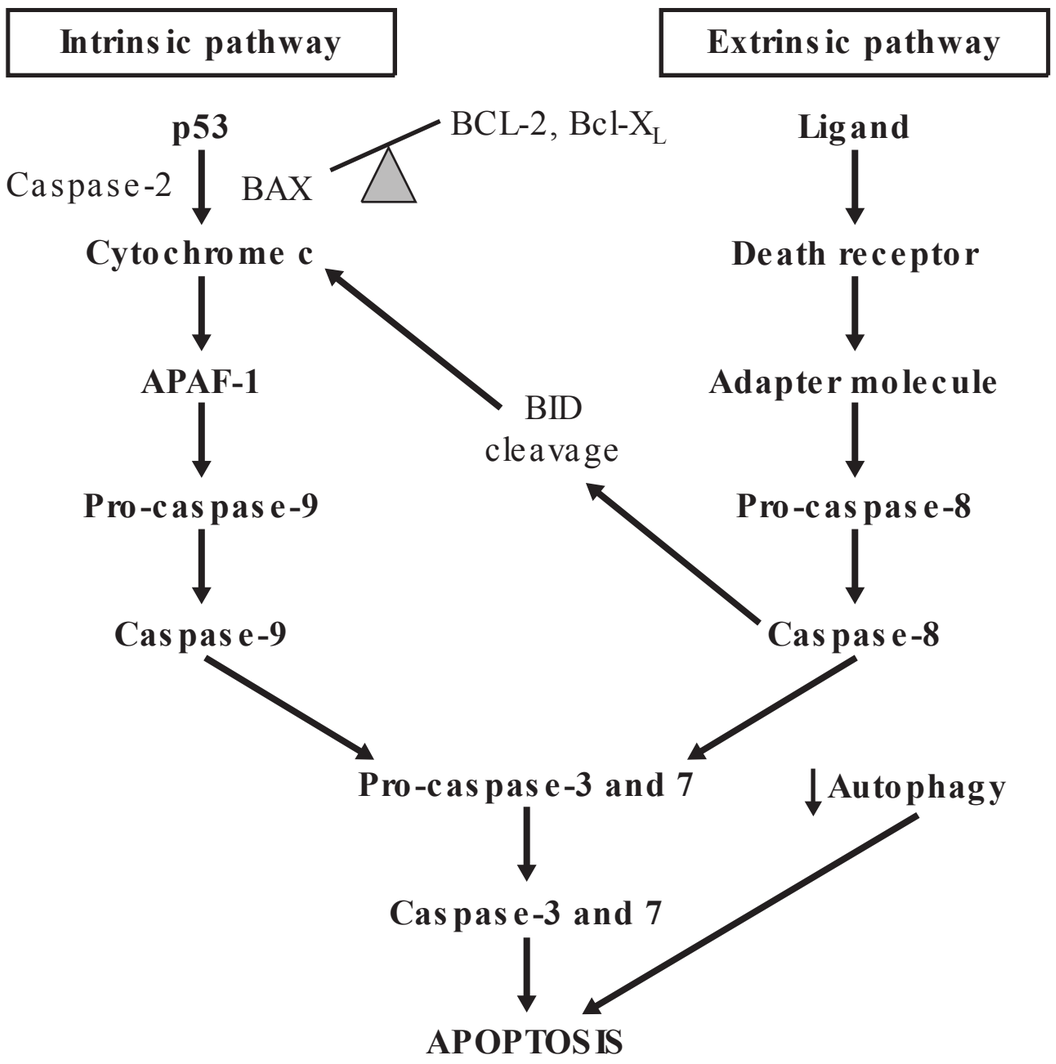


FIGURE 29.7 The major pathways of apoptosis. There are two major pathways of caspase-mediated apoptosis.[461] In the mitochondrial or intrinsic pathway, stress-induced signals (e.g., p53) act via Bcl-2 proteins to cause cytochrome c release from mitochondria. In the intrinsic pathway, there is the binding of a ligand (e.g., Fas ligand) to its death receptor (e.g., Fas) that recruits an adaptor protein. In the intrinsic pathway, the death receptors are a subset of the tumor necrosis factor (TNF) receptor family of cell surface molecules. Cytochrome c binds to a protein called apoptotic protease activating factor-1 (APAF-1). This binding allows APAF-1 to activate caspase-9, an initiator caspase, which then activates caspase-3 and -7. The presence of an excess of the antiapoptotic protein Bcl-2 on mitochondria inhibits cytochrome c release. Caspase-2 is a recently discovered caspase that is a critical initiator of the mitochondrial apoptosis pathway.[463] The activation and increased activity of caspase-2 is required for the permeabilization of mitochondria and the release of cytochrome c.[463] In the extrinsic pathway, the death receptors (CD95/Fas/APO-1, TNFR1, DR3/WSL-1/TRAMP, DR4/TRAIL-R1, DR5/TRAIL-R2, and DR6) are a subset of the TNF/NGF receptor family of cell surface molecules that possess a common motif within their cytoplasmic tails, called the death domain. The death domains of these receptors recruit adapter molecules that, in turn, recruit caspases to the receptor complex. For example, Fas antigen (CD 95) is engaged by its ligand (FasL) resulting in apoptosis. The activation of procaspase-8 requires association with its cofactor Fas-associated death domain (FADD). The pathways may be linked because caspase-8 may cleave a member of the Bcl-2 family, BH3-interacting domain death agonist (BID), which can release cytochrome c. Apoptosis mediated by both pathways has been described during renal ischemia–reperfusion in rats.[464,465] Both caspase-3 and -7 play a crucial and extensively studied role in the promotion of all forms of apoptotic cell death.[160] Caspase-7, like caspase-3, is an executioner caspase and is downstream of the initiators caspase-8 and -9. In general, cells may be more sensitive to apoptosis if autophagy is inhibited.[273] Bax, Bcl-2-associated X protein.

Numerous recent studies have demonstrated that erythropoietin (EPO) protects against ischemic AKI by affecting apoptotic cell death.[286–288] A single dose of EPO either pre-ischemia or just before reperfusion improves renal function and tubular injury; prevents the activation of caspase-3, 8, and 9; and reduces apoptotic tubular cell death.[286] EPO also protects against hypoxia-induced apoptosis in human proximal tubule cells.[287] In the same study, EPO functionally protected against ischemic AKI in rats in vivo and reduced outer medullary thick ascending limb apoptosis while potentiating tubular mitosis and proliferation.[287] In another study in rats with ischemic AKI, EPO decreased serum creatinine, decreased tubular apoptosis and necrosis, decreased tubular cell proliferation, increased antiapoptotic Bcl-2 protein expression, decreased caspase-3 activity, and increased heat shock protein 70 (HSP70) expression.[288] In a model of endotoxemia-induced AKI in mice, EPO significantly decreased renal superoxide dismutase and attenuated the renal dysfunction as assessed by inulin-GFR.[289] EPO receptors are expressed on mesenchymal stem cells (MSCs).[290] EPO results in an expansion of MSCs in bone marrow and the spleen that may mediate protection against ischemic AKI.[290] Thus, EPO may be a potential new therapy for AKI in humans.

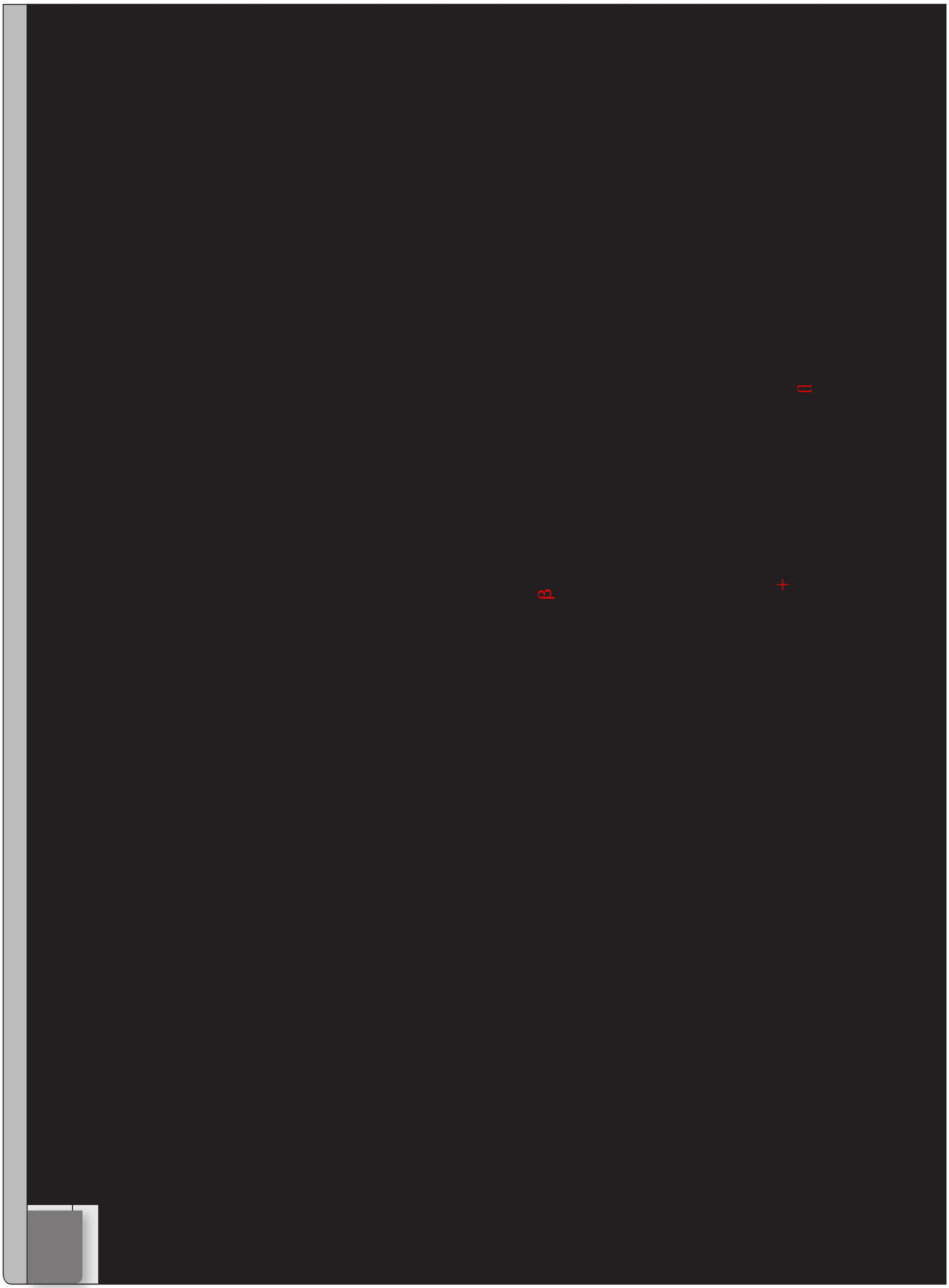
Complement

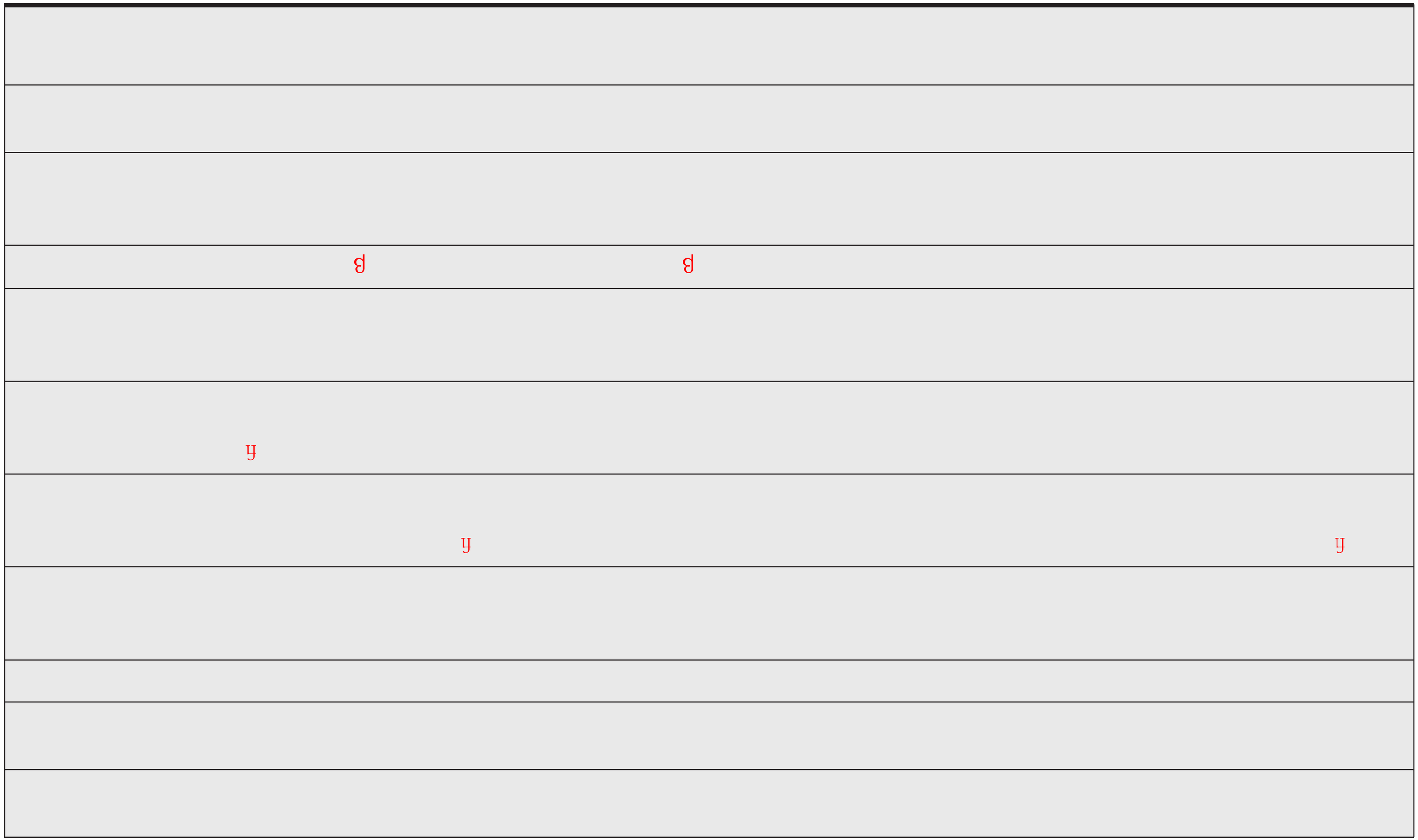
The complement system is a mediator of ischemia–reperfusion injury in the heart, lung, brain, intestines, and muscle.[291] A predominant role for C5b-9 in renal ischemia–reperfusion injury has been demonstrated.[292] In this study, the primary damaging effect of complement was on parenchymal cells rather than vascular endothelial cells. In another study, lack of a functional alternative complement pathway ameliorated ischemic AKI in mice.[293] In this study, mice deficient in factor B, an essential protein in the alternative complement pathway, were functionally and histologically protected against ischemic AKI. Treatment of mice with an inhibitory antibody to mouse factor B prevented the deposition of C3b on the tubular epithelium and systemic generation of C3b and protects against apoptosis and AKI in mice.[294] Loss of polarity of complement receptor 1–related protein y (Crry) in the tubular epithelium precedes activation of the alternative pathway along the basolateral aspect of the tubular cells, and heterozygous gene-targeted mice that expressed lower amounts of Crry are more sensitive to ischemic injury.[295] Also, the inhibition of Crry expression in proximal tubular epithelial cells in vitro resulted in alternative pathway–mediated injury to the cells.[295] Alternative complement pathway activation after renal ischemia reperfusion induces the production of macrophage inflammatory protein-2 (MIP-2) and mouse homolog of IL-8 (KC) by proximal tubule epithelial cells.[296] In addition, the alternative complement pathway, an innate immune system, recognizes hypoxic injury and triggers a systemic inflammatory response through the generation of C3a and a subsequent activation of the NF-κB system.[296]

29.7 Apoptosis in Hypoxic/Anoxic Tubular Injury In Vitro (Cultured Cells)

Cell Type	Type of Injury	Signals	Comment	Reference
MDCK and primary culture rat proximal tubules	Hypoxia		Necrosis also observed	485
Primary culture mouse proximal tubules	Partial ATP depletion (Antimycin A)	Renal growth factors did not ameliorate apoptosis	Severe ATP depletion caused necrosis	36
LLC-PK1 (proximal) and MDCK cells	ATP depletion		Same pattern in LLC-PK1 and MDCK	486
MDCK (distal)	Partial ATP depletion (Antimycin A)	Fas, FADD, caspases, PARP	Severe ATP depletion caused necrosis	487
Opossum kidney (proximal)	ATP depletion (cyanide, 2-deoxy-D-glucose)	Bcl-2/Bax	Prior heat shock attenuated apoptosis	246
Rat proximal tubules	Hypoxia	Caspases Bcl-2	Serine protease inhibitors suppressed caspase-9 activation and apoptosis	488
Rat proximal tubules	Antimycin A	Mitochondrial Ca^{2+} and permeability	L-type calcium channel blocker decreased apoptosis	489
Human tubular cells	Fas transfection	Fas	High but not basal Fas expression caused apoptosis	490
MDCK	Overexpression of ankyrin death domain	Fas	Inhibition of ankyrin Fas interaction decreased apoptosis	491
Rat proximal tubules	Hypoxia Cisplatin Staurosporine	Mitochondrial cytochrome c release	Minocycline upregulates Bcl-2 and decreases apoptosis	492
Rat proximal tubules	ATP depletion	Bid cleavage	Bid cleavage and apoptosis blocked by antiapoptotic Bcl-2 overexpression and caspase-9 inhibition	493
HK-2 cells	Mitochondrial dysfunction	Apoptosis antagonizing transcription factor (AATF)	Silencing of AATF worsened mitochondrial dysfunction	494

MDCK, Madin Darby canine kidney; LLC-PK1, cultured proximal tubule; FADD, fas-associated death domain; PARP, poly ADP ribose polymerase domain; Bax, Bcl-2-associated X protein; HK-2, human kidney-2.





Klotho

Klotho is a transmembrane protein that provides some control over insulin sensitivity and aging. It is increased in the kidney, blood, and urine.[297] Ischemic AKI reduced Klotho in the kidney, blood, and urine. Klotho deficiency resulted in worse AKI, and Klotho overexpression resulted in less AKI. This study suggests that AKI is a state of reversible Klotho deficiency.[297]

The Relative Importance of Proximal Versus Distal Tubular Injury

There is an ongoing debate regarding which nephron segments are most severely injured in ischemic AKI.[84] The target zone for hypoxic injury has also been extensively studied in the isolated perfused rat kidney (IPRK). In this model the target zone predominantly involves the S3 segments of the proximal tubule and also the distal tubules located within the outer stripe of the outer medulla and their cortical equivalent, the medullary rays, those straight sections of the proximal and distal tubules draining the superficial cortical glomeruli. Although the sensitivity of the proximal tubules to injury is well recognized in all models of AKI, the debate over whether the proximal or distal nephron segments are the primary target for hypoxic/ischemic injury has been well reviewed.[84] Of interest in the IPRK model of injury is the presence of a consistent artifact as a result of the absence of an oxygen carrier during erythrocyte-free perfusion. This artifact is the necrosis of mTAL cells first described by Alcorn et al.[298] It had been observed by Leichtweiss et al.[299] that tissue oxygen tension fell sharply in the region of the corticomedullary junction. Studies by Brezis et al.[300] demonstrated that the mTAL lesion resulted from hypoxia and provided support for the hypothesis that countercurrent diffusion of oxygen from descending to ascending limbs of the vasa recta is responsible for the prevailing low oxygen tension of the renal medulla. Subsequently, this group was able to induce a similar lesion in a number of models of renal injury. They have championed the notion that the mTAL segment lies on the brink of hypoxia as a result of the unique architecture of the kidney, which facilitates the countercurrent multiplier required for the formation of concentrated urine.[301] Most recently, it has been demonstrated that the induction of apoptosis targeted only in TAL cells in the absence of neutrophil inflammation in the mouse kidney results in renal failure, oliguria, and an impaired urine concentrating ability.[302]

Other groups have also suggested that the arteriovenous diffusion of oxygen between adjacent parallel arteries and veins is responsible for lowering tissue PO_2 in the corticomedullary region and for maintaining the very low medullary PO_2.[303,304] However, Endre et al.[305] demonstrated in the IPRK in the presence of low concentrations of erythrocytes that mTAL injury was prevented both under control conditions with high perfusate oxygen tension and in the presence of hypoxia.[305] The proximal tubule continued to be injured by hypoxia in the presence of erythrocytes, confirming that mTAL necrosis is an artifact of cell-free perfusion in this model. Nevertheless, coupled with the evidence for preglomerular arteriovenous diffusion of oxygen, which reduces average cortical PO_2 to subvenous levels,[306] the IPRK studies suggest that an even greater amount of the kidney is under threat from hypoxia when renal perfusion is reduced. Such widespread borderline hypoxia may be adaptively useful in priming the oxygen sensor in renal erythropoietin producing cells. However, when there is reduced renal perfusion, critically low levels of oxygen can be reached in tubular regions, particularly where there is high energy demand from transepithelial transport. Clearly, both proximal straight tubules (S3) and mTAL exist in such a region under constant threat of hypoxia. Magnetic resonance (MR) microscopy studies of the IPRK have demonstrated swelling of the cells in these interbundle regions in the outer medulla and their cortical equivalent, the medullary rays, restricting flow through the vascular bundles.[307] These MR observations complement the earlier observations by Thiel et al.,[308] Mason et al.,[309,310] and others that there is erythrocyte aggregation and stasis in the outer stripe of the model after reperfusion following ischemia. This reduction in perfusion may in part mediate the continued injury in this area.

Of greater interest is the observation that frank necrosis of mTAL cells is rarely seen in vivo. However, several studies in the IPRK have observed DNA fragmentation in mTAL cells after brief hypoxia[54] and after 15 or 60 minutes of reperfusion after ischemia.[311] DNA fragmentation has been observed after 24 hours reperfusion following ischemia in vivo in rats,[312,313] although little or no morphologic evidence of apoptosis has been observed in any of these studies. Similar DNA fragmentation was observed in human autopsy specimens after renal hypoperfusion.[314] Recent studies of the Bcl-2 multigene family and growth factors by Gobé et al.[312,314] in a 30 minute bilateral arterial clamp model of ischemia–reperfusion have suggested a way of reconciling the observations of proximal cell necrosis and DNA fragmentation without apoptosis in nearby mTAL. After 24 hours of reperfusion, distal tubules showed a marked increase in the expression of antiapoptotic Bcl-2 and a moderate increase in antiapoptotic Bcl-X_L and proapoptotic Bax. Proximal tubules showed a marked increase in Bax expression and a moderate increase in Bcl-X_L. Twenty-four hours after the expression of the Bcl-2 proteins was increased, IGF-1 and epidermal growth factor (EGF) protein levels were increased in the distal tubule, similar to the Bcl-2 antiapoptotic proteins, and were also detected in the adjacent proximal tubules, suggestive of paracrine action in these tubules. TGF-β expression was moderately increased in regenerating proximal tubules, but no relationship was seen with the pattern of expression of the Bcl-2 genes. An explanation of these results is that the distal tubule is adaptively resistant to ischemic injury via the promotion of survival by antiapoptotic Bcl-2 genes, and its survival allows for the expression of growth factors critical not only to the maintenance and regeneration of its own cell population (autocrine action), but also to the adjacent ischemia-sensitive proximal tubular cells (paracrine action).

Therefore, the hypothesis has been proposed that both the S3 proximal tubule and mTAL cells reside in regions where oxygen availability is borderline. Hypoxia induces both necrosis and apoptosis in proximal tubular cells. Hypoxia triggers apoptosis in mTAL cells but the presence of antiapoptotic Bcl-2 genes prevents the completion of programmed cell death and the DNA fragmentation is repaired. The induction of the growth factors EGF and IGF in these mTAL and distal tubule (DT) cells then provides both autocrine and paracrine mechanisms, respectively, for the recovery of the mTAL and proximal tubules. Because proximal cells are necrotic or have sloughed due to a loss of cell adhesion, proximal tubule recovery is delayed compared to the mTAL. This hypothesis also provides a mechanism for tubular obstruction by casts because viable mTAL cells are the source of Tamm-Horsfall protein.

Tubuloglomerular Feedback

Tubuloglomerular feedback ("tubular communication with the glomerulus") operates within the juxtaglomerular apparatus (JGA) of each nephron where changes are sensed in the salt content of fluid at the luminal macula densa and that information is transmitted to the afferent arteriole to cause compensatory changes in single nephron GFR.[315] nNOS (NOS 1) is expressed in the macula densa and may influence tubuloglomerular feedback. However, micropuncture experiments using NOS antagonists have shown that NO may modulate tubuloglomerular feedback.[315] Local NOS blockade causes the curve that represents tubuloglomerular feedback to shift leftward and become more steep. Changes in macula densa NO production may underlie the resetting of tubuloglomerular feedback, which is required in order to keep the tubuloglomerular feedback curve aligned with ambient tubular flow as tubular flow changes to accommodate physiologic circumstances. Also, macula densa NO production may be substrate limited and dissociated from NOS protein content. The importance of NO to tubuloglomerular feedback resetting and the substrate dependence of NO production have both been found during changes in dietary salt consumption.[316,317] In addition, nNOS inhibition sensitizes the tubuloglomerular feedback mechanism after volume expansion.[318] Macula densa cells detect changes in distal sodium chloride concentration, at least in part, through an apical Na:2Cl:K cotransporter.[319] Macula densa NO directly inhibits Na:2Cl:K cotransport, and NO and angiotensin (AT)II independently alter cotransporter activity.[319] To determine the role of the local renin–angiotensin system on tubuloglomerular feedback, mice with absent renal tissue expression of angiotensin converting enzyme (ACE) were studied.[320] Tubuloglomerular feedback was absent in mice without ACE in the kidney, suggesting that renal tissue ACE is an important contributor to tubuloglomerular feedback.[320] Mice deficient in adenosine A_1 receptors lack tubuloglomerular feedback.[321] Mice deficient in ecto-5′nucleotidase/CD73, the enzyme responsible for adenosine formation from AMP, have an impairment of the tubuloglomerular feedback regulation of GFR.[322]

Taken together, the proximal tubular injury and resultant dysfunction could contribute to the drastic fall in GFR, the hallmark of ischemic AKI. One potential mechanism is increased tubuloglomerular feedback. Specifically, in AKI, decreased proximal tubule reabsorption would increase solute delivery to the macular densa with the resultant constriction of the afferent arteriole and a fall in GFR.[323] In normal nephrons, the maximal fall in GFR with increased solute delivery to the macular densa is approximately 50%. Thus, increased tubuloglomerular feedback could be a major factor in mediating the pathway whereby proximal tubule damage could lower GFR. However, because clinical AKI or ischemic AKI is associated with a 90% fall in GFR, either additional factors or increased sensitivity of tubuloglomerular feedback postischemic injury to the kidney must occur. In that regard, dissected afferent arterioles from ischemic kidneys have been shown to have increased cytosolic calcium concentrations and enhanced vasoconstriction responses to AT-II and endothelin.[324,325] It is thus theoretically tenable that the sensitivity of the tubuloglomerular feedback is indeed enhanced postischemia. However, the role of tubuloglomerular feedback in ischemic AKI remains controversial.[326]

In support of a pathogenic role of tubuloglomerular feedback in ischemic AKI are studies by Brian Meyer and colleagues[55,56] that demonstrated the following: (1) the translocation of NaK-ATPase to the cytoplasm results in depolarization confined to the proximal tubule; (2) the fractional excretion of lithium, a surrogate measure for the fraction of filtered sodium that is delivered to the macula densa, the site of tubuloglomerular feedback, is massively increased; and (3) that these abnormalities persist for the duration of the maintenance phase of postischemic AKI. This study provides evidence for decreased proximal reabsorption of sodium, resultant increased sodium delivery to macula densa, tubuloglomerular feedback, and a resultant filtration failure that accompanies ischemic AKI.

Another pathway whereby tubular injury can contribute to a fall in GFR is by causing intraluminal cast formation and tubule obstruction. This will be the next topic discussed.

TUBULAR CAST FORMATION AND OBSTRUCTION

The classic radiologic findings in early AKI, prior to the realization that contrast is nephrotoxic, was an early dense nephrogram not followed by a pyelogram. Because the nephrogram phase represents contrast entering the tubules by filtration, a persistent nephrogram suggests a tubular obstruction with ongoing glomerular filtration.

Kidneys with ischemic AKI are swollen and, therefore, it was suggested that interstitial edema may lead to tubular collapse secondary to extraluminal-mediated compression.

However, it is clear that recovery from AKI can occur when the kidneys are still enlarged and swollen. An increased excretion of tubular epithelial casts are, however, a hallmark of recovery from AKI.[24] The presence of tubular casts on a renal biopsy, as well as urinary casts, has provided morphologic support for a role in tubular obstruction due to intraluminal cast formation in the pathogenesis of ischemic AKI.[327] As noted previously, although earlier micropuncture studies failed to consistently demonstrate increased tubular pressures postischemia, several subsequent studies provided convincing evidence for the presence of tubular obstruction in experimental ischemic AKI. Arendhorst et al.,[328] using micropuncture techniques during saline loading, demonstrated clear evidence of increased tubular pressures in postischemic, as compared to normal, kidneys. Renal vasodilation to restore renal blood flow also demonstrated increased tubular pressures in ischemic AKI in the rat. Perhaps the most compelling studies, however, were those micropuncture experiments performed by Tanner and Steinhausen.[329] They found that perfusing the proximal tubule with artificial tubular fluid at a rate that did not increase tubule pressure in normal animals increased tubule pressures in animals after a renal ischemic insult. Moreover, venting those obstructed tubules led to improved nephron filtration rates. Burke et al.[330] also demonstrated that the prevention of ischemic AKI in dogs with mannitol led to a decrease in intratubular pressures, suggesting that the induced-solute diuresis led to the relief of the cast-mediated tubular obstruction.

Although it is clear that brush border membranes, necrotic cells, viable cells, and perhaps apoptotic tubular epithelial cells enter tubular fluid after an acute renal ischemic insult, the actual process and predominant location of the cast formation is less clear. It is known that the casts uniformly stain for Tamm-Horsfall protein.[327]

Integrins

Integrins are heterodimeric glycoproteins consisting of different combinations of alpha and beta subunits; they recognize the most common universal tripeptide sequence, arginine-glycine-aspartic acid (RGD), which is present in a variety of matrix proteins.[12] These integrins can mediate cell–cell adhesion via an RGD inhibitable mechanism.[11]

In normal kidneys, proximal tubular cells are stained by the RGD peptide, RhoG-RGD, basolaterally in a punctate pattern and with Bt-RGD only minimally. On the other hand, ischemic kidneys labeling with RhoG-RGD and Bt-RGD occurred at the basolateral and apical aspect of tubular cells as well as on desquamating or desquamated cells within the tubular lumen and also on the vasa rectae.[331] In ischemic kidneys, antibodies to β1 and αV subunits of integrins stained glomeruli and the apical aspect of the proximal and distal tubules. Desquamated cells and cellular conglomerates obstructing the tubular lumina were intensely stained with RGD peptides.[332] Dual labeling experiments with Bt-RGD and antibodies against integrin receptors demonstrated αVβ3 binding sites for RGD peptides in the vasculature and some desquamated cells, whereas the majority of the desquamated cells bind Bt-RGD via β1 integrins.[331]

Experimental results support a role for adhesion molecules in the formation of casts. It has been shown that a translocation of integrins to the apical membrane of tubular epithelial cells may occur with ischemia.[11,226,227] Possible mechanisms for the loss of the polarized distribution of integrins include cytoskeletal disruption, state of phosphorylation, activation of proteases, and the production of NO.[333,334] These integrins are known to recognize RGD tripeptide sequences.[13,332] Thus, viable intraluminal cells could adhere to other luminal or paraluminal cells. The Goligorsky group provided experimental evidence for this cell–cell adhesion process as a contributor to tubule obstruction in ischemic AKI.[335–337] Synthetic cyclical RGD peptides were infused prior to the renal ischemic insult in order to block cell-to-cell adhesion as a component of tubule obstruction.[14,335–338] Using micropuncture techniques, the cyclic RGD tripeptides blocked the rise in tubular pressure postischemic insult.[13] An in vivo study of RGD peptides (cyclic RGDDFLG and RGDDFV) in ischemic AKI in rats demonstrated the attenuation of renal injury and an accelerated recovery of renal function.[14] The systemic administration of fluorescent derivatives of two different cyclic RGD peptides, a cyclic Bt-RGD peptide and a linear RhoG-RGD peptide, infused after the release of a renal artery clamp ameliorated ischemic AKI in rats.[14,337] The staining of these peptides suggests that cyclic RGD peptides inhibited tubular obstruction by predominantly preventing cell-to-cell adhesion, rather than cell-to-matrix adhesion.[332]

In addition to cell–cell adhesion, it is worthy to note that Zuk et al.[339] have demonstrated increased fibronectin in the tubular lumen after an ischemic insult, and fibronectin is known to possess arginine-glycine-aspartic acid (RGD) sequences that are recognized by cellular integrins. Moreover, Tamm-Horsfall protein (THP) is known to possess an RGD sequence, which may or may not be in a position to be recognized by integrins. This possibility, however, led to in vitro cellular adhesion studies in which $LLCPK_1$ cell adhesion to several different matrices (i.e., collagen I and collagen IV) was examined.[333] Interestingly, THP diminished cell adhesion in artificial fluid, mimicking distal tubular fluid but not tubular fluid similar to AKI or collecting duct fluid, which have significantly higher ionic concentrations.[340] In this regard, it has been suggested that THP becomes a polymeric gel in the presence of high ionic strength fluid, but is a non-gel monomeric substance in low ionic strength fluid. Studies have documented that the gel formation by THP is an active process that can be abolished by boiling. A role of the oligosaccharide component of THP in the gel formation was demonstrated because N-glycanase treatment to remove the oligosaccharide abolished the gel formation.[340]

Thus, the intraluminal presence of brush border membranes and viable and nonviable cells in association with the extracellular matrix (eg, fibronectin, THP, adhesion molecules) support their involvement in cast formation in ischemic AKI. The actual tubular obstruction by the casts, however, may only occur in the presence of the impaired vascular responses to renal ischemia. More specifically, if net glomerular filtration pressure was normal, the majority of the tubular casts may be excreted in the urine rather than lodging in the collecting duct and other nephron sites. Tubular factors in the pathogenesis of ischemic AKI are shown in Figure 29.7. The various perturbations in the renal vasculature that occur in association with a renal ischemic insult will now be discussed.

VASCULAR PERTURBATIONS

Ischemic AKI is associated with renal vasoconstriction with a resultant decrease in glomerular hydrostatic pressure and renal plasma flow.[341–343] Not only are circulatory vasoconstrictors, such as catecholamines, AT-II and endothelin, as well as renal sympathetic tone frequently increased in the setting of ischemic AKI,[68] but the renal vascular response to vasoconstrictors has been shown to be enhanced. This increased response to vasoconstrictors is due in part to the earlier mentioned increase in cytosolic calcium concentration in the afferent arterioles of the glomerulus. Endothelial damage is also associated with a diminution of the renal vasodilators, which oppose the action of vasoconstrictors. In experimental sepsis, the NO secondary to iNOS has been suggested to downregulate renal eNOS.[344] Moreover, the renal clamp model of AKI in the rat has been shown to be associated with downregulation of endothelial-derived nitric oxide (eDNO).[324,345] Recent studies have also shown that endothelin receptor antagonists ameliorate the diminution in renal hemodynamics associated with renal ischemia in the isolated perfused rat kidney[346,347] Further support for endothelin as an important mediator of ischemia–reperfusion-induced renal injury is the protective effect of an endothelin-A receptor antagonist in rats after clamping of the renal arteries.[348] Impairment of prostaglandin synthesis by the damaged endothelium can also profoundly enhance renal vascular resistance associated with renal ischemia. In this regard, infusion of prostaglandin E1 may protect against ischemic AKI.[349] Also, inhibition of thromboxane A2 improves renal function in rats exposed to warm ischemia–reperfusion.[350]

Intravital two-photon microscopy is an ideal method to study the microvascular events within the functioning kidney in vivo.[351–354] Intravital two-photon microscopy enables investigators to follow functional and structural alterations with subcellular resolution within the same field of view over a short period of time. Endothelial cell dysfunction within the microvasculature was observed and quantified using the infusion of variously sized, differently colored dextrans or proteins. Movement of these molecules out of the microvasculature and accumulation within the interstitial compartment are readily observed during AKI. The FVB-TIE2/GFP mouse, in which the endothelium is fluorescent, has been used to study morphologic changes in the renal microvascular endothelium during ischemia–reperfusion injury in the kidney.[355] Alterations in the cytoskeleton of renal microvascular endothelial cells correlated with a permeability defect in the renal microvasculature as identified using fluorescent dextrans and two-photon intravital imaging. Also, proximal tubule cell injury was increased in areas adjacent to areas of reduced endothelial injury and dysfunction.

Acute and chronic microvascular alterations are seen in a mouse model of ischemic AKI.[358] Three-dimensional reconstructions of microvascular networks obtained 24 hours following an acute ischemic injury demonstrate an intact endothelial monolayer in areas of increased microvascular permeability. There was no terminal deoxynucleotidyl transferase (TdT) mediated nick-end labeling (TUNEL) staining in microvascular endothelial cells despite the activation of caspase-3. This study demonstrates that detachment and a subsequent loss of endothelial cells following ischemic injury is not a major contributor to altered microvascular permeability.

In a more recent study, it was demonstrated that impaired endothelial proliferation and mesenchymal transition contribute to vascular rarefaction following AKI.[356] In the kidney of Sprague-Dawley rats after ischemic AKI, proliferating endothelial cells were undetectable for up to 2 days following ischemia/reperfusion (I/R). Endothelial mesenchymal transition states were identified.

Toll-like receptor 4 (TLR4) regulates early endothelial activation during ischemic acute kidney injury.[357] Increased TLR4 expression was seen on endothelial cells of the vasa recta of the inner stripe of the outer medulla of the kidney 4 hours after reperfusion.[357] The addition of hydrogen peroxide increased TLR4 expression in MS1 microvascular endothelial cells in vitro. TLR4 was localized to proximal tubules in the cortex and the outer medulla after 24 hours of reperfusion.

Acute alterations of the renal microvasculature, including altered microvascular permeability, are important contributors to the overall pathophysiology of AKI.[358,359] These acute microvascular alterations may have chronic consequences that result in the progression of AKI to chronic kidney disease (CKD).

Oxygen Free Radicals

Studies supported indirect evidence for injury induced by oxygen free radicals (OFRs) during reperfusion.[360,361] Although some studies have demonstrated that activated neutrophils produce OFR injury after ischemia,[362,363] other studies in isolated proximal tubules[364] and the studies in the cell-free isolated perfused rat kidney[365] indicated that OFRs were generated and contributed to the injury process even in the absence of neutrophils. The identification

of the actual species of OFRs involved in reperfusion injury required direct methods of detection rather than a reliance on scavengers.

The direct detection of hydroxyl radicals was initially achieved in isolated proximal tubules by using biochemical traps.[364] Similar studies were subsequently performed in the intact kidney using 0.5 mM salicylate to react with hydroxyl radicals during reperfusion for 15 minutes after an ischemia of 15 minutes.[366] An increase in 2,5 dihydroxybenzoic acid was observed using high performance liquid chromatography (HPLC) with electrochemical detection. Subsequent studies by Kadkhodaee et al.[367] used electron paramagnetic resonance (EPR) and 5,5-dimethyl-l-pyrroline N-oxide (DMPO) as a spin trap and confirmed that hydroxyl radicals were generated during a brief 3 minute reperfusion period following 20 minutes of ischemia. Interestingly, both studies demonstrated a significant generation of hydroxyl radicals in control kidneys, which was abolished by the addition of the scavenger, dimethyl-2-thiourea (DMTU). An increase in an unidentified carbon-centered radical was also identified during reperfusion in the EPR study and could represent an early lipid peroxidation product.[367]

With 60 minutes of reperfusion after 20 minutes of ischemia in the IPRK, tubular damage was prominent in both the cortical and medullary proximal tubule and in the mTAL.[311] Pretreatment with either allopurinol, which acts both to inhibit xanthine oxidase and acts as an OFR scavenger, or DMTU, reduced both the morphologic features of injury and the extent of DNA fragmentation in the mTAL. Taken together, these results suggest that hydroxyl radicals formed during reperfusion after ischemia play a significant role in both necrotic and apoptotic cell injury.

On the background of these postischemic vascular perturbations is the observation that a decrease in renal perfusion pressure is not associated with autoregulation of either GFR or renal blood flow.[324,341,342,368–370] In fact, rather than renal vasodilation, renal vasoconstriction occurs with a fall in renal perfusion pressure in the postischemic kidney. Thus, a degree of hypotension, which is of no clinical significance in the normal kidney, may cause renal damage in the kidney during the recovery phase of AKI. The same increased sensitivity in the postischemic kidney has also been shown to occur with nephrotoxic agents such as aminoglycosides.

Studies to examine the role of Ca^{2+} and calcium channel blockers (CCBs) in the vascular perturbations in experimental ischemic AKI have been performed. These studies demonstrate that intrarenal CCBs can reverse the increased sensitivity to renal nerve stimulation as well as the loss of renal autoregulation, both of which characterize experimental AKI.[371] In addition, other studies in the rat showed that atrial natriuretic peptide (ANP), which attenuates vasoconstrictor-induced increases in $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells,[372] is also protective against ischemic AKI[373] despite the fact that its systemic administration causes a fall in arterial pressure.

Cisplatin-induced or ischemic AKI in vivo and hypoxia/reoxygenation of tubular epithelial cells in vitro induces the production of reactive oxygen metabolites (ROM).[374] The generation of ROM results in tubular epithelial cell death, which is mediated by caspases and/or endonucleases. The inhibition of ROM protects the tubular epithelium from caspase and endonuclease activation and from cell death.

Endotoxemia-Induced Acute Kidney Injury

Recent experimental studies in septic mice have incriminated still another mediator of renal vasoconstriction. The intraperitoneal administration of lipopolysaccharide (LPS) as an endotoxin was associated with a profound decrease in GFR and renal blood flow both in wild-type and iNOS knockout mice. A soluble receptor of TNF, however, was associated with profound improvement in renal hemodynamics in both wild-type and iNOS knockout mice.[375] Because septic patients with renal failure have a high mortality, this observation has potential therapeutic importance.

Sepsis in mice has also been shown to be associated with an impaired response of NO-mediated cyclic GMP in the renal cortex, the agent's secondary messenger for vasodilation. The renal nerves and the activation of the renin–angiotensin system contribute to renal vasoconstriction during sepsis. In this study, renal denervation decreased the high plasma renin levels during endotoxemia and was protective against the decreased GFR and renal blood flow in a normotensive model of endotoxemia-induced sepsis.[376]

Oxygen radicals may contribute to vasoconstriction in endotoxemia-induced AKI.[377] AKI during sepsis is associated with increased NO and oxygen radicals, including superoxide. Renal extracellular superoxide dismutase (EC-SOD) is decreased in endoteoxemia.[378] Antioxidant therapy with chemically dissimilar antioxidants, metalloporphyrin and tempol, preserved GFR and renal blood flow during endotoxemia.[378] This protective effect was reversed by the inhibition of iNOS, suggesting the importance of the bioavailability of NO for the preservation of renal function during endotoxemia.[378]

The demonstration of global renal vasoconstriction in sepsis may depend on the model used. In a nonlethal hyperdynamic model of sepsis in sheep injected with Escherichia coli, renal failure developed despite markedly increased renal blood flow.[379]

In another study in endotoxemia-induced AKI, the role of renal inflammation and apoptosis was determined.[380] In this study, LPS acted on extrarenal TLR4, leading to systemic TNF release and subsequent AKI. Mice with a mutation in TLR4 were resistant to LPS-induced AKI and had less neutrophil infiltration and renal cell apoptosis.[380]

The role of caspase-1 and its associated cytokines was investigated in a nonhypotensive model of endotoxemic AKI.[381] In mice with endotoxemic AKI, the GFR measured by fluorescein isothiocyanate (FITC)-labeled inulin was significantly higher in caspase-1-/- versus wild-type mice at 16 and 36 hours. IL-1 β and IL-18 protein were significantly increased in the kidneys of mice with endotoxemic AKI versus vehicle-treated mice. However, the inhibition of IL-1β with L-1Ra, or the inhibition of IL-18 with IL-18–neutralizing antiserum-treated or combination therapy with IL-1Ra plus IL-18–neutralizing antiserum did not improve the GFR in mice with endotoxemic AKI. These results suggest that neither IL-1β nor IL-18 are the mediators on endotoxemic AKI.[381]

Ghrelin is a stomach-derived growth hormone secretagogue. Ghrelin has been shown to have anti-inflammatory properties. Serum ghrelin levels were increased in endotoxemia AKI accompanied by increased ghrelin receptor (GHSR-1a) protein expression in the kidney.[382] Ghrelin administration significantly decreased serum cytokine levels (TNF-α, IL-1β, and IL-6), serum endothelin-1 levels, serum NO levels, and renal iNOS expression in endotoxemic AKI. When administrated before LPS, ghrelin protected against the fall in glomerular filtration rate. In another study, ghrelin was shown to improve renal function in mice with ischemic AKI.[383]

INFLAMMATION IN ISCHEMIC ACUTE KIDNEY INJURY

Ischemic AKI has been described as an inflammatory disease.[20] This is evidenced by numerous studies demonstrating endothelial injury, leukocyte infiltration in the kidney, and the generation of inflammatory mediators by tubular cells.[20]

Endothelial Cell Injury

Silver nitrate staining of blood vessels was studied in rats at 4 hours after the release of a renal artery clamp.[384] Ischemic AKI resulted in disorganization of endothelial integrity with areas of denudation, partial disappearance of cell–cell borders, and distortion of cell–cell contacts most prominent in the renal microvasculature. Intravital microscopy of blood flow in peritubular capillaries provided direct evidence for the existence of a no-flow phenomenon caused by endothelial injury.[384,385] The administration of endothelial cells or surrogate cells expressing endothelial NOS, either intravenously or intra-arterially, resulted in functional protection against ischemic AKI.[384] These studies suggested that endothelial cell injury is the primary cause of the no-flow phenomenon and that, when ameliorated, there is attenuation of renal function.

It has been demonstrated in mice that injury of renal microvascular endothelium alters the barrier function after ischemia.[355] In this study, circulating von Willebrand factor (vWF), a marker of endothelial injury, was increased in the circulation 24 hours after ischemia. In FVB-TIE2/GFP mice, in which the microvasculature can be visualized, there were alterations in the cytoskeleton and in the integrity of adherens junctions that correlated with a permeability defect identified using fluorescent dextrans and two-photon intravital imaging.[355]

The extension phase of AKI is marked by continued hypoxia and an inflammatory response, which are more marked in the corticomedullary junction.[19] Severely reduced blood flow, stasis, and the accumulation of red blood cells has been documented in the corticomedullary region.[19] Endothelial cell injury is thought to play an important role in the initiation and extension phase of ischemic AKI.[19,386]

Neutrophil Activation

Renal ischemia–reperfusion injury is associated with an increase in infiltrating neutrophils.[387] The adherence of neutrophils to the vascular endothelium is an essential step in the extravasation of these cells into ischemic tissue.[29] Therefore, leukocyte adhesion molecules have been studied in renal injury.[388] After adherence and chemotaxis, infiltrating leukocytes release reactive oxygen species and enzymes that damage the cells.[29] The infusion of normal neutrophils accentuates severe ischemia–reperfusion injury and decreases GFR during ischemia. Activated neutrophils have been shown to further decrease GFR in response to renal ischemia at least in part due to the release of oxygen radicals.[363,389–391] In contrast, the infusion of oxygen radical–deficient neutrophils from patients with chronic granulomatous disease did not worsen the course of ischemic injury.[390] The mechanism by which adherent leukocytes cause ischemic injury is unclear, but likely involves both the release of potent vasoconstrictors including the prostaglandins, leukotrienes, and thromboxanes,[392] as well as direct endothelial injury via the release of endothelin and a decrease in NO.[68,393]

Intracellular adhesion molecule 1 (ICAM-1) has been suggested to play an important role in the pathophysiology of ischemic AKI.[387,394] Increased systemic levels of the cytokines, TNF-α, and IL-1 may upregulate ICAM-1 after ischemia and reperfusion in the kidney.[394] ICAM-1 on endothelial cells promotes the adhesion of neutrophils to these cells and causes tissue damage. The administration of a monoclonal antibody against ICAM-1 protected against ischemic AKI in rats.[387,390] Pretreatment with an ICAM-1 antisense oligodeoxyribonucleotide ameliorated the ischemia-induced infiltration of granulocytes and macrophages and resulted in less cortical renal damage as assessed by a quantitative pathologic grading scale.[395] In parallel, ICAM-1–deficient mice are protected against renal ischemia.[394] Thus, ICAM-1 is a mediator of ischemic AKI, probably by potentiating neutrophil–endothelial interactions.

Red blood cell swelling has been suggested to cause the medullary blood flow congestion, which occurs after renal ischemia and worsens the relative hypoxia in that region of the kidney. The restoration of renal blood flow in experimental renal ischemia in dogs, however, occurs with either an isotonic or hypertonic mannitol induced–diuresis. There is now evidence that upregulation of adhesion molecules may contribute to this impaired medullary blood flow postischemic injury.[388,396,397]

P-selectin, an important molecule involved in the adherence of circulating leukocytes to tissue in inflammatory states, also seems to be involved in the infiltration of the leukocytes during ischemic injury. In fact, renal ischemia has also been shown to be associated with upregulation of endothelial P-selectin with enhanced adhesion of neutrophils.[398] A soluble P-selectin glycoprotein ligand prevented the infiltration of leukocytes and ameliorated ischemia-induced renal dysfunction.[334] In contrast to P-selectin, L-selectin does not appear to mediate tubular damage in the postischemic kidney.[388] After adherence and chemotaxis, neutrophils release reactive oxygen species or oxygen free radicals. IL-17 produced by neutrophils regulates interferon (IFN)-γ–mediated neutrophil migration in the ischemic AKI in mice.[399]

There is evidence that neutrophils mediate tubular injury in AKI.[400] This evidence is derived from studies that show an accumulation of neutrophils in ischemic AKI and studies demonstrating a beneficial role of anti–ICAM-1 therapy in AKI.[394] Also, mice depleted of peripheral neutrophils by antineutrophil serum were protected against ischemic AKI.[394] However, in another study, rats depleted of peripheral neutrophils by antineutrophil serum were not protected against ischemic AKI.[401] In another study, mice were injected with 0.1 mg of the rat IgG2b monoclonal antibody RB6-8C5 (BD Pharmingen Inc., San Diego, CA) intraperitoneally 24 hours before a renal pedicle clamp.[191] This results in the depletion of neutrophils in the peripheral blood and in the kidney during ischemic AKI. In this study, there was slight functional protection and no histologic protection against ischemic AKI in neutrophil-depleted mice.[191]

The kinetics of margination and transmigration of neutrophils in vivo in the kidney and lungs following renal ischemia–reperfusion has been studied.[402] At 24 hours after an ischemic AKI, kidney neutrophil content increased threefold. The neutrophils were found primarily in the interstitium and, to a lesser degree, marginated to the vascular endothelium. Interstitial neutrophils had significantly lower levels of intracellular IFN-γ, IL-4, IL-6, and IL-10, and thus, a tendency for decreased amounts of IL-4 and TNF-α compared to the marginated neutrophils. Transmigration sites of neutrophils were directly associated with areas of increased vascular permeability. The activation of the adenosine 2A receptor significantly decreased both kidney neutrophil transmigration by about half and vascular permeability by about a third. This study suggests that there is a sequential recruitment and transmigration of neutrophils from the vasculature into the interstitium of the kidney at the site of tissue injury in ischemic AKI.

Lymphocytes

The role of lymphocytes in ischemic AKI is an ongoing area of study.[403,404] Lymphocytes have been examined in genetically altered immune-deficient mice. In one study, mice with a combined deficiency of both CD4 and CD8 cells were protected against ischemic AKI at 48, but not at 24 hours postischemic reperfusion.[405] In a follow-up report by the same investigators, nu/nu mice that are athymic and deficient in both CD4 and CD8 T cells were protected against ischemic AKI 24- and 48-hours postischemic reperfusion.[406] To determine the pathogenic T-cell type, mice with targeted genetic deficiencies of either CD4 or CD8 T cells were also studied. CD4-deficient mice, but not CD8-deficient, are protected against ischemic AKI.[406] Therefore, it appears that the pathogenic T-cell subtype in ischemic AKI is the CD4 T cell. However, RAG-1-/- mice, which lack mature T and B lymphocytes, are not protected against ischemic AKI despite lacking both CD4 and CD8 T cells.[407] In a recent study,[408] mice deficient in B lymphocytes alone were protected against ischemic AKI. In summary, CD4 T-cell deficient, nu/nu (lacking mature T cells), and B-cell deficient mice are protected against ischemic AKI, whereas CD8 T-cell and RAG-1-/- (lacking mature B and T cells) mice are not protected.

The effect of a complete depletion of CD4 T cells with a monoclonal antibody in ischemic AKI is not known. In one report,[409] the use of GK1.5 antibody alone to deplete CD4 T cells did not protect against ischemic AKI; however, the complete depletion of CD4 T cells as judged by fluorescence activated cell sorting (FACS) analysis did not occur. Protection did occur when GK1.5 antibody was used with two other antibodies, which resulted in the depletion of both CD4 and CD8 T cells.[409] In another study,[410] the complete depletion of CD4 T cells using the GK1.5 antibody was not protective against ischemic AKI in mice. CXCR3 plays an important role in the recruitment of Th1 cells into the kidney in ischemic AKI.[411] In summary, it is believed that CD4 T cells are important in the pathogenesis of ischemic AKI and that very few T cells in the kidney are enough to contribute to injury.[404,406,409]

Regulatory T cells (Tregs) are known to blunt the immune response. Foxp3+ regulatory T cells play a role in kidney repair after an ischemic AKI.[412] Tregs also contribute to the protective effect of ischemic preconditioning in the kidney.[413] Treatment of mice with a Treg cell–depleting antibody reversed the protective effect of preconditioning on kidney neutrophil infiltration, function, and histology. Sphingosine-1-phosphate receptor (S1PR) agonists reduce ischemic AKI in mice that lack T and B lymphocytes (Rag-1 knockout mice).[414] S1PR agonists also reduce hypoxia-induced

apoptosis in cultured mouse proximal tubule cells. This study shows that the protective effect of S1PR agonists is independent of T cells.

Natural killer (NK) cells are lymphocytes that mediate innate immunity against pathogens and tumors via their ability to secrete cytokines.[415] NK cells are unique in their constitutive expression of receptors for cytokines (eg, IL-18), which are produced by activated macrophages.[416] NK cells are activated by IL-18 independently of IL-12.[417] A model of NK cell activation in injured tissues has been proposed.[418] In this model, it is hypothesized that NK cells are recruited to sites of injury from the bloodstream. Once in the tissue, NK cells become activated and release cytokines like IL-18.[418] In support of this hypothesis, it is known that NK cells play a role in numerous disease processes.[419] NK cell depletion in wild-type C57BL/6 mice is protective against ischemic AKI.[420] An adoptive transfer of NK cells worsened injury in NK-, T-, and B-cell–null Rag2(-/-)γ(c)(-/-) mice with ischemic AKI. NK cell–mediated kidney injury was perforin (PFN) dependent because PFN(-/-) NK cells had a minimal capacity to kill tubular epithelial cells in vitro compared with NK cells from wild-type mice. Alternatively, B cells limit repair after ischemic AKI.[421]

Monocyte/Macrophages

Another inflammatory cell that is a potential mediator of injury in ischemic AKI is the monocyte/macrophage.[404] Macrophages infiltrate the postischemic rat kidney.[422] Macrophage chemoattractants (e.g., monocyte chemoattractant protein 1 [MCP-1]) are increased in the postischemic rat kidney.[404] In a model of macrophage depletion using liposomal clodronate, it was demonstrated that macrophages contribute to tissue damage during acute rejection.[423] It was determined that macrophages are a source of injurious IL-18 in ischemic AKI in mice.[424] Macrophage depletion in the kidney was achieved by using a tail vein injection of liposomal-encapsulated clodronate (LEC). The adoptive transfer of RAW 264.7 cells, a mouse macrophage line that constitutively expresses IL-18 mRNA, reversed the functional protection against AKI in LEC-treated mice. In addition, the adoptive transfer of peritoneal macrophages in which IL-18 function was inhibited also reversed the functional protection in macrophage-depleted mice, demonstrating that IL-18 from the adoptive transfer of macrophages is not sufficient to cause ischemic AKI. Possible sources of injurious IL-18 in AKI include the proximal tubule and lymphocytes. In this regard, freshly isolated proximal tubules from mice release IL-18 into the medium when exposed to hypoxia, and proximal tubules from caspase-1–deficient mice are protected against hypoxic injury.[178] An anti-B7-1 antibody blocks mononuclear cell adherence in the vasa recta of rats and attenuates ischemic AKI both functionally and histologically.[425] Gene therapy in rats expressing an amino-terminal truncated MCP-1 reduced macrophage infiltration and AKI.[426] Two recent studies have demonstrated that macrophage depletion using liposomal clodronate is protective against ischemic AKI in mice.[427,428]

Dendritic cells act as antigen-presenting cells and as messengers between the innate and adaptive immunity. The kidney has a rich network of resident dendritic cells.[429] Dong and colleagues[430] have identified the surveying renal dendritic cell network as the predominant source of TNF-α during the early stages of ischemic AKI.[430]

Proinflammatory cytokines increase the expression of the CX_3C chemokine, fractalkine, on injured endothelial cells. The fractalkine receptor (CX_3CR1) is expressed on NK cells, monocytes, and some CD8+ T cells.[431] Fractalkine has a mucinlike stalk that extends the chemokine domain away from the endothelial cell surface, enabling the presentation of the CX_3C-chemokine domain to leukocytes. Fractalkine serves the dual function of an adhesion molecule and a chemoattractant.[431] Fractalkine is a major chemoattractant for NK cells and monocytes, but not for neutrophils.[432] Fractalkine expression is increased in patients with renal tubulointerstitial inflammation, with the strongest expression localized to vascular sites near to macrophage inflammation.[433] Fractalkine is a strong candidate for directing mononuclear cell infiltration induced by vascular injury.[433] Fractalkine expression is increased in the endothelium of large blood vessels, capillaries, and glomeruli in ischemic AKI.[427] Fractalkine receptor inhibition is protective against ischemic AKI.[427]

Inflammatory Mediators

In renal ischemia–reperfusion, tubular epithelial cells produce TNF-α, IL-1, IL-6, IL-8, IL-18, TGF-β, MCP-1, RANTES, and fractalkines.[20,190,191] Leukocytes produce TNF-α, IL-1, IL-8, MCP-1, reactive oxygen species, and eicosanoids.[20] The anti-inflammatory cytokine, IL-10, inhibits TNF-α, ICAM-1, and iNOS and protects against ischemic and cisplatin-induced renal failure and AKI.[434]

Statins are potent anti-inflammatory drugs.[435] Both in vitro and in vivo studies suggest lipid lowering–independent anti-inflammatory functions of statins.[435] After adhesion to the vascular endothelium, inflammatory cells migrate to the site of inflammation.[435] Statins act independently of lipid lowering to selectively inhibit leukocyte adhesion by direct interactions with the leukocyte-function antigen 1 (LFA-1).[435] Statins reduce macrophage influx and chemokine expression in rat kidneys.[436] Statins are known to decrease the expression of proinflammatory cytokines by inflammatory cells.[435] Simvastatin reduces the expression of IL-6 and MCP-1 in monocytes from hypercholestrolemic patients and in cultured endothelial cells.[435] Pravastatin downregulates TNF-α and MCP-1[437] in human monocytes in vitro. Some of the anti-inflammatory effects of statins are mediated by NO.

Rats were treated with cerivastatin or a vehicle for 3 days before the induction of ischemic AKI.[438] Statin

treatment reduced the increase in serum creatinine by 40% and protected against tubular necrosis. In addition, monocyte and macrophage infiltration was almost completely prevented, ICAM-1 upregulation was decreased, and iNOS expression was reduced.[438] In another study, atorvastatin improved the course of ischemic AKI in aging rats by enhancing NO availability and improving renal hemodynamics.[439]

Proinflammatory cytokines are increased in the serum of animal models of AKI.[66] Data in animal models of AKI suggest that the inflammatory response in AKI is dysregulated. The effect of AKI on the production and elimination of proinflammatory cytokines may be a key mechanism by which patients with AKI have increased distant organ dysfunction and increased mortality. In animals with AKI, TNF-α,[440] IL-1β,[440] IL-6, KC, and granulocyte colony stimulating factor (GCSF) increase in the serum after AKI.[66] Cytokine production also increases in the kidney.[66] Renal cytokine production may contribute to renal injury and cause the increase in serum cytokines. Circulating cytokines may contribute to extrarenal organ injury.

Besides IL-18, there are other cytokines that play a role in ischemic AKI. The TNF-like weak inducer of apoptosis (TWEAK, TNFSF12) is a member of the TNF superfamily. TWEAK activates the Fn14 receptor and regulates apoptosis, proliferation, and inflammation. TWEAK and Fn14 expression was increased in experimental AKI induced by folic acid.[441] The IL-6/IL-6R axis plays a critical role in AKI.[442] High-mobility group box 1 (HMGB1), a nuclear factor released extracellularly as an inflammatory cytokine, is an endogenous ligand for TLR4. A neutralizing anti-HMGB1 antibody protected against ischemic AKI and reduced levels of IL-6, TNF-α, and MCP1.[443] Alternatively, the administration of recombinant HMGB1 after reperfusion exacerbated ischemic AKI. TLR4-deficient mice were protected against AKI and the administration of an anti-HMGB1 antibody or a rHMGB1 did not affect this renoprotection. This study concluded that endogenous HMGB1 promotes kidney damage after IRI, possibly through the TLR4 pathway.

RECOVERY FROM ISCHEMIC ACUTE KIDNEY INJURY

It has been demonstrated in rats that severe ischemic AKI results in a permanent alteration in renal capillary density that contributes to a urinary concentrating defect and renal fibrosis.[444,445] However, in contrast to other organs, the kidney can recover from tubular necrosis, at least in the short term. There are two potential mechanisms of recovery other than cell repair: (1) the dedifferentiation and proliferation of the surviving nonnecrotic tubular cells,[446] and (2) the mobilization and delivery of bone marrow stem cells to the injured kidney.[447]

After an ischemic AKI, there is a proliferation of tubular cells that mimic events in the developing kidney.[448] Epithelial cells are also dedifferentiated during the recovery period.[446] In the postischemic kidney, there is also an expression of genes that encode growth factors.[263,265,449] The dedifferentiation and the proliferation of tubular epithelial cells may result in the spreading of cells over the damaged basement membrane. Cell adhesion molecules like neural cell adhesion molecule (NCAM), cytokines, and KIM-1[59,450] may play a role in these processes. The targeted delivery of hepatocyte growth factor (HGF) to the proximal tubule in transgenic mice resulted in a marked protection against ischemic AKI.[451] Understanding the physiology of repair and the recovery of surviving tubular cells may lead to therapies to hasten the recovery process in humans.

Bone marrow stem cells have the capacity to migrate to other organs.[447] Bone marrow–derived cells can populate and contribute to the turnover of both the normal and injured renal tubular epithelium.[452] Cells from the bone marrow of adult mice are mobilized into the circulation by transient renal ischemia and home specifically to the injured kidney where they differentiate into tubular epithelial cells.[447] It was investigated whether an increase in circulating stem cells, using pharmacologic mobilization from the bone marrow, would improve renal function in mice with ischemic AKI.[453] The pharmacologic increase in stem cells was associated with marked granulocytosis and worsening of renal failure. In the future, therapies aimed at stimulating the proliferation, mobilization, and targeting of stem cells may enhance recovery from ischemic AKI.[447] However, in another study, it was shown that intrarenal cells, not bone marrow–derived cells, are the major sources for regeneration in postischemic AKI. Furthermore, a single injection of bone marrow cells did not make a significant contribution to renal functional or structural recovery.[454]

Mesenchymal stem cells (MSCs) play a role in regeneration and immunomodulation. The administration of MSCs protects against ischemic AKI in rats.[455] In this study, the expression of IL-1β, TNF-α, IFN-γ, and iNOS was significantly reduced by the intravenous administration of MSCs. In addition, the beneficial effects of MSCs were found to be mediated by paracrine actions and not by their differentiation into target cells. Human MSCs improve renal function and survival in mice with cisplatin-induced AKI.[456] The treatment of mice with autologous and allogeneic MSCs after AKI was safe and reduced renal fibrosis in mice that survived an AKI.[457] CD44 and hyaluronic acid interactions recruit exogenous MSCs to injured tissues to improve renal regeneration.[458] Also, MSCs exert beneficial effects on tubular cell repair in an ischemic AKI by the production of the prosurvival and mitogenic growth factor IGF-1.[459] A phase 1 study of MSCs in patients at risk for AKI after cardiac surgery is under way.

Colony stimulating factor (CSF-1) signals directly to renal tubular epithelial cells to mediate repair in mice.[460] Macrophages have been implicated in tissue repair, and CSF-1,

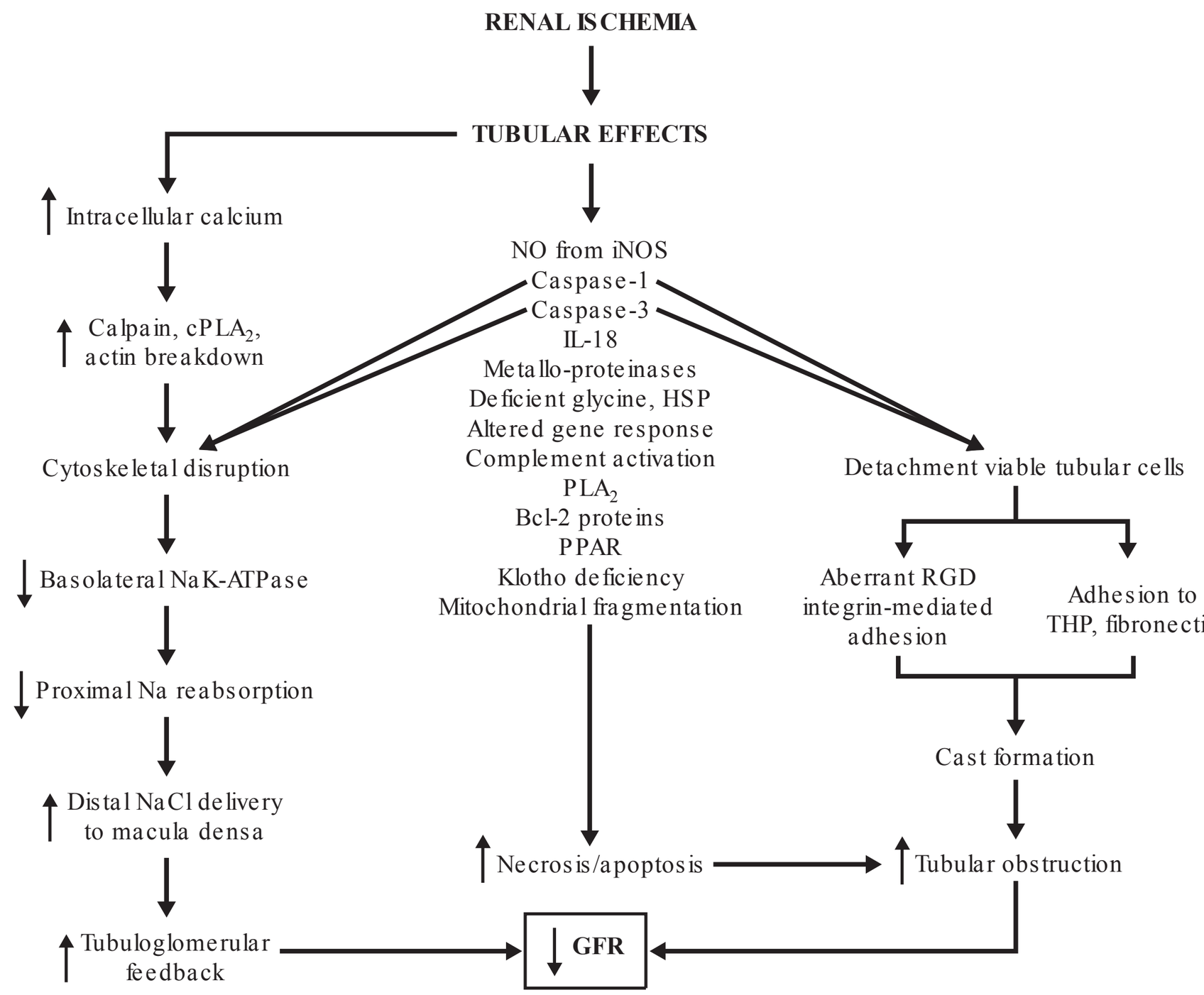


FIGURE 29.8 The tubular factors in the pathogenesis of ischemic acute kidney injury. NO, nitric oxide; iNOS, inducible nitric oxide; IL-18, interleukin 18; HSP, heat shock protein; PLA_2, phospholipase A_2; RGD, arginine-glycine-aspartic acid; THP, Tamm-Horsfall protein; PPAR, peroxisome proliferator–activated receptors; GFR, glomerular filtration rate. Reproduced from Kribben et al.[466] with permission.

the principal macrophage growth factor, is expressed by tubular epithelial cells. Mice injected with CSF-1 had decreased tubular pathology and improved renal function in an ischemic AKI. The study further demonstrated that CSF-1 mediates renal repair by both a macrophage-dependent mechanism and a direct autocrine/paracrine action on tubular epithelial cells (TECs).

SUMMARY

Tubular and vascular perturbations and inflammation combine to cause ischemic AKI. The tubular and vascular events in ischemic AKI are summarized in Figures 29.8 and 29.9. The inflammatory events are summarized in Figure 29.10. Recent laboratory studies using in vivo, cellular, and molecular approaches have provided substantial insight into the pathogenesis of the syndrome. These studies have identified several potential therapeutic interventions, which need to be tested with prospective clinical trials. Interventions that have attenuated experimental ischemic/hypoxic proximal tubule damage include cysteine protease inhibitors, melanocyte stimulating hormone (MSH), specific iNOS inhibition,

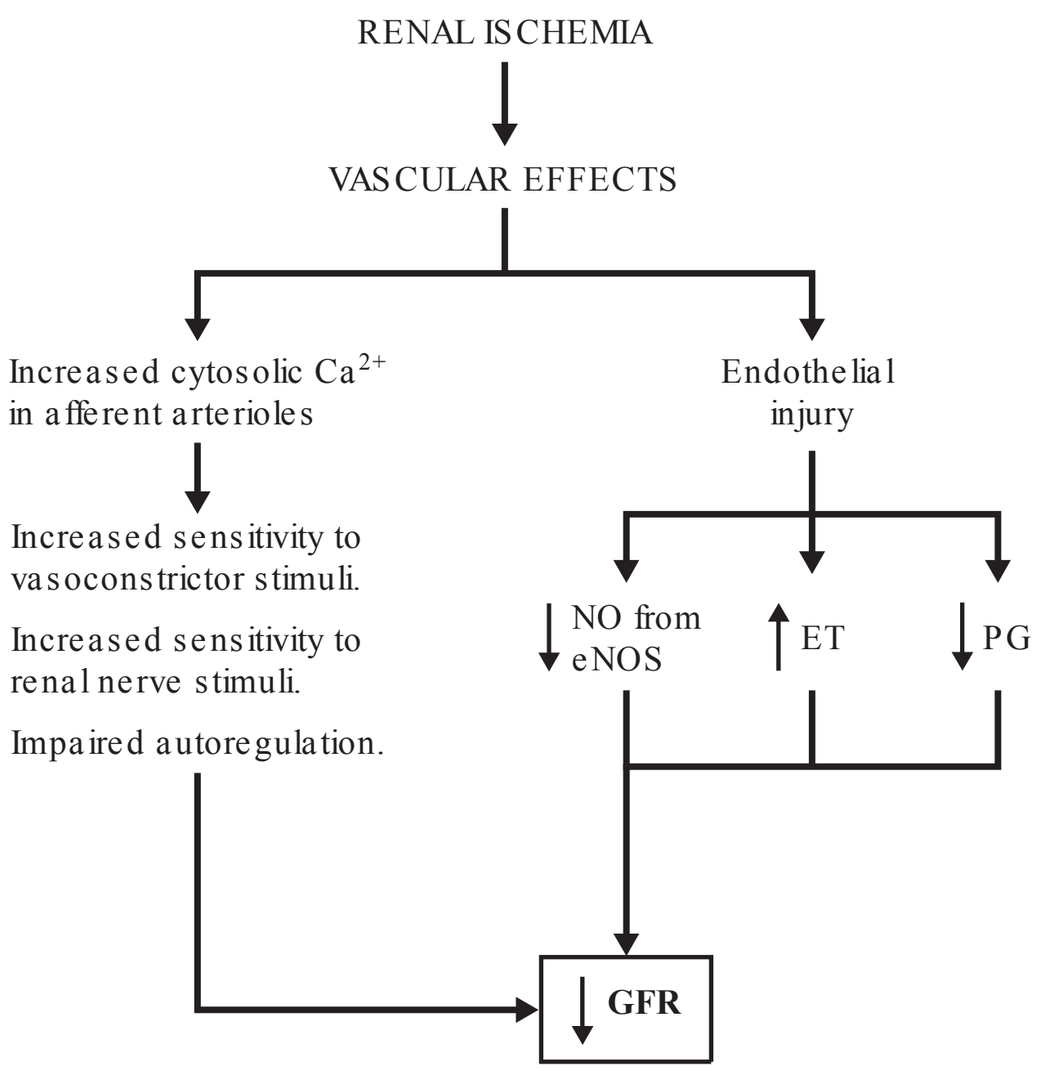


FIGURE 29.9 Vascular factors in the pathogenesis of ischemic acute kidney injury. NO, nitric oxide; eNOS, endothelial nitric oxide; ET, endothelin; PG, prostaglandins; GFR, glomerular filtration rate. Reproduced from Kribben et al.[466] with permission.

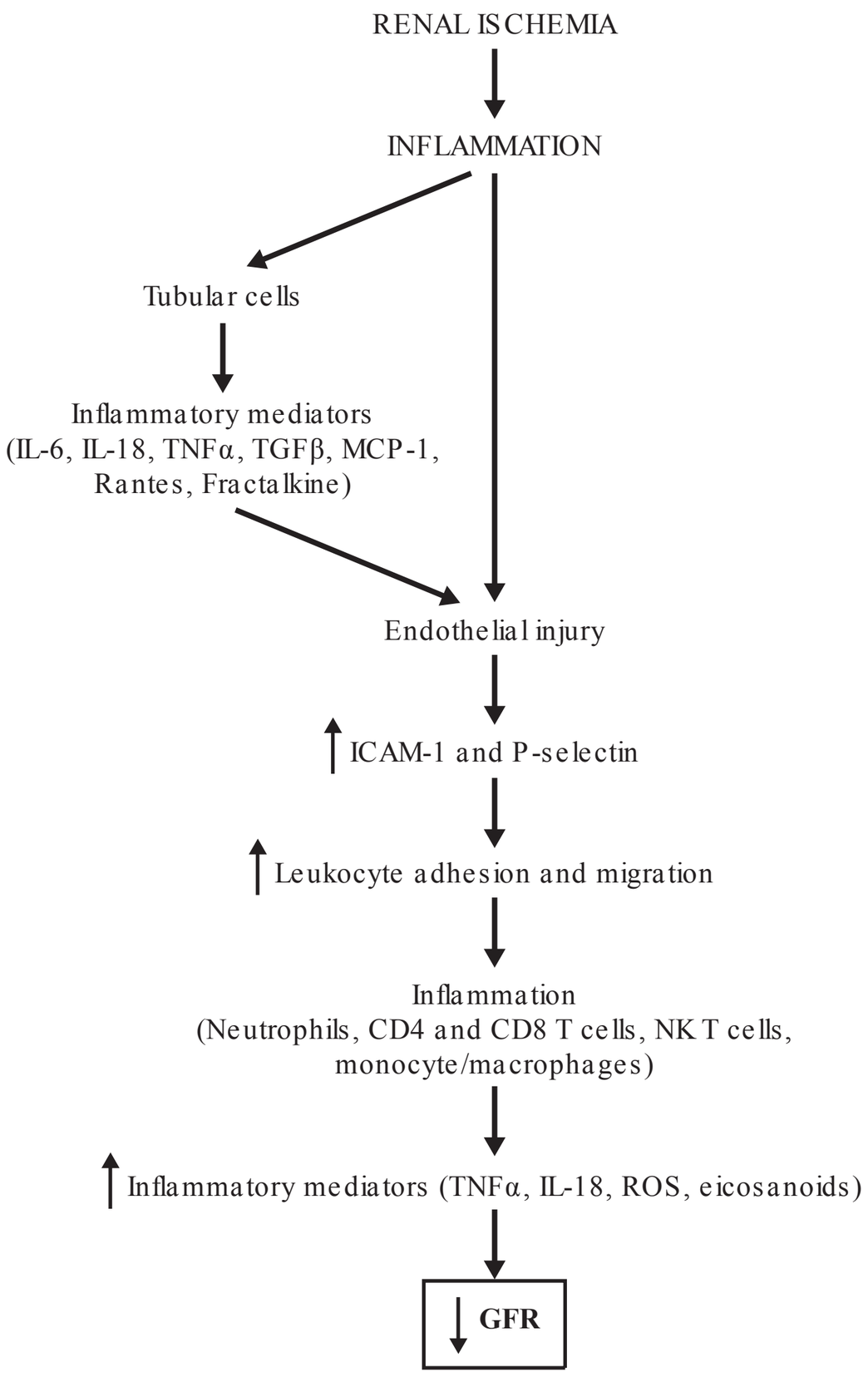


FIGURE 29.10 The inflammatory response in ischemic acute kidney injury. IL, interleukin; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; MCP-1, monocyte chemoattractant protein 1; RANTES, regulated upon activation, normal T cell expressed and secreted; ICAM-1, intracellular adhesion molecule 1; NK, natural killer; ROS, reactive oxygen species; GFR, glomerular filtration rate .

synthetic cyclical arginine-glycine-aspartic acid (RGD) sequences, mannitol, oxygen radical scavengers, TNF soluble receptors, inducers of HSP-70 and anti-ICAM antibodies, endothelin antagonists, IL-18 antiserum, IL-6 inhibition erythropoietin, NGAL, CTLA4 immunoglobulin, fractalkine receptor inhibition, CSF-1, MSCs, IGF-1, macrophage stimulating protein, macrophage inhibition, hemeoxygenase-1, PPAR-α, β/δ activators, CXCR3 inhibition, sphingosine-1-phosphate receptor agonists, siRNA to p53, soluble thrombomodulin, IFN regulatory factor (IRF-1), fibrates, HMGB1 inhibition, ghrelin, Klotho, HIF-1 activation, complement factor B inhibition, and adenosine A1 receptor activation, to mention a few (Table 29.9).

29.9 Some Emerging Therapies for Ischemic Acute Renal Failure

Cysteine protease inhibitors
Caspase inhibitors
IL-18 inhibition
IL-6 inhibition
áMSH
Specific iNOS inhibition
Synthetic cyclical RGD sequences
Oxygen radical scavengers
TNF soluble receptors
Inducers of HSP
Anti-ICAM antibodies
Endothelin antagonists
Endothelial cell infusion
Mannitol with natriuretic peptides or calcium channel blockers
Erythropoeitin
NGAL
CTLA4 immunoglobulin
Fractalkine receptor inhibition
CSF-1
MSCs
IGF-1
Macrophage stimulating protein
Macrophage inhibition
Hemeoxygenase-1
Peroxisome proliferator–activated receptor (PPAR) α, β/δ activators
CXCR3 inhibition
Sphingosine-1-phosphate receptor agonsists
IFN regulatory factor (IRF-1)
Fibrates
High mobility group box (HMGB1) inhibition
Ghrelin
Klotho
HIF-1 activation
Complement factor B inhibiton
Adenosine A1 receptor agonist
Adenosine A2B receptor agonist
siRNA to p53
Soluble thrombomodulin

IL, interleukin; MSH, melanocyte stimulating hormone; iNOS, inducible nitric oxide; RGD, arginine-glycine-aspartic acid; TNF, tumor necrosis factor; HSP, heat shock protein; anti-ICAM, intercellular adhesion molecule-1; NGAL, neutrophil-gelatinase-associated lipocalin; CTLA4, cytotoxic T-lymphocyte antigen 4; CSF-1, colony stimulating factor; MSC, mesenchymal stem cell; IGF-1, insulin-like growth factor 1; CXCR3, chemokine CX-C-motif receptor 3; HIF-1, hypoxia-inducible factor 1; siRNA, small interfering RNA.

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CHAPTER

30 Pathophysiology of Nephrotoxic Cell Injury

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Nephrotoxic epithelial renal cell injury is induced by a variety of stimuli, including chemical exposure, which can lead to acute kidney injury (AKI). Chemicals can induce cell injury either directly or indirectly. Examples of chemicals that directly induce renal cell injury include chemotherapeutics, antibiotics, oxidants, metals, and cysteine conjugates. In contrast, indirect chemical insults are initiated at sites removed from renal epithelial cells. Processes that induce indirect renal epithelial cell injury include decreased renal blood flow, renal ischemia, and reperfusion-induced cell injury and death. Inflammatory cells also indirectly and secondarily induce renal epithelial cell injury in a number of models.[1–4]

A secondary effect of nephrotoxicant-induced cell death is the generation of "backleak." After injury, epithelial cells can be released from the basement membrane and adhere to each other via integrins.[5–8] These cellular aggregates form tubular casts that block the flow of filtrate and increase intraluminal pressure, decreasing the single nephron glomerular filtration rate.[5] In addition, the loss of epithelial cells leaves gaps in the basement membrane, allowing tubular filtrate to backleak into the circulation, further decreasing the single nephron glomerular filtration rate. Thus, backleak and the loss of epithelial cells contribute to decreased renal function (Fig. 30.1). Tubular cast formation can be induced by both direct and indirect chemical injury.

After either direct or indirect injury, renal epithelial cells can die or repair and regenerate. The processes involved in renal cell regeneration have been reviewed,[9] and they are categorized into four different mechanisms that include dedifferentiation, proliferation, migration, and redifferentiation—each having defined morphologic characteristics and activation of differential cell signaling pathways. Processes of renal cell regeneration are somewhat similar to epithelial-mesenchymal transition (EMT) in embryonic development and cancer[9] and EMT is hypothesized to mediate fibrosis during chronic kidney injury induced by multiple stimuli.[10,11]

Significant controversy exists concerning the source of postinjury regenerating renal epithelial cells. Although some researchers have suggested that the majority of regenerating epithelial cells are derived from stem cells, present in either the kidney or the bone marrow, most recent studies provide convincing evidence that extratubular cells do not appreciably contribute to epithelial repair and regeneration after AKI.[12] Furthermore, there was no evidence of intratubular "progenitor cells."

The process of renal cell repair begins when cells adjacent to the injured area dedifferentiate, proliferate, and migrate into the denuded areas. Ultimately, the cells differentiate, and tubular structure and function are restored. Of course, such renal cell regeneration is not applicable for all nephrotoxicants. For example, repair of renal proximal tubule cell necrosis induced by the aminoglycoside tobramycin is initiated 4 days after treatment, with cells resuming normal morphology after 14 days.[13] In contrast, a 4-day regimen of the anticancer agent cisplatin also resulted in renal dysfunction with proximal tubular necrosis; however, renal dysfunction persisted.[14]

Several chemicals mediate AKI by inhibiting repair via alteration of differentiation, migration, and proliferation or dedifferentiation. Inhibition of repair with these compounds occurs at concentrations that do not induce overt cell injury. For example, Counts and colleagues studied renal repair and regeneration in vitro in renal proximal tubular cells (RPTCs) using a model that involved mechanical injury[15] and showed that $HgCl_2$; the mycotoxin fumonisin B_1; and the haloalkene cysteine conjugate, S-(1,2)-dichlorovinyl-L-cysteine (DCVC), inhibited the normal proliferative and migratory renal cell responses in the absence of overt cytotoxicity. Thus, mechanisms involved in the pathophysiology of nephrotoxic-induced AKI are not always directly related to cell death.

The goal of this chapter is to review mechanisms by which chemicals produce renal epithelial cell injury and death. Other chapters in this volume, and several excellent reviews, discuss renal cell repair and regeneration, as well as mechanisms of renal cell death and AKI produced by specific chemicals.

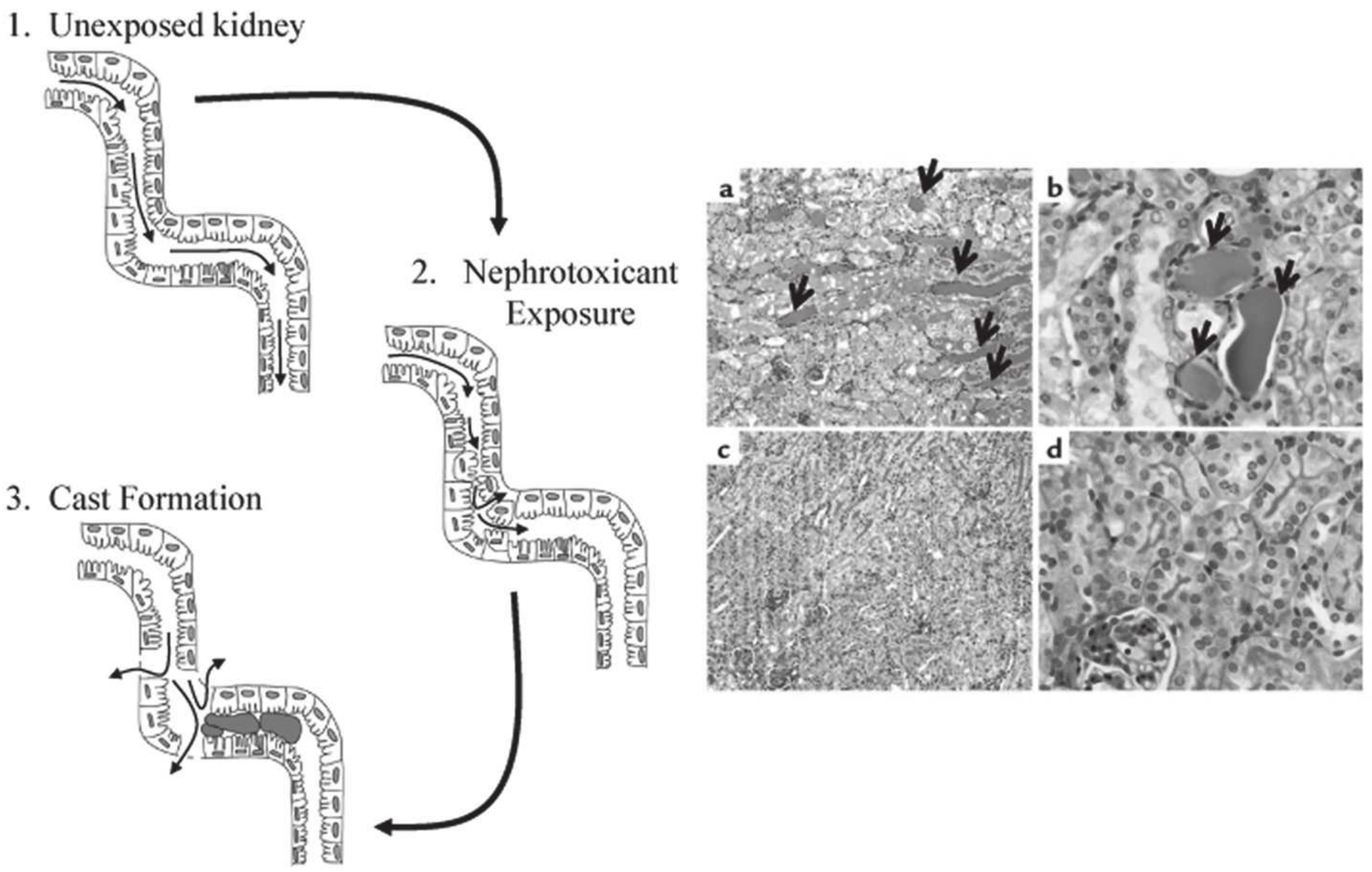

FIGURE 30.1 Cast formation in the nephron. **Left:** *1.* Filtrate flow (as represented by the *small arrows*) through the tubules is constant and unobstructed in unexposed kidneys. *2.* Exposure of the kidney to nephrotoxicants results in cell injury and death, and can induce detachment of the cells from the basement membrane. *3.* Detached cells can adhere to each other and form casts (*pink*), which obstruct filtrate flow and increase intraluminal pressure. This increases permeability in the basement membrane and back leak of filtrate into the interstitium. **Right:** Cisplatin-induced cast formation in wild-type mice (*a* and *b*) and mice mutant for tumor necrosis factor (TNF)-α (*c* and *d*) as determined by PAS staining. The magnification in panels *a* and *c* is ×100, and that in panels *b* and *d* is ×400. Cast formation is visible in panels *a* and *b* as indicated by the *pink/purple* aggregates between the tubules (*arrows*). In contrast, little cast formation can be seen in TNF-α knockout mice (panels *c* and *d*). (Adapted from Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest*. 2002;110(6):835–842, with permission.) (See Color Plate.)

SUSCEPTIBILITY OF THE KIDNEY TO INJURY

The kidney is highly susceptible to numerous agents because of several functional properties of this organ. These include: (1) receiving 20% to 25% of the cardiac output, ensuring high levels of toxicant delivery over a period of time; (2) extensive reabsorptive capacity with specialized transporters promoting cellular uptake of the toxicant; (3) concentrating abilities resulting in high concentrations of toxicants in the medullary lumen and interstitium; (4) biotransformation enzymes for the formation of toxic metabolites and reactive intermediates; (5) high metabolic rate and workload of renal cells causing increased sensitivity to toxicants; and (6) sensitivity of the kidney to vasoactive agents.

Nephrotoxicants can target specific nephron segments. The proximal tubule epithelial cell is typically the primary target; however, other parts of the nephron can also be affected by chemicals with a specificity that is concentration-dependent. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen target the collecting ducts at low concentrations, but also induce damage to the proximal tubules at higher concentrations.[16,17] Furthermore, different segments of the proximal tubule (S_1, S_2, and S_3) are targets for different nephrotoxicants. For example, aminoglycoside antibiotics, chromate, cadmium chloride, and the mycotoxin citrinin primarily target the S_1 and S_2 segments, whereas cyclosporine, $HgCl_2$, uranyl nitrate, cisplatin, bromobenzene, and cysteine conjugates of halogenated hydrocarbons target the S_3 segment.[17,18] Interferon-α, gold, and penicillamine can target cells in the glomeruli, whereas angiotensin-converting enzyme (ACE) inhibitors can target cells in the renal vasculature.[17] Clostridium perfringens B and D and trichloroethylene can target distal tubules, and radiocontrast media, triethanolamine, amphotericin, and nystatin tend to target the loop of Henle. Studies suggesting that trichloroethylene targets the distal tubules are derived from in vitro models only at high doses,[19] whereas agents that target the loop of Henle also can also affect the proximal tubules.[16]

These segmental differences in chemical sensitivity may be attributed to: (1) differences in toxicant delivery to a given segment, (2) differences in transport and uptake among segments, and (3) differences in biotransformation enzymes among segments. Once again, concentration may be a deciding factor.

NEPHROTOXICANT TRANSPORT

Many nephrotoxicants require transport into epithelial cells to induce injury, either by passive diffusion or by active or facilitated transport. Increased accumulation typically correlates to increased injury and decreased cellular function, which leads to AKI.

Several transporters are expressed in the kidney for the purpose of ensuring renal cell homeostatic functions, such as reabsorption and secretion; however, these proteins can also transport nephrotoxicants.[20] Major transporters found in renal cells include, but are not limited to, the organic cation transporters (OCTs),[21,22] organic anion transporters (OATs),[23,24] the organic anion transporting polypeptide (OATP) family,[25,26] and transporters involved in multidrug resistance (MDR) such as P-glycoprotein.[27]

Organic Anion Transporters and Organic Cation Transporters

OATs and OCTs are members of the solute carrier superfamily group 22A (SLC22A: human nomenclature) as assigned by the human genome organization (HUGO) nomenclature committee.[28] Several members in the SLC22A family have homologs with human, mouse, rat, and rabbit kidneys, in addition to having overlapping substrate specificity with each other and with other transporter families. Other than physiologic substrates,[28,29] these proteins also transport drugs, natural products, industrial chemicals, and pollutants.[20,23,30–32]

The OCT family of proteins typically transports small, hydrophobic, positively charged chemicals. Major isoforms include OCT1 (SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3), OCT6 (SLC33A16), OCTN1 (SLC22A4), OCTN2 (SLC22A5), and OCTN3 (SLC22A21).[28] Nephrotoxicants transported by OCTs include the chemotherapeutic cisplatin, which is a substrate for OCT2 in humans and rats.[28,33–35] OCT2 may also mediate proximal tubule cell death induced by paraquat, a commonly used herbicide known to induce AKI. OCT1 mediates the toxicity of platinum compounds including cisplatin, oxaliplatin, and carboplatin in Madin-Darby canine kidney (MDCK) cells,[22] and both OCT1 and OCT2 mediate the transport of 1-methyl-4-phenyl-pyridinium, disopyramide, and chlorpheniramine into renal cells.[36] OCT2 was also demonstrated to mediate the nephrotoxicity of ifosfamide both in vitro and in vivo.[37]

OCT1 and OTC2 were recently reported to mediate the transport and toxicity of several antiretroviral drugs used to treat human immunodeficiency virus (HIV) in human embryonic kidney 293 (HEK293) cells.[38] Furthermore, OCT1, OCT2, and OCT3 were reported to mediate the transport of tyrosine kinase inhibitors (TKIs), such as imatinib, in HEK293 cells.[39] More in vivo studies are needed to fully determine the role of OCTs in nephrotoxicity induced by both antiretroviral drugs and TKI.

The OAT families of proteins typically transport small organic anions into cells. Major isoforms include OAT1 (SLC22A6), OAT2 (SLC22A7), OAT3 (SLC22A8), OAT4 (SLC22A11), OAT5 (SLC22A19), OAT6 (SLC22A20), OAT7 (SLC22A9), OAT8 (SLC22A25), OAT10 (SLC22A13), and URAT1 (SLC22A12).[28] Nephrotoxicants reported to be transported by OATs include the mycotoxin ochratoxin A, which is transported into renal tubular cells by OAT1, OAT 3, and OAT 5.[30,31,40] Ochratoxin A transport into renal cells is inhibited by probenecid,[41] an inhibitor of most OAT proteins. Recent studies also suggest that aristolochic acid, an inducer of both acute renal failure (ARF) and cancer, is transported into HEK293 cells via OAT1, OAT3, and OAT4.[42] Other nephrotoxicants whose toxicity is mediated by OCTs include methotrexate (OAT1, OAT2, and OAT3), uremic toxins such as hippuric acid and indoleacetic acid (OAT1 and OAT3), and NSAIDs (OAT1, OAT2, OAT3, and OAT4).[28]

The ability of Hg^{+2} and its cysteine conjugates to induce cell death in vivo and in MDCK cells is altered by inhibitors or substrates of OAT proteins, suggesting that the nephrotoxicity of this environmental contaminant is regulated by these transporters.[23,24] This hypothesis was confirmed by studies demonstrating that overexpression of human OAT1 in MDCK cells altered the nephrotoxicity of these compounds.[43] Other compounds for which toxicity is suggested to be mediated by OATs include the trichloroethylene metabolite DCVC, some chlorinated phenoxyacetate-based herbicides, antiviral drugs, and β-lactam-based antibiotics.[28,44]

Organic Anion Transporting Polypeptides

OATPs are members of the solute carrier O family (SLCO, formerly referred to as the SLC21 family[45]). Endogenous substrates for these proteins include bile acids, hormones, and eicosanoids.[26] Currently, genes for 11 human OATPs, 15 rat OATPs, and 15 mice have been identified.[25,45] Not all of these are expressed in kidney. Furthermore, several OATPs expressed in humans are not expressed in rodents, such as OATP1A2, OATP1B1, and OATP1B3.[45] Additionally, there are several rodent OATPs that do not have a human homolog. Such differences should be taken into account when assessing the role of OATPs in nephrotoxicity.

OATPs demonstrated to be expressed in human kidneys include OATP1A2, OATP2A1, OATP2B1, OATP3A1, OATP4A1, and OATP4C1.[45] Rat and mouse kidneys are reported to express Oatp1a1, Oatp1a6, Oatp2a1, Oatp2b1, Oatp3a1, Oatp4a1, and 4c1[29,46,47] (the lowercase denotes rodent genes). Oatp1a3 is reported to be a rat specific isoform.[29] The expression of several mouse kidney oatp, such as Oatp1a1, Oatp3a1, and Oatp4c1, are reported to differ depending on gender,[47] but it is not known if this trend is replicated in human kidneys.

Several studies demonstrate that OATPs mediate the transport of nephrotoxicants. For example, ochratoxin A (OATP1A2, Oatp1a1), methotrexate (OATP1B1, Oatp1a3), and digoxin (OATP4C1) are known substrates.[29] Studies also suggest that the expressions of OATPs are altered by nephrotoxicants. For example, treatment of mice with nephrotoxic doses of cisplatin for 4 days increases the expression of Oatp2a1 and Oatp2b1 mRNA.[48] Future studies are needed to fully understand the role of OATPs in the pathophysiology of nephrotoxic renal cell injury.

Maillard Reaction Products

Maillard reaction products (MRPs) are members of the ATP-binding cassette super family (ABCC).[29] Substrates for these proteins include hydrophobic molecules, such as

the chemotherapeutics vincristine and doxorubicin. At least six different MRP genes have been identified (designated MRP1-6, and MDR1) and all are expressed in the kidney.[49]

P-glycoprotein is perhaps the most well known MRP. Localized to the apical membrane of proximal tubule cells, MRP is believed to mediate the efflux of organic anions from the kidney. Known substrates for P-glycoproteins include methotrexate and cisplatin.[49,50] The nephrotoxicity of cisplatin is altered by overexpression of P-glcyoprotein.[50] P-glycoprotein may also mediate the nephrotoxicity of diallyl disulfide and S-allyl-cysteine, $HgCl_2$, calcineurin, and cyclosporine.[51–54]

CELL DEATH

The mechanisms by which chemicals induce epithelial cell death are as varied as the chemicals themselves; nevertheless, some commonalities do exist. For example, many chemicals require transport into cells to induce death. Furthermore, regardless, of how nephrotoxicants gain entry into cells, cell death is thought to occur through one of three mechanisms: apoptosis (type I cell death), autophagy (type II cell death), or necrosis (type III cell death).[55,56] Other commonalities that exist in injured and dying cells include activation of proteases, increases in cytosolic Ca^{2+}, changes in mitochondrial function and morphology, and changes in nuclear morphology and chromatin/DNA structure.

Necrosis, apoptosis, and autophagy can be identified by assessing differences in cellular and nuclear morphology. In fact, some suggest that morphology is standard for delineating mechanisms of cell death; however, it is becoming evident that morphology alone is not the best way to identify the mechanisms of cell death.[55] This reflects the fact that the mechanism of death induced by a given chemical is dependent on multiple factors such as the cell type being injured, the time of compound exposure, and the compound dose. Such multiple dependencies are typified by arsenic, which can induce all three types of cell death in a given cell.[55] Additionally, the cell death mechanism may change midway through any series of postinsult events (Fig. 30.2). Thus, a chemical may initiate autophagy, but this pathway may switch to apoptosis as the dose and time of exposure increases, or if p53 is released to the cytosol. Furthermore, apoptosis may switch to necrosis as ATP decreases or if cytosolic Ca^{2+} increases high enough to activate select proteases or induce membrane rupture. Thus, a particular mechanism of cell death cannot always be directly linked to a specific morphology and, possibly, multiple cell death pathways may be activated in a single cell.[55]

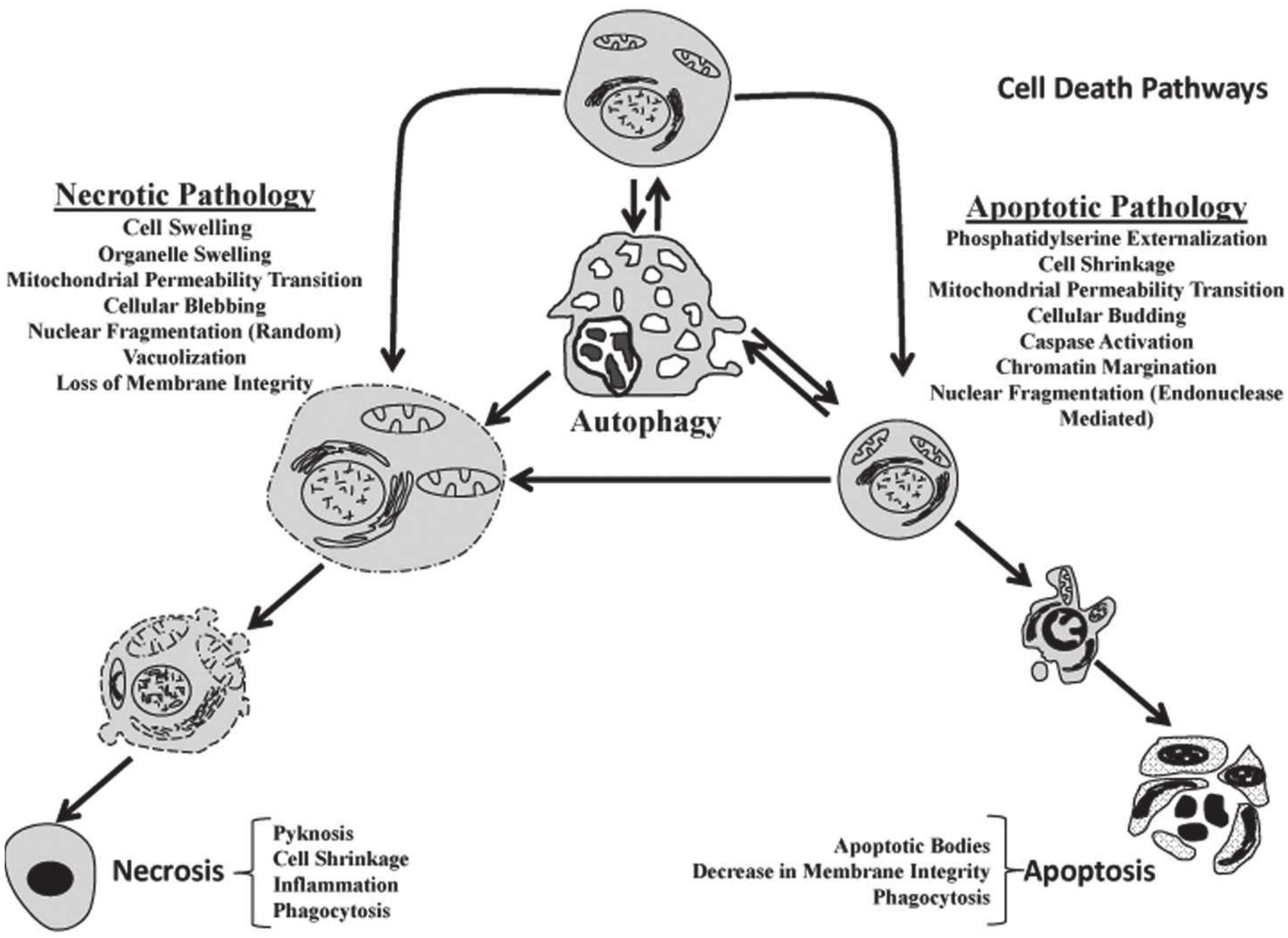

FIGURE 30.2 Schematic comparing the pathologic and morphologic features of necrosis, apoptosis, and autophagy. At the top middle, a normal cell is shown; below the normal cell is an autophagic cell demonstrating mass vacuolization; and autophagosomes (not shown). **Left:** Cell and organelle swelling, followed by vacuolization, blebbing, and increased membrane permeability (lysis) and finally necrotic changes (i.e., coagulation, shrinkage, and karyolysis). **Right:** Cell shrinkage followed by budding and karyorrhexis and finally necrotic changes (i.e., breakup into cluster of apoptotic bodies). The pathologies necrosis and apoptosis are listed. *Double arrows* represent the hypothesis that select pathways can switch. For example, autophagy can lead to cell survival and also progress to apoptosis.

Necrosis

Necrosis affects masses of contiguous cells and is characterized by swelling of organelles and increases in cell volume, after which the cell membrane becomes more permeable and ruptures with the release of cellular contents, followed by inflammation. Historically, necrosis has been used to describe drastic tissue changes occurring after cell death. These include karyorrhexis, karyolysis, pyknosis, condensation of the cytoplasm, and intense eosinophilia.

Morphologic markers for cellular necrosis include a loss of membrane and organelle integrity, cell swelling, and swelling of the endoplasmic reticulum (ER) and mitochondria (Figs. 30.2 and 30.3). Nuclear morphology in necrotic cells is usually typified by pyknosis (nuclear condensation without fragmentation); however, DNA fragmentation can occur in some cases, especially when agents that target the DNA are used. This can give rise to chromatin margination. Cellular blebs also form, but unlike apoptosis, necrotic cell blebs do not typically contain organelles. Necrosis usually induces inflammation, often with the infiltration of neutrophils and inflammatory cells in vivo (Fig. 30.3).

Biochemical markers for necrosis include a drastic and rapid loss of ATP (greater than 70%–80%), rapid and sustained increases in cytosolic Ca^{2+}, leakage of intracellular constituents such as lactate dehydrogenase, DNA fragmentation, and protease activation. A hallmark of necrosis is that it does not require ATP, separating it from both apoptosis and autophagy. DNA fragmentation also occurs in apoptosis, but DNA fragmentation that occurs during necrosis is random and not usually inhibited by caspases. Proteases activated during necrosis include select types of calpains, which are usually activated due to high concentrations of cytosolic Ca^{2+}. Evidence exists that calpains can also be activated during apoptosis (see below); thus, calpain activation alone is not a valid marker for necrosis.

Apoptosis

Apoptosis usually affects scattered individual cells and, morphologically, the cell shrinks whereas organelle integrity is initially retained (Figs. 30.2 and 30.3). Next, chromatin become pyknotic and marginate against the nuclear membrane and, ultimately, the cell shrinks to a dense, round mass

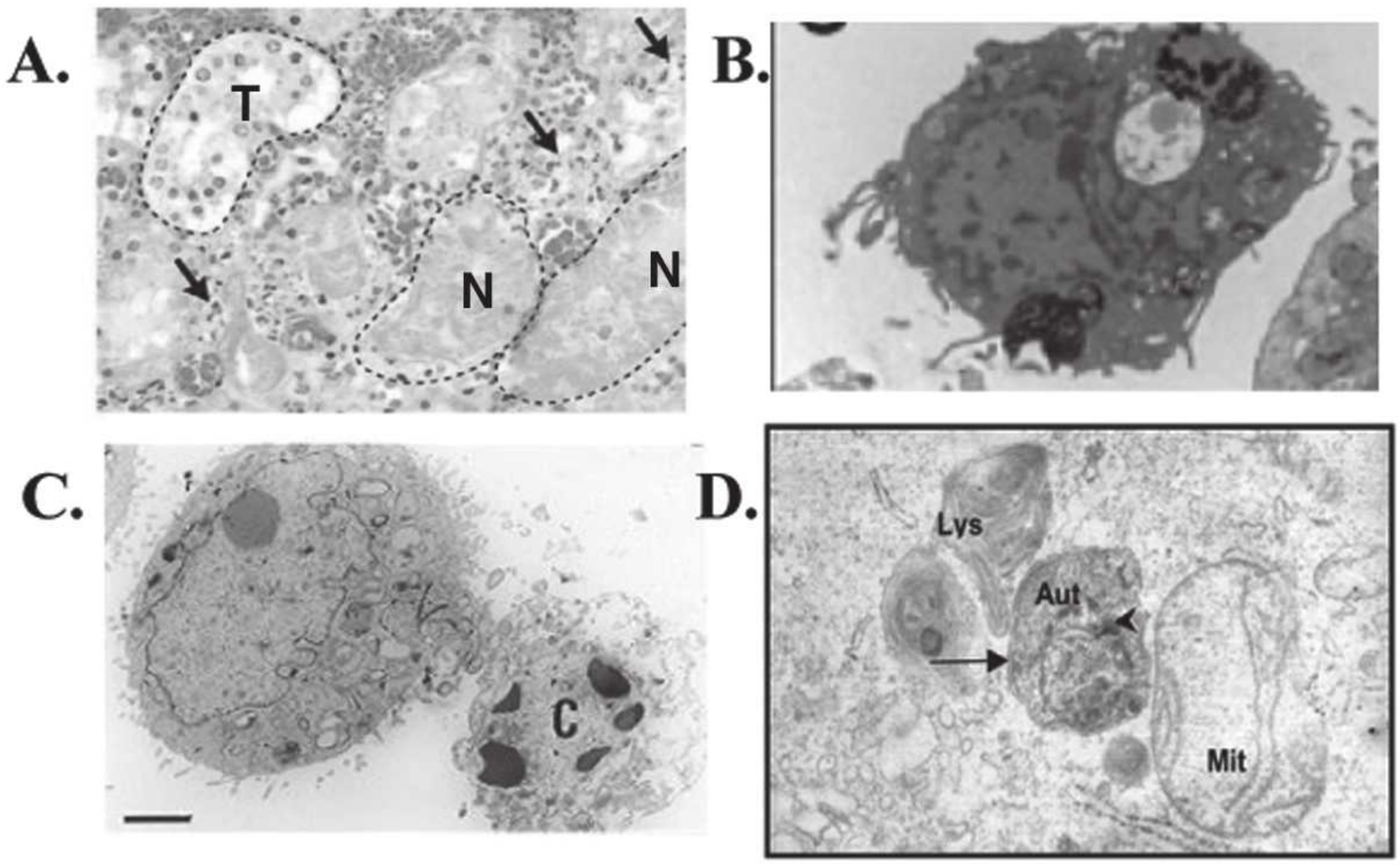

FIGURE 30.3 Comparison of the morphologic features of necrosis, apoptosis, and autophagy in tissues and cells. **A:** Hematoxylin and eosin staining of human kidney tissue after arterial embolization for treatment of renal cancer demonstrating intact tubules (*T*) and necrotic tubules (*N*). Arrows represent neutrophils and mononuclear inflammatory cells. (Modified from Hotchkiss RS, Strasser A, McDunn JE, et al. Cell death. *N Engl J Med.* 2009;361:1570, with permission.) **B:** Transmission electron microscopy (TEM) of necrotic human embryonic stem cells showing loss of membrane integrity without chromatin margination, cytosolic vacuolization, and spilling out of intracellular constituents. (Modified from Heng BC, Vinoth KJ, Lu K, et al. Prolonged exposure of human embryonic stem cells to heat shock induces necrotic cell death. *Biocell.* 2007;31(3):405, with permission.) **C:** TEM human HEK293 cells undergoing lysosomal mediated apoptosis. The cell on the right is relatively healthy whereas the cell on the left has shrunken and exhibits chromatin and nuclear condensation and is beginning to lose the membrane integrity (late apoptosis). (Modified from Heng BC, Vinoth KJ, Lu K, et al. Prolonged exposure of human embryonic stem cells to heat shock induces necrotic cell death. *Biocell.* 2007;31(3):405, with permission.) **D:** TEM of autophagic primary cultures of normal human renal cells exposed to cyclosporine demonstrating formation of the autophagosomes (*Aut*) with a double membrane (*long arrow*), next to a lysosomes (*Lys*) and a mitochondria (*Mit*). The *arrowhead* represents a cytoplasmic organelle. (Pallet N, Bouvier N, Legendre C, et al. Autophagy protects renal tubular cells against cyclosporine toxicity. *Autophagy.* 2008;4(6):72, with permission.)

(apoptotic body) or forms pseudopodia (i.e., buds) containing nuclear fragments and/or organelles that break off into small fragments (apoptotic bodies). In either case, adjacent cells or macrophages phagocytize the apoptotic bodies, and inflammation typically does not occur.

A key difference between necrosis and apoptosis is the activation of caspases in the latter. Caspases are cysteinyl aspartate-specific proteases that belong to an 18-member family.[57–59] Caspases can be divided into three groups based on structural differences and substrate preferences: initiator caspases (caspase -2, -8, -9, -10, and possibly -12), executioner caspases (caspases -3, -6, and -7), and cytokine processors (caspases -1, -4, -5, -13, and -14). Caspases-15 to 18 have been identified in numerous mammals, but not in humans, with the exception of caspase 16.[59]

Initiator caspases are activated by numerous processes including receptor-directed mechanisms and chemical exposure. They mediate chemical-induced apoptosis in numerous cell types, including proximal tubular cells,[60–62] glomerular cells,[63] medullary cells,[64,65] and cells present in the collecting ducts.[66–68] Activation of initiator caspases results in the activation of executioner caspases, which leads to several of the biochemical characteristics of apoptosis. Initiator caspases can also be substrates for executioner caspases.[69]

Caspase-8 is an initiator caspase that plays an integral role in receptor-mediated apoptosis.[69–71] It is activated by membrane receptors such as Fas-ligand and tumor necrosis factor (TNF)-α receptors[71,72] and, in turn, cleaves the Bcl-2 family protein Bid to form tBid.[70] tBid acts on mitochondria to cause the release of pro-apoptotic proteins and results in the activation of caspase-9 and caspase-3. In contrast, caspase-8 can directly activate caspase-9 or caspase-3, independently of the mitochondria (Fig. 30.4).

Caspase-8 can be activated by nephrotoxicants independent of receptor-mediated mechanisms. For example, cisplatin and etoposide activate caspases-8, -9, and -3 in LLC-PK1 cells in the absence of receptor-stimulation.[73] In contrast, cisplatin and cyclosporine activate caspase-3 in the absence of caspase-8 in mouse and rabbit RPTC.[74–76] Thus, the role of caspase-8 in chemical-induced renal cell apoptosis is variable.

Executioner caspases cleave numerous substrates that ultimately result in the morphologic features of apoptosis. Perhaps the most important substrates are proteins that control DNA degradation (DNAase). Caspases are known to mediate the activation of the nuclease DNA fragmentation factor (DFF). DFF is composed of two subunits: a 40-kDa DNAase subunit (CAD/DFF40) and a 45-kDa inhibitor of caspase-activated deoxyribonuclease (ICAD/DFF45).[69] Caspase-3 cleaves ICAD/DFF45 during apoptosis, which results in the release and activation of CAD/DFF40. Active CAD/DFF40 results in double-stranded DNA breaks in chromosomes, giving rise to the characteristic nonrandom DNA-ladderlike pattern seen with apoptosis on agarose gels.[69]

Caspases can also mediate DNA degradation by cleaving poly(ADP-ribose) polymerase (PARP). PARP is involved in DNA repair and maintenance of stability, and regulates DFF40 activity.[69] Caspases-3 and -7 can cleave and inactivate PARP.[77] PARP cleavage is used as a marker for apoptosis in renal cells, including apoptosis induced by antimycin A and DCVC.[78,79] Cleavage of DNA repair enzymes (such as PARP) by caspases is thought to prevent cells from making a futile repair attempt.

Caspases have numerous other substrates other than DNAases. Initiator caspase substrates include other caspases, the pro-apoptotic protein Bid, α-tubulin, and vinculin.[58] Cytokine caspase substrates include inflammatory mediators such as such Il-18, Pro-IL-1B, and Il-17, whereas executioner caspase substrates include protein kinase C (PKC), focal adhesion kinases (FAK), and the cell cycle regulator p21.[58] Cleavage of these proteins is believed to inhibit futile repair attempts, facilitate apoptosis signaling cascades, and allow for cytoskeleton reorganization and packaging of cell constituents into apoptotic bodies.[80]

Caspase-3, perhaps the best studied executioner caspase, can also cleave receptors, such as type 1 inositol(1,4,5) P4 receptor, Ca^{2+}-ATPase, the Na^{+}/Ca^{+} exchanger, and the Na^{+}/K^{+}-ATPase pump.[55] The Na^{+}/K^{+} ATPase may also be cleaved by initiator caspases, such as caspase-8 and -9.[81] Cleavage of these receptors is believed to alter ion homeostasis and facilitate decreases in intracellular K^{+}, which further promotes caspase activation. Cleavage of these receptors also leads to cell size alterations, such as cell shrinkage, early during apoptosis after cleavage of Na^{+}/K^{+} ATPase, or cell rupture due to swelling after Ca^{2+}-ATPase inactivation during late-stage apoptosis/secondary necrosis.

Role of Mitochondria in Apoptosis

The role of mitochondria in cell death cannot be understated, especially for apoptosis. Mitochondria regulate apoptosis by at least two major processes: maintenance of ATP production and release of pro-apoptotic proteins, such as cytochrome c, Bcl-2 family proteins, and DNAases. In addition, mitochondria regulate apoptosis by participating in Ca^{2+} signaling cascades and mediating protease activation.[55]

ATP is considered to be a requirement for both the initiation and execution of apoptosis.[55] It is required for formation of an apoptosome protein complex (see later), which facilitates the activation of caspase-9. It may also be required for transport of pro-apoptotic proteins into the nucleus.[55] ATP may also represent an important switch point between apoptosis or necrosis; depleting ATP below 30% transforms apoptotic liver cell death to necrotic death patterns.[82] In addition, ATP is needed to maintain Na^{+}/K^{+} ATPase pumps on the plasma membrane, and pump inactivation will eventually lead to cellular swelling, pathologic increases in intracellular Ca^{2+}, and cellular lysis, which is typical of necrosis.

Cytochrome c is a heme protein bound to the inner mitochondrial membrane, transferring electrons between complexes III and IV of the electron transport chain. Release of cytochrome c from mitochondria activates the intrinsic pathway of apoptosis. Cytosolic cytochrome c will bind

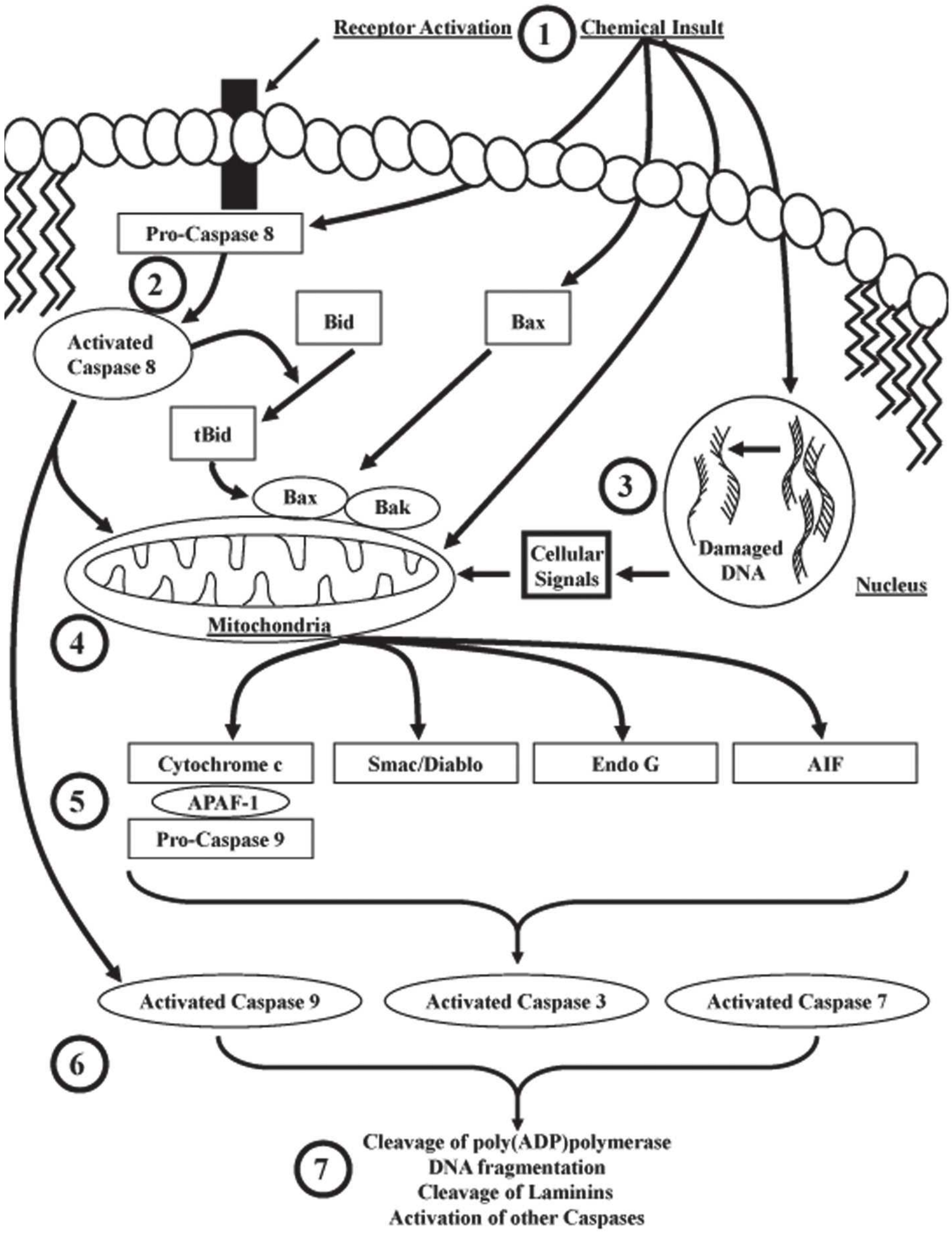

FIGURE 30.4 Cell signaling cascades involved in the activation of caspases and apoptosis. **1:** Receptor-mediated death signals or chemicals can initiate apoptosis through multiple mechanisms. **2:** Pro-caspase 8 is activated by receptor-mediated signals at the cellular membrane or directly by chemicals. Once activated, caspase-8 cleaves Bid to t-Bid, which interacts with Bax/Bak to induce mitochondrial-mediated apoptosis or directly activates caspase-9 and other caspases. **3:** Some chemicals cause DNA damage that signals the release of pro-apoptotic proteins from the mitochondria. **4:** Receptor-mediated signals, direct chemical injury, or signals resulting from DNA damage can all cause cytochrome c, Smac/Diablo, Endo G, and AIF release from the mitochondria. **5:** Released cytochrome c forms a complex with APAF-1 and pro-caspase 9, resulting in caspase-9 activation. **6:** Activated caspase-9 cleaves and activates pro-caspase-3 and -7, which can also be activated by caspase-8 independently of cytochrome c. **7:** Activated caspases (e.g., 3 and 7), AIF, and Endo G cause the classical markers of apoptosis such as cleavage and activation of poly(ADP)polymerase, inactivation of inhibitors of DNases leading to DNA fragmentation, cleaved laminins, and the activation of other caspases.

to apoptotic protease activating factor 1 (APAF-1), which promotes the binding and proteolytic cleavage of pro-caspase-9 to caspase-9 (the apoptosome),[83] and then activated caspase-9 cleaves and activates executioner caspases (i.e., caspases-3, -6, and -7) (Fig. 30.4). Nephrotoxicants known to induce cytochrome c release in correlation with apoptosis include cisplatin and DCVC.[71,84] Cytochrome c release from the mitochondria is associated with a decrease in the mitochondrial inner membrane potential and the accumulation of several pro-apoptotic proteins such as Bad, Bax, and Bax at the mitochondria (Fig. 30.4). Other pro-apoptotic proteins released from the mitochondria include apoptosis-inducing factor (AIF), Smac/Diablo, Omi, and Endo G (Fig. 30.4).[70,71,85–92]

Bad, Bak, Bax, and Bid belong to the Bcl-2 family of pro-apoptotic proteins, which are characterized by specific regions of homology, termed Bcl-2 homology domains.[93] Under nonstressed conditions, these proteins exist bound to proteins in the mitochondria and cytosol.[70] After toxicant exposure, Bax, Bid, or Bak can dissociate and translocate to the mitochondria which initiates the formation of a pore complex that causes membrane rupture[55] and subsequent loss of mitochondrial membrane potential, facilitating the release of cytochrome c, Endo G, Smac/Diablo, Omi, and AIF (Fig. 30.4).[68,70,74] Bid mediates apoptosis induced by hypoxia and ATP depletion in cultures of rat RPTC[94]; Bax mediates proximal tubular apoptosis in mice treated with cisplatin in vivo[65]; and Bak is elevated during apoptosis in primary bovine glomerular endothelial cells induced by TNF-α or lipopolysaccharide (LPS[95]) or during ischemia-reperfusion-induced renal cell apoptosis in mice.[96]

In contrast to Bax, Bid, and Bak, Bcl-2 is an anti-apoptotic protein.[60] Increased Bcl-2 prior to toxicant exposure protected numerous cells, including renal cells,[96] from toxicant-induced apoptosis.[96] The protective effect of Bcl-2 may be the result of its ability to bind Bax, Bid, and Bak, preventing them from inducing mitochondrial pore formation, altering mitochondrial membrane permeability, initiating the release of mitochondrial pro-apoptotic proteins, and activating caspases.[97] Overexpression of Bcl-2 protected against ATP-depletion-induced apoptosis in cultures of rat RPTC,[94] and upregulation of Bcl-2 protected kidney epithelial cells both in vitro and in vivo against apoptosis induced by hypoxia, azide, cisplatin, and staurosporine.[98]

AIF is released from mitochondria in response to decreases in the mitochondrial membrane potential induced by ATP depletion[86,99]; ischemia-reperfusion; anti-fas antibodies[100]; or exposure to high concentrations of Ca^{2+},[101] t-butyl hydroperoxide,[101] or atractyloside.[101] Cellular pathologies associated with AIF release are similar to those seen with caspases (chromatin condensation and oligonucleosomal DNA fragmentation).[100] Recent studies suggest that increases in cytosolic Ca^{2+} and calpain activation also facilitate the release of AIF from mitochondria[102]; and studies in LLC-PK1 cells support this hypothesis.[103]

AIF is a protease with properties similar to caspases, including being inhibited by N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-fmk), a commonly used broad spectrum caspase inhibitor.[86] Thus, the decrease in renal cell death observed in the presence of Z-VAD-fmk may be a result of AIF or caspase inhibition. AIF can induce DNA fragmentation independently of caspases.[69] AIF is released in opossum kidney (OK) cells after ATP depletion-induced by sodium cyanide and 2-deoxy-D-glucose.[87,99] AIF is also activated in HEK293 cells after exposure to cadmium,[104] in LLC-PK1 cells after exposure to cisplatin,[105] and in OK cells after exposure to the peroxisome proliferator-activated receptor agonist ciglitazone.[106]

Smac/Diablo is a pro-apoptotic protein released from the mitochondria to the cytosol during apoptosis. It blocks anti-apoptotic activity of inhibitors of apoptosis proteins (IAP), which increase apoptosis.[89] The ability of Smac/Diablo to promote apoptosis is not exclusively a result of its ability to bind IAP.[107] Smac/Diablo functions at the same level of executioner caspases, but downstream of the Bcl-2 family of proteins.[90]

Smac/Diablo is expressed in the mouse kidney and in several renal cell models.[108] It mediates apoptosis in vivo in mice after treatment with high concentrations of folic acid or after exposure of cultures of renal epithelial cells to TNF-α.[89] Increased expression of Smac/Diablo potentiates TNF-α- and etoposide-induced apoptosis in HEK293 cells[107]; however, similar to several other pro-apoptotic proteins, expression of Smac/Diablo is not essential for apoptosis in kidney cells. For example, acetaminophen-induced renal cell apoptosis proceeds in a caspase-dependent manner in the absence of Smac/Diablo activity.[109]

Omi is a mammalian serine protease homologous to bacterial HtrA endoprotease.[110] Omi localizes to the mitochondria and is expressed ubiquitously in a number of cell types including RPTC.[91] Omi is released from the mitochondria after exposure to apoptotic stimuli and binds to, and cleaves, IAP.[91] Omi-directed degradation of IAP facilitates caspase activation and the subsequent biochemical and morphologic features of apoptosis. In addition, Omi can translocate to the nucleus and activate the transcription factor p73, which induces pro-apoptotic proteins such as bax.[69] Omi participates in both caspase-independent and caspase-dependent cell death,[69,111] an event that has been proven using either siRNA against Omi, or a synthetic inhibitor, called ucf-101, in in vitro and in vivo models, including primary cultures of mouse RPTC.[91,111] More work is needed to determine if Omi can mediate cell death induced by other nephrotoxicants.

Autophagy

Autophagy is essentially "a cell eating itself."[112,113] This process was originally thought to be a cell survival pathway activated to produce energy during times of metabolic stress, such as starvation.[113] Some cells undergoing autophagy can recover; however, ample evidence exists that autophagy itself leads to cell death, specifically referred to as type II cell death.[55,112,113]

Significant evidence shows that autophagy mediates renal cell death.[56,114,115] The role of autophagy in renal cell death differs depending on the experimental conditions.[56] Further, it is difficult to determine if autophagic cells are a result of a cell death mechanism, or a failure in repair or survival mechanism.[56] Complicating this issue is that induction of autophagy is cell- and toxicant-dependent. Nevertheless, clear correlations exist between nephrotoxicity and autophagy. Autophagic cells are present in vivo in rat renal cells after ischemia-reperfusion and after treatment of mice with tunicamycin, a stimulant of ER Ca^{2+} release.[56] In vitro, autophagy was identified in HK-2 cells after exposure to H_2O_2, in RPTC cultures after exposure to cisplatin, and in primary cultures of human renal cells treated with cyclosporine A.[56,116]

Morphologic markers for autophagy include the presence of autophagic vacuolization of the cytoplasm and the autophagosome, which forms near the lysosome and can contain cytosolic organelles (Figs. 30.2 and 30.3).[55] This occurs in the absence of chromatin condensation. Double-membrane vesicles, autophagosomes, are formed and fuse with lysosomes to facilitate protein degradation[112] and other morphologic changes that are best identified using transmission electron microscopy.

Biochemical markers of autophagy include expression of microtubule-associated protein-1 light chain 3 (LC3), and degradation of the cell signaling adaptor p62.[112] LC3 is only considered an autophagic cell marker when it is cleaved to a lower molecular weight protein called LC3II. Cleavage allows LC3 to bind to phosphatidylethanolamine, which facilitates the formation of autophagosomes. Autophagosome formation is also facilitated by two kinases: autophagy-specific phosphatidylinositol 3-kinase (PI3K) Vps34 (also called human class III PI3K) and target of rapamycin (TOR) kinase.[56,112]

Beclin-1 is another protein whose expression is critical for autophagy. Beclin-1 facilitates formation of autophagosomes by regulating Vps34 (human class III PI3K[56]). It contains a BH-3 only domain, and is inhibited by other BH-3 only domain containing proteins called Bcl-2 and Bcl-X_L.[55] Proteins containing BH3-only domains are typically pro-apoptotic; however, beclin-1 does not induce apoptosis. In fact, beclin-1 is cleaved by caspases. Cleavage of beclin-1 by caspases is believed to be an important switch point used by cells to inhibit autophagy and stimulate apoptosis.[55]

p53, another regulator of autophagy, is a tumor suppressor protein found in the cytosol of living cells in an inactivated state and bound to the co-repressor Mdm2 (see later). The release of p53 from Mdm2 is stimulated by ionization radiation, DNA damage, oxidative stress, and several other death-inducing stimuli. Released p53 can translocate to the nucleus and induce apoptosis, cell cycle alterations, and the transcription of several proteins, including those that mediate autophagy.[55] Interestingly, cytosolic p53 (unbound to Mdm2) appears to inhibit autophagy in nonrenal cells.[117] It is not known if p53 can regulate autophagy in renal cells using similar mechanisms.

INITIATORS OF CELLULAR INJURY

Nephrotoxicants initiate renal cell injury by a variety of mechanisms. Some initiate toxicity directly because of their reactivity with selected cellular macromolecules, such as observed with the antifungal drug amphotericin B, which increases the permeability of the plasma membrane to cations,[118] the mycotoxin fumonisin B_1 that inhibits sphinganine (sphingosine) N-acyltransferase,[119] and aminoglycosides that bind initially to cellular anionic phospholipids.[120] Other nephrotoxicants initiate toxicity following biotransformation to a reactive intermediate or a stable metabolite, and nephrotoxicants can initiate toxicity indirectly through the production of reactive oxygen species.

Role of Biotransformation

Renal xenobiotic metabolism contributes significantly to whole-body metabolism and/or renal toxicity of numerous chemicals because of the role of the kidney as a primary route of xenobiotic excretion. Some chemicals require metabolism or biotransformation to a toxic reactive intermediate for cellular injury to occur (Fig. 30.5). Then the reactive intermediate binds covalently to critical cellular macromolecules, which are thought to interfere with the normal functioning of the macromolecules and thereby initiate cellular injury. Often, these reactive intermediates or "alkylating" agents are electrophiles that bind to cellular nucleophiles. The renal xenobiotic-metabolizing enzymes found in experimental animals and humans have been reviewed by Lock[121] and are summarized in Table 30.1. These include cytochrome P-450, flavin containing monooxygenase (FMO), and glutathione S-transferase (GST).

Cytochrome P-450

The kidney contains many of the xenobiotic-metabolizing enzymes found in the liver; however, in general, their concentration within the kidney is lower. For example, renal cytochrome P-450 ranges between 0.1 and 0.2 nmol per mg microsomal protein across a variety of species, which represents approximately 10% of hepatic cytochrome P-450.[121] The distribution of cytochrome P-450 also varies along the nephron, with the highest levels typically found in the S_2 segment, followed by the S_3 and S_1 segments, with the other tubular segments having less than 10% of that of the S_1 segment.[121]

The renal cytochrome P-450 system is active against a variety of endogenous and exogenous compounds, and numerous cytochrome P-450 isoforms have been identified in renal tissue. For example, cytochromes P-450 1A1, 1A2,1B1, 2A, 2B1, 2B2, 2B6, 2B9, 2B10, 2C2, 2C11, 2E1, 2J2, 2J3, 2J5, 2J9, 3A1, 3A4, 4A1, 4A2, 4A3, 4A5, 4A6, 4A8, 4A11, 4F1, 4F4, 4F5, 4F6, 4F11, and 4F12 have been identified in renal cells of the human, mouse, rat, and rabbit kidney.[121–125]

Cytochrome P-450 expression depends on the species and sex being studied, as well as the site along the nephron. For example, cytochrome P-450 2A, 2C, and 2E are present in male mouse kidneys but are barely detectable in female mouse kidneys.[121] Several studies suggest differences in the expression of cytochrome P-450 isoforms between human and rodent kidneys. An important example is the expression of cytochrome P-450 2E1, which has been detected in renal proximal and distal tubular cells of mice and rats, but not human kidneys.[121,122,126] In contrast, both human and rodent kidneys express high amounts of cytochrome P-450 4A isoforms. However, rat kidneys express 4A1, 4A2, and 4A3, whereas the human kidney appears to express 4A11.[122,127] Such differences in xenobiotic expression must be considered when assessing the role of biotransformation in chemical-induced nephrotoxicity.

In contrast to the liver, fewer compounds are documented to produce nephrotoxicity through renal cytochrome P-450

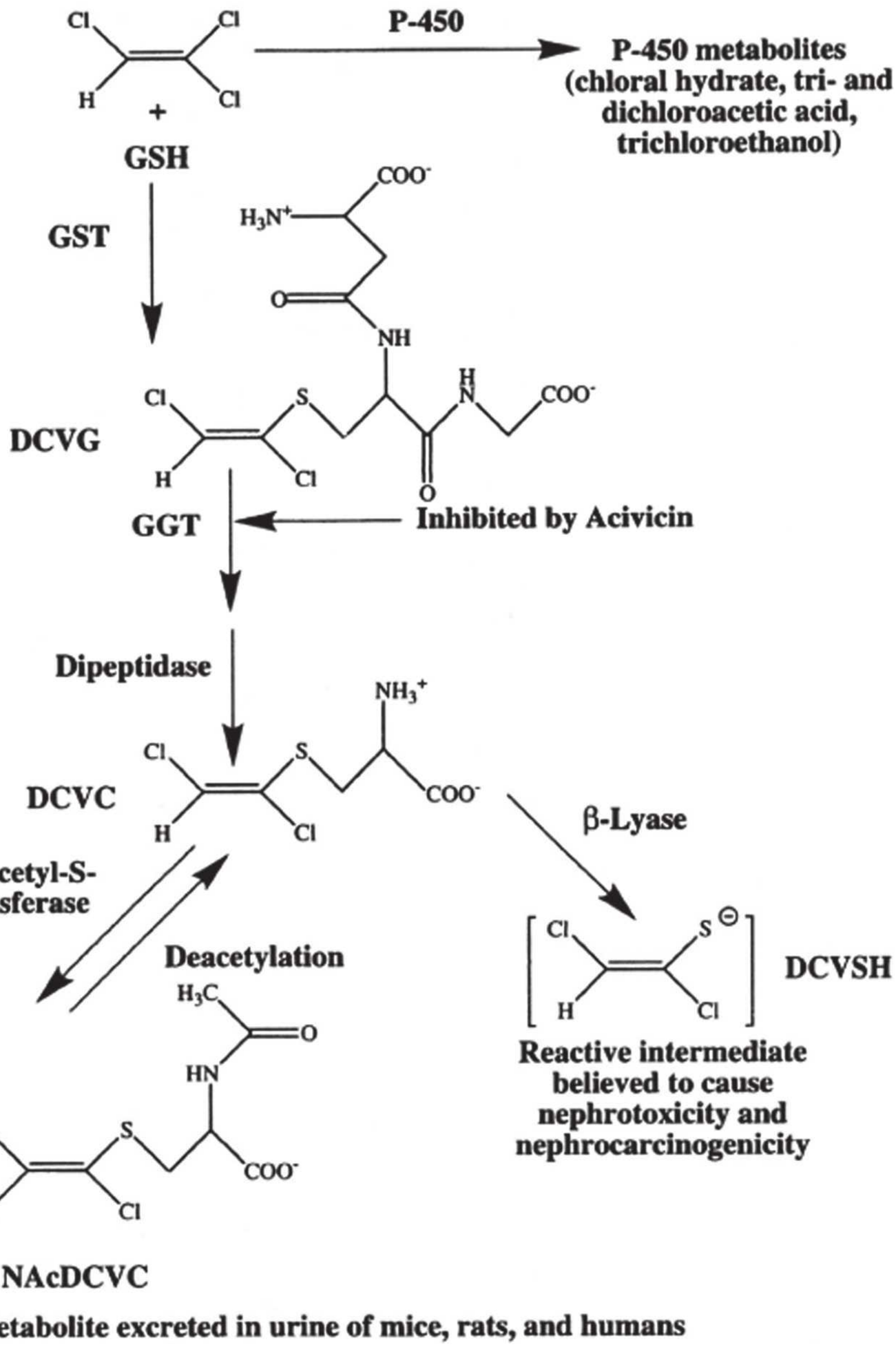

FIGURE 30.5 The bioactivation of trichloroethylene by the glutathione-(GSH-) conjugation pathway. Trichloroethylene (*top left*) can be metabolized by either cytochrome P-450 to the compound listed (*top right*) or be conjugated to GSH by the glutathione S-transferase (GST) to form S-(1,2)-dichlorovinyl-glutathione (DCVG). These reactions can occur either in the liver or in the kidney. DCVG formed in the liver is delivered to the kidney via the bile or the blood where the high concentrations of γ-glutamyltransferase (GGT) and dipeptidase in the kidney results in the cleavage of the GSH moiety and the formation of S-(1,2)-dichlorovinyl-L cysteine (DCVC). Metabolism of DCVC by *N*-acetyl-S-transferase produces *N*-acetyl-S-(1,2)-dichlorovinyl-L-cysteine (NAcDCVC), which is excreted in the urine of mice, rats, and humans exposed to trichloroethylene. NAcDCVC also can be deacetylated back to DCVC. Metabolism of DCVC by cysteine-conjugate β-lyase results in the formation of a reactive thiol that can rearrange to form a protein acylating species. (From Cummings BS, Parker JC, Lash LH. Role of cytochrome P450 and glutathione S-transferase alpha in the metabolism and cytotoxicity of trichloroethylene in rat kidney. *Biochem Pharmacol.* 2000;59:531, with permission.)

bioactivation, although renal cytochrome P-450 contributes to the nephrotoxicity of chloroform[128,129] by metabolizing it to the unstable trichloroethanol, which releases HCl to form phosgene. Phosgene reacts with: (1) two molecules of glutathione to produce diglutathionyl dithiocarbonate, (2) water to produce two molecules of HCl and CO_2, (3) cysteine to produce oxothizolidine-4-carboxylic acid, or (4) cellular macromolecules to initiate toxicity.[128,130,131]

Chloroform bioactivation by renal cytochrome P-450 is sex- and species-dependent. The marked sex difference in the nephrotoxicity of chloroform is reversed by castration of males or treatment of females with testosterone, suggesting that the renal cytochrome P-450 responsible for chloroform bioactivation is under androgenic control.[130,132] Because cytochrome P-450 isozymes 2B1 and 2E1 are present in male mice and are expressed in female mice treated with testosterone, these isozymes may be responsible for renal chloroform bioactivation.[131]

Acetaminophen is metabolized in the mouse kidney by cytochrome P-450 2E1 to the reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQ), which binds to cellular proteins.[132,133] In the liver, NAPQ binds to a selenium binding protein (58 kDa),[134,135] microsomal glutamine synthetase (44 kDa),[136] cytosolic N-10-formyl tetrahydrofolate dehydrogenase (100 kDa),[135,137] and mitochondrial glutamate dehydrogenase (50 kDa).[137] It is possible that similar protein binding may occur in renal cells.

Studies also suggest that acetaminophen mediates renal cell death in mouse RPTC by inducing ER stress.[109] In this model, acetaminophen treatment increased the expression of GADD153, an ER stress protein, and induced caspase-12 cleavage and apoptosis—independently of caspase-3, -9,

30.1 Expression of Selected Xenobiotic Biotransformation Enzymes in the Kidney

Enzyme	Cell Type	Species	References
Cytochrome P450 monooxygenases			
IA	Proximal tubules	Rat, mouse, human	390, 391
IA2	Proximal tubules	—	123
IIB	Proximal tubules	Rat and mouse	122
	Distal tubules	Rat and mouse	122
IIC2	Proximal tubules	—	
IIC9	Unknown[a]	Human but not rat	390
IIC11	Distal tubules	Male rat	122
IID	Proximal tubules	—	125
IIE1	Proximal tubules	Rat, mouse, not human	122, 127, 392
	Distal tubules	—	122
IIJ	Proximal tubules	Human, rat, mouse	123, 393–395
IIIA1	Glomerulus	Rat, mouse, not human	122, 390, 391
IIIA4	Proximal tubules	Human, not rat or mouse	390
IVA2	Proximal tubules	Rat, mouse, not human	122, 390
	Distal tubules	—	122, 390
IVA3	Proximal tubules	Rat, mouse, not human	122, 390
	Distal tubules	—	122, 390
IVA11	Proximal tubules	Human, not rat or mouse	126, 127
IVF	Proximal tubules	Human and mouse	124, 127
	Distal tubules	Mouse	124
Flavin-containing monooxygenases		—	
FMO1	Unknown[a]	Rat, mouse, and human	141, 390
FMO3	Unknown[a]	Rat, mouse, and human	141, 390
FMO5	Unknown[a]	Human	141
Glutathione S-transferases			
GST α	Proximal tubules	Rat, mouse, and human	19, 126, 148
	Distal tubules	—	
GST μ	Proximal tubules	Rat, mouse, not human[b]	19, 126, 148, 396
GST π	Proximal tubules	Rat, mouse, and human	19, 126
GST θ	Proximal tubules	Human	126

[a]Activity and expression have been measured in kidney microsomes only.
[b]GSTμ is expressed in some human kidney malignancies.

or the release of the mitochondrial pro-apoptotic protein Smac/Diablo.

Flavin-containing Monooxygenase

Flavin-containing monooxygenase (FMO) oxidizes the nucleophilic nitrogen, sulfur, and phosphorus moieties of a number of chemicals, including DCVC, tamoxifen, and cimetidine.[121,138,139] The role of FMO in nephrotoxicity has received less attention than cytochrome P-450, but several FMO are expressed in the kidney. Like cytochrome-P450, renal FMO expression and activity is species- and sex-dependent. For example, rabbit kidneys express FMO1, 2, 4, and 5, but not 3, and FMO1 is expressed in the female, but not the male kidney.[121] FMO3 activity is detected in the kidneys of rats, dogs, mice, rabbits, and humans.[140] Rat kidneys appears to have two- to sixfold greater activity levels (as determined by methionine S-oxidase activity) than other species, including humans.[141] Studies in human kidney microsomes demonstrate that FMO1, FMO3, and FMO5 are all expressed, but at different levels.[141] Furthermore, samples from African American patients had significantly more FMO1 activity compared to their Caucasian counterparts, suggesting that the expression of renal FMO isoforms may differ depending on race.[141] Studies in mice

suggest that sex- and age-dependent differences exist for the expression of FMO mRNA in the kidney[142]; however, no differences in the expression of FMO1, FMO3, or FMO5, and overall FMO activity were detected between human male and female kidney microsomes.[141] Thus, more work is needed to determine if FMO expression is sex-dependent in human kidneys.

In vitro, FMO1, FMO3, FMO4, and FM05 metabolize cysteine S-conjugated S-allyl cysteines, whereas FMO3 metabolizes DCVC.[141] However, little DCVC was metabolized in human kidney microsomes, even though FMO3 was expressed in these tissues, suggesting that FMO may not contribute to the nephrotoxicity of this compound in human renal cells. In contrast, treatment of human proximal tubular cells with the FMO inhibitor methimazole decreased DCVC-induced apoptosis.[143] Studies also suggest that FMO catalyzed sulfoxidation of the sevoflurane (a commonly used anesthetic) degradation product fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether mediates its renal toxicity.[144] Other nephrotoxicants suggested to be metabolized by FMO include 4-amino-2,6-dichlorophenol.[145] 4-amino-2,6-dichlorophenol is a metabolite of 3,4-dichloroanaline, a common industrial manufacturing intermediate. Finally, FMO may also mediate the nephrotoxicity of some pesticides such as organophosphate thioether compounds.[146]

Glutathione S-Transferase

The conjugation enzymes glucuronosyltransferases, sulfotransferases, and glutathione S-transferases (GST) are located in the kidney where they conjugate both endogenous and exogenous compounds. These enzymes increase the water solubility, excretion, and elimination of several nephrotoxicants.[121] Although this typically decreases renal cell injury, some nephrotoxicants are bioactivated by these enzymes.

GST mediates the conjugation of the tripeptide glutathione (GSH, γ-glutamylcysteinlyglycine) to compounds with electrophilic centers.[121] They are considered phase II biotransformation enzymes and are divided into cytosolic, membrane associated, and mitochondrial members. There are seven different cytosolic subfamilies (A, alpha; M, mu; P, pi; T, theta; O, omega; S, sigma; and Z, zeta). Microsomal GST is referred to as membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG), whereas mitochondrial GST is called K (kappa) GST.[121,147] All of these GSTs, with exception of GST S, are expressed in rat and human kidneys,[19,121,126,147] and GST expression in normal human RPTC appears to be similar to those observed in rat RPTC.[126]

GST expression can differ among nephron segments. For example, in the rat kidney, GST A is expressed primarily in proximal and distal tubules, whereas GST M and P are primarily expressed in the distal tubules.[19,121,126] These expression patterns differ from a previous study, especially with regard to GST A in distal tubules.[121,148] Thus, more research is needed to resolve this discrepancy.

As mentioned previously, conjugation of toxicants to GSH is normally a detoxification pathway in which electrophiles are neutralized and made more amenable for excretion. Unfortunately, numerous extrarenally formed glutathione conjugates are nephrotoxic. For example, the extrarenal conjugation of GSH is important for the nephrotoxicity of $HgCl_2$,[149] halogenated alkenes, and aromatics, and possibly acetaminophen.[131,150,151] The nephrotoxicity of the halogenated alkene trichloroethylene in rats and humans is believed to be a direct result of its conjugation with GSH to form S-(1,2)-dichlorovinyl-glutathione, and the subsequent processing of the glutathione-conjugate to DCVC in RPTC (Fig. 30.5).[19]

In vivo, trichloroethylene is conjugated with GSH in the liver and delivered via the bile or blood to the kidney. The expression of enzymes, such as γ-glutamyl transferase and dipeptidase in the RPTC and biliary and intestinal tract, results in the cleavage of the γ-glutamyl and glycyl moieties, respectively, and the formation of DCVC. Metabolism of DCVC by N-acetyl-S-transferase produces N-acetyl-S-(1,2)-dichlorovinyl-L-cysteine, which is excreted in the urine of mice, rats, and humans exposed to trichloroethylene.[152] N-acetyl-S-(1,2)-dichlorovinyl-L-cysteine also can be deacetylated back to DCVC. Metabolism of DCVC by cysteine-conjugate β-lyase results in the formation of a reactive thiol that can rearrange to form a protein acylating species. A strong correlation exists between increases in markers of renal injury (proteinuria, creatinine clearance, glucosuria) and GSH metabolites of trichloroethylene in the blood and urine of humans exposed to high amounts of trichloroethylene.[153] Key determinants in the nephrotoxicity of trichloroethylene and similar chemicals, such as sevoflurane, isoflurane, and desflurane, which utilize this common pathway of biotransformation,[144,154] appear to be dependent on γ-glutamyl transferase, dipeptidase, and cysteine-conjugate β-lyase activity found in the kidney.

Role of Reactive Oxygen Species

Reactive oxygen species (ROS) mediate cellular injury during inflammatory responses, ischemia-reperfusion, and after nephrotoxicant exposure. Cellular ROS are generated during the normal function of the mitochondrial and microsomal electron transport chains as a result of the incomplete reduction of O_2 to water (Fig. 30.6).[155] Superoxide anion free radical is produced by a one-electron reduction of O_2, and H_2O_2 is produced by a two-electron reduction of O_2. Superoxide anion can dismutate to form H_2O_2, or H_2O_2 can be formed directly. The hydroxyl radical is formed from H_2O_2, and the superoxide anion free radical is formed via the metal-catalyzed Haber-Weiss reaction or the superoxide-driven Fenton reaction. Ferrous iron (Fe^{2+}) appears to be the major intracellular initiator of the reaction, but cuprous ions may participate as well. The precise source and form (e.g., ferritin) of the ferrous iron is still unclear.

One source of Fe^{2+} may be the heme-moiety that resides in the active site of cytochrome P-450 isoforms.[156]

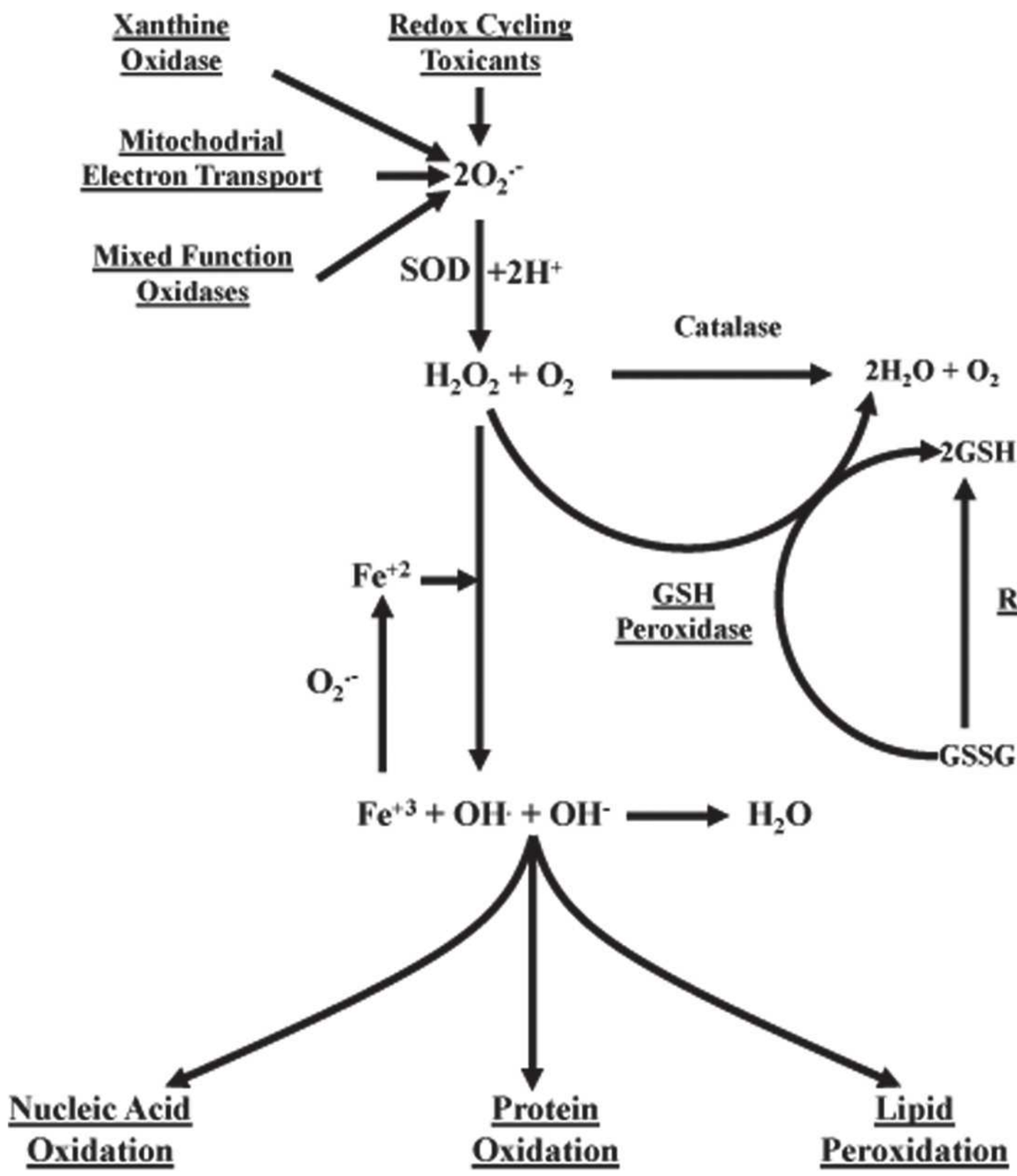

FIGURE 30.6 A schematic representation of the major pathways and possible intracellular targets and oxidants. Detoxification pathways and protective agents are also shown. See text for details. *GSH,* glutathione; *GSSG,* glutathione disulfide; *SOD,* superoxide dismutase.

This hypothesis is supported by the observation that rats treated intraperitoneally with cisplatin for 4 days had significantly lower renal cytochrome P-450 compared to control rats, and the decrease in P-450 correlated with increases in bleomycin-detectable iron content in the kidney. Piperonyl butoxide (a cytochrome P-450 inhibitor) decreased cisplatin-induced release of iron in the kidney and the functional and morphologic markers of kidney toxicity.[157] These same effects were observed in LLC-PK_1 cells; thus, P-450 may serve as one source of Fe^{2+} to initiate the formation of ROS.

Superoxide anion acts as a reductant for Fe^{3+}, and the Fe^{2+} generated reduces H_2O_2 to the hydroxyl radical, which reacts rapidly with adjacent molecules. Superoxide anion and H_2O_2 are less reactive, and H_2O_2 may diffuse away from the initial site of formation to produce injury at a distant site within the cell. Although H_2O_2 readily crosses cell membranes, superoxide anion and hydroxyl radicals do not. Because ROS production is a natural byproduct of metabolically active cells, such as the kidney, significant defenses exist against the normal production of ROS or those produced under pathologic conditions (Fig. 30.6).

The term "oxidative stress" is commonly used to describe conditions that lead to increased ROS formation. Chemicals may initiate oxidative stress indirectly by augmenting ROS production. For example, Walker and Shah[158] showed that gentamicin enhances H_2O_2 generation in isolated rat renal cortical mitochondria, and Lund and associates[159] showed that mitochondria isolated from rats treated with $HgCl_2$ have elevated H_2O_2 production.

Another mechanism by which chemicals produce oxidative stress is through "redox cycling." Certain compounds, especially quinones, can undergo a one-electron reduction to a semiquinone radical and a second one-electron reduction to the hydroquinone. The hydroquinone is oxidized to the quinone, and the cycle begins again, hence the term "redox cycling." During the reduction process, superoxide anion is formed from O_2, and oxidative stress ensues. For example, Brown and colleagues[160] demonstrated that menadione (2-methyl-1,4-naphthoquinone) produces toxicity in isolated rat renal epithelial cells through its ability to undergo redox cycling and cause oxidative stress. It should be recognized that the ability of quinones to undergo redox cycling varies with the quinone, and some quinones produce toxicity through their ability to arylate cellular macromolecules, particularly protein sulfhydryls.[160,161]

ROS can induce lipid peroxidation, inactivate enzymes by directly oxidizing protein sulfhydryl or amino groups, depolymerize polysaccharides, and induce DNA strand breaks. Lipid peroxidation results from the interaction of free radicals with polyunsaturated fatty acid side chains of membrane phospholipids to form free radicals and relatively stable lipid hydroperoxides.[162] Transition metals can catalyze the decomposition of lipid hydroperoxides, which results in the formation of alkoxyl and peroxyl free radicals that propagate the reaction. Lipid breakdown products such as hydroxylated fatty acids, 2-alkenyls, and 4-hydroxyalkenyls, are toxic, and may contribute to organelle and cellular dysfunction. Thus, ROS-induced degradation of membrane lipids

decrease cellular membrane integrity, alter enzymatic activity and transport properties, and induce isotropy.[163] The oxidation of protein sulfhydryl and amino groups by ROS can alter enzyme activity and membrane structure and function to the point that cell death will ensue. Finally, ROS can produce DNA strand breaks. Although H_2O_2 does not directly damage DNA, its stability and ability to diffuse throughout the cell allows it to come into contact with metal-based enzymes, like cytochrome P-450, which catalyze the formation of hydroxyl radicals, which can damage DNA.

A variety of structurally diverse nephrotoxicants produce renal cell injury by mechanisms involving oxidative stress, including $HgCl_2$,[159,162] haloalkene cysteine conjugates,[164,165] cyclosporine A,[165] and cisplatin.[76,166,167] The diversity of these nephrotoxicants highlights the critical and common roles that ROS play in the mechanism of renal cell death.

MEDIATORS OF CELL INJURY

Numerous common cellular pathways that mediate cell death have been identified. It is generally thought that upon initial exposure, nephrotoxicants will activate at least one, if not more, of these pathways. For necrosis, apoptosis, and possibly autophagy, a point exists along the sequence, yet to be identified, referred to as the point of no return. Here, the cell will die irrespective of any intervention. Also, along this sequence are switch points at which the cell death pathways may change from one mechanism to another. Investigators have tried to identify the sequence of deleterious events, the point of no return, and switch points for years and these efforts have led to the identification of numerous intracellular mediators critical in the generation of renal cell injury and death.

p53 and p21

The tumor suppressor protein p53 and the cell cycle inhibitor protein p21 can mediate renal cell death and acute renal failure.[76,168,169] Activation of p53 typically induces cell death and cell cycle arrest.[76,169] Activation of p21 is protective against numerous types of nephrotoxic events, including ischemia and cisplatin exposure.[168–171] p53 can induce p21 during renal cell injury, but p21 can also be activated independently of p53.[172] The mechanisms of p53-meidated activation of p21 are under study, but may involve increases in transcription. Transcription and signaling kinases are believed to mediate mechanisms by which p21 expression is increased independently of p53.[168,173]

p53 is activated in renal cells by agents that that induce DNA damage, like cisplatin,[172,174] and it is activated in renal cells after ischemia and oxidative stress subsequent to exposure to bromate, Fas, antimycin A, histone deacytelylase (HDAC) inhibitors, and aristolochic acid.[175,176] The mechanism of p53 activation involves its phosphorylation at numerous serine residues, followed by its release from the regulator protein Mdm2. Other regulators of p53 include ataxia telangiectasia mutated kinase (ATM), ataxia telangiectasia and Rad-3 related kinase (ATR), checkpoint kinase (ChK), and NF-kB.[169]

After activation, p53 can translocate to the nucleus or the mitochondria, or remain in the cytosol. In the nucleus, p53 induces the transcription of a number of genes, including p21,[172] and can activate several apoptotic pathways such as those involving caspases.[75] Translocation of p53 to the mitochondria results in its interaction with the outer mitochondrial membrane and binding with the anti-apoptotic proteins Bcl-2 and Bcl-xL.[169] This interaction releases the pro-apoptotic proteins Bax and Bak, which induce mitochondrial pore formation in mitochondria and facilitate the release of the pro-apoptotic proteins cytochrome c, Omi, Smac/Diablo, and Endo G.[55] Both nuclear and cytosolic p53 may regulate autophagy, and studies suggest that p53 localization represents a key switch point between autophagy and apoptosis.[55]

Pharmacologic and molecular studies demonstrate that inhibition of p53 decreases cisplatin-induced apoptosis in rabbit RPTC through a mechanism that includes caspase inhibition of.[75,76,169] In support of this hypothesis, others have demonstrated that inhibition of p53 nuclear translocation inhibits AKI and renal cell death in vivo after renal ischemia in rats.[177] Of note, p53 inhibition in these studies did not totally inhibit cell death; thus, quite possibly, p53-independent mechanisms contribute to nephrotoxicity induced by cisplatin, oxidative stress, ischemia, and other DNA damaging agents.

Induction of p21 occurs in response to DNA damage and p53-induced cell cycle arrest.[170] p21 is also activated independently of p53 by transcriptional-mediated mechanisms and by mechanisms involving mitogen activated protein kinases (MAPK).[168,173] p21 is a cyclin-dependent kinase (cdk) inhibitor that interacts with numerous cdk, such as cdk2, to control cell cycle.[168,170,178] Activation of p21 decreases renal cell death induced by cisplatin and ischemia reperfusion.[168] The mechanism of protection is linked to its ability to alter the cell cycle and allow for cell repair. In support of this hypothesis, knockout mice lacking p21 exhibited increased renal cell cycle activity and apoptosis, and were more susceptible to cisplatin and ischemia-induced ARF compared to wild type controls.[170] The majority of studies with p21 and renal cell death have focused on cisplatin and ischemia reperfusion. More work is needed to determine if p21 can mediate other forms of chemical-induced nephrotoxicity.

Signaling Kinases

Signaling kinases alter the activity, expression, or localization of another protein by altering its phosphorylation, including other signaling kinases. Signaling kinases differ in terms of the amino acids targeted for phosphorylation (serine/threonine/tyrosine), the location within a cell (membrane-bound or cytosolic), and the protein targeted for phosphorylation.

Table 30.2 lists several signaling kinases identified in the kidney, the site within the kidney or cell involved, the nephrotoxicant involved, and several references to studies that suggest critical roles for signaling kinases both in the development of renal cell death and in the recovery of renal cells after toxicant-induced injury.

Protein Kinase C

Protein kinase C (PKC) is a family of serine/threonine kinases, and at least 12 different mammalian isoforms have been identified. These are divided into conventional PKC (cPKC: α, $\beta_{1/2}$, and γ), novel PKC (nPKC: ϵ, ϵ', δ, η, θ, and μ), and atypical PKC (aPKC: τ, λ, and ζ).[179] The isoforms differ in terms of preferred substrates and mechanisms of action. Activation of cPKC is Ca^{2+}- and diacylglycerol-dependent, whereas activation of nPKC is Ca^{2+}-independent.[180] In contrast, activation of aPKC is independent of both Ca^{2+} and diacylglycerol. RPTC have been reported to express α, β_1, β_2, ζ, δ, λ, and ϵ,[181,182] and several other isoforms are expressed in the kidney of rats, mice, and humans.[183]

PKC is reported to mediate the toxicity of cisplatin, ischemia, oxidants, and TNF-α in multiple renal cell models.[184–189] The exact role of PKC in renal cell death depends on the toxicant and the specific isoform(s) involved.[190,191] For example, activation of PKC-α in rabbit RPTC during cisplatin treatment results in mitochondrial dysfunction and cell death,[189] and similar findings have been reported for PKC-ϵ in RPTC after oxidative stress.[192] Interestingly, PKC-ϵ targeted several mitochondrial proteins including complexes I and IV, and F(0)F(1)-ATPase in RPTC.[192] In contrast, activation of PKC-ζ after exposure to t-butylhdyroperoxide mediates cellular repair.[190,191]

MAPK (ERK1/2, p38 and JNK)

MAPK are serine-threonine kinases activated by a cascade of protein-protein interactions. They mediate cell growth, adhesion, differentiation, gene expression, and apoptosis. They can also mediate activation of p53.[193,194] ERK1/2 (p42/44MAPK), p38 MAPK, and c-jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) are three of the

30.2 Selected Signaling Kinases Involved in Renal Cell Injury, Survival, or Repair

Kinase	Location	Nephrotoxicant	Reference
Protein Kinase C (PKC)			
Conventional PKC			
PKCα	Proximal tubules	Cisplatin	189, 397
	DCVC[a]		
Novel PKC			
PKCϵ	Proximal tubules	Oxidative injury	192
Atypical PKC			
PKCζ	Proximal tubules	t-Butylhydroperoxide	191
Mitogen Activated Protein Kinase (MAPK)			
ERK1/2	Proximal tubules	Cisplatin	189, 198, 205,
		H_2O_2	208, 398
		TGHQ[c]	
JNK/SAPK[a]	Proximal tubules	Cisplatin	205
P38	Proximal tubules	Cisplatin	198, 205, 208
		H_2O_2	
		TGHG[c]	
Other Kinases			
Protein kinase B[b]	LLC-PK1	Cisplatin	398, 399
	Proximal tubules	Mechanical injury	
		H_2O_2	
Phosphoinositide-3-kinase	LLC-PK1	Cisplatin	399
	Proximal tubules	Mechanical injury	

[a]S-(1,2)-dichlorovinyl-L-cysteine.
[b]Also known as AKT.
[c]2,3,5-tris-(glutathion-s-yl)hydroquinone.

most studied MAPK. These kinases are activated by additional kinases: MAPK/extracellular signal-regulated kinase or MEK.[195]

ERK1/2, also referred to as extracellular regulated kinases,[196] are activated through epidermal growth factor receptor (EGFR), which is mediated by a small G-protein Ras.[197,198] Studies show that ERK1/2 is activated in renal cells after exposure to cisplatin, oxidants, bromate, and aminoglycosides.[115,189,198,199] ERK1/2 activation in these studies is reported to be protective, which correlates with the role of this kinase in survival and proliferation.[197,200,201] The ability of ERK1/2 to act as prosurvival signals is partially due to their ability to translocate to the nucleus and activate transcription factors such as AP-1, Elk-1, and c-Myc.[202] In addition, ERK1/2 can phosphorylate and inhibit caspase-9, which can inhibit apoptosis.[69]

The role or ERK1/2 in renal cell injury is toxicant- and cell-dependent, and some studies suggest that ERK1/2 can mediate apoptosis.[203,204] For example, Arany et al.[205] demonstrated that ERK activation mediated cisplatin-induced renal cell death in vivo and in vitro in mouse models. However, the same investigators observed that ERK activation protected against oxidant-induced (H_2O_2) cell death.[205] Interestingly, cisplatin-induced ERK activation and renal cell death was dependent on EGFR and c-Src activation. Studies by Zhuang and colleagues[206,207] also showed that exposure of RPTC to H_2O_2 activates Src, EGFR, and ERK1/2, as well as protein kinase B (Akt) and phosphoinositide-3-kinase (PI3K). ERK1/2 activation in this model was proposed to mediate apoptosis by activating caspase-3.[207] EGFR-induced activation of ERK1/2 is also believed to mediate 2,3,5-tris-(glutathione-S-yl)hydroquinone (TGHQ)-induced death in LLC-PK1 cells.[198,208] Thus, activation of ERK1/2 in renal cells after toxicant exposure may not always result in survival.

p38 MAPK and JNK/SAPK are activated in response to cellular stress, inflammation irradiation, heat shock, ROS, LPS, TNF-α, and IL-1.[209–211] These proteins are activated by Rac, a small G protein that activates distinct MEK rather than those involved in ERK1/2 activation.[210] p38 and JNK are generally thought to mediate cell death and cytostasis; however, like ERK1/2, the role of p38 and JNK in renal cell injury is toxicant- and cell-dependent. For example, activation of p38 by nephrotoxicants such as cisplatin and bromate in renal cell lines activates p53 and induces cell death.[169,199] In contrast, activation of p38 in primary cultures of RPTC after oxidant induced injury mediates dedifferentiation.[206] The detailed mechanisms of Src, EGF-R, PI3K, ERK1/2, and p38/SAPK activation, the targets of these kinases, and their role in cell death produced by diverse toxicants remain to be determined.

Altered Calcium Homeostasis

The role of Ca^{2+} in the pathophysiology of nephrotoxic cell injury cannot be understated. Intracellular Ca^{2+} homeostasis is necessary for cell viability because Ca^{2+} is a second messenger that plays a critical role in a variety of cellular functions.[55,212] With regard to cell death Ca^{2+} can mediate necrosis, apoptosis, and autophagy.[55,112] Initially, it was thought that only high, supraphysiologic increases in Ca^{2+} would induce cell death; however, it is now accepted that even small changes in Ca^{2+} signaling can significantly affect cell death, especially with regard to apoptosis.[55,213]

Ca^{2+} interacts with many key organelles known to participate in cell death, including the ER and the mitochondria. Ca^{2+} also mediates the activation of several proteins involved in cell death, such as calpains, endonuclease, phospholipase A_2, and protein kinases. Recent studies suggest that interaction of Ca^{2+} with calpains mediates activation of the pro-apoptotic protein AIF.[102] There is also a well-documented requirement of Ca^{2+} for autophagy[112] that directly links the release of ER Ca^{2+} stores and activation of AMP activated proteins kinases and calcium/calmodulin kinase β.[112] The ability of Ca^{2+} to stimulate autophagy in response to nephrotoxicant-induced renal cell exposure has not been heavily studied.

Cytosolic free Ca^{2+} is ~100 nM and is tightly regulated in the face of a large extracellular-intracellular gradient (10,000:1) by a series of pumps and channels located on the plasma membrane and ER. Mitochondria were not originally thought to participate in Ca^{2+}-mediated cell signaling processes under normal conditions. The advent of more advanced techniques for measuring low Ca^{2+} has changed this view, and strong evidence supports the idea that mitochondria are integral to Ca^{2+} signaling under both normal and pathologic conditions.[55]

Increases in intracellular/cytosolic free Ca^{2+} can induce cell injury and death after nephrotoxicant exposures. The source of this Ca^{2+} is typically the ER or the extracellular space (i.e., from the plasma membrane). Release of Ca^{2+} from the ER increases cytosolic free Ca^{2+} from ~100 nM to 300 nM within seconds.[214] Such increases are typically transient and buffered by transport of Ca^{2+} back into the ER, to the extracellular space or into the mitochondria. As mentioned above, release of ER Ca^{2+} can induce autophagy, and buffering of ER Ca^{2+} release by the mitochondria can induce apoptosis. Entry of Ca^{2+} from the extracellular space can increase the cytosolic free Ca^{2+} to μM levels—and even mM levels—if membrane integrity is lost. Such increases typically induce necrosis.

Decreasing the extracellular Ca^{2+} concentration or blocking extracellular Ca^{2+} influx will decrease cell death.[215–217] For example, increases in cytosolic free Ca^{2+} were observed in a hypoxia model using rat RPTC and in a mitochondrial inhibitor model using rabbit RPTC, and chelating intracellular Ca^{2+}, or decreasing the influx of extracellular Ca^{2+}, decreased cell death.[216–219] Nephrotoxicants that increase cytosolic free Ca^{2+} include $HgCl_2$,[220,221] pentachlorobutadienyl-glutathione,[167] pentachlorobutadienyl-L-cysteine,[222] tetrafluoroethyl-L-cysteine,[223] DCVC,[224–226] oxidants,[227,228] sevoflurane, miconazole,[229,230] cyclosporine A,[231] and gentamicin.[158]

Ca^{2+} is proposed to mediate apoptosis by both direct and indirect methods.[213] The direct pathway involves Ca^{2+}-mediated activation of calpains, which can induce caspases and.[55,232] Apoptosis may ensue, provided that ATP is maintained above 20% to 30%.[82] Furthermore, as mentioned above, the mitochondria can buffer ER Ca^{2+} release. Transport of Ca^{2+} into the mitochondria can activate Ca^{2+}-mediated matrix dehydrogenases, which can stimulate ATP and ROS production. Furthermore, Ca^{2+} uptake into the mitochondria can result in permeabilization of the outer mitochondrial membrane, which leads to opening of the permeability transition pore and release of pro-apoptotic proteins such as cytochrome c and AIF.[55,233,234]

The indirect pathway of Ca^{2+}-mediated apoptosis involves the activation of the phosphatase calcineurin,[213] which results in activation of the nuclear factor protein of activated T-cells (NFAT), which then increases the expression of pro-apoptotic Fas and TRAIL. Calcineurin activation may be mediated by calpain, which cleaves an endogenous inhibitor of calcineurin called cain/cabin 1.[235] The contribution of calcineurin to nephrotoxicant-induced epithelial renal cell injury has not received much attention; however, calcineurin inhibitors used during transplants surgeries induce significant renal proximal tubule injury,[236] suggesting that calcineurin acts to protect renal cells from damage.

Proteinases

Nonphysiologic activation of proteinases in the cytosol, organelles, or membranes can disrupt the normal function of these structures, leading to cell death and ARF. Proteases known to mediate nephrotoxic-induced renal cell injury include those found in the lysosomes (serine and cysteine proteases), along with calpains and caspases.

Lysosomal Proteases

Proteases found in lysosomes include serine and cysteine proteases that are acidic hydrolases. These require a lower pH to facilitate degradation (~pH 5) and thus will not typically function if released into the cytosol. They can mediate cell death during extreme cases of hypoxia and ischemia reperfusion, which induce lysosomal rupture in correlation with acidosis.[237] In contrast, less evidence exists that these proteases mediate nephrotoxicant-induced cell death.[238] In support of this hypothesis, studies with cysteine and serine proteinase inhibitors revealed these compounds to be ineffective in protecting rabbit RPTC segments from antimycin A, tetrafluoroethyl-L-cysteine, bromohydroquinone, and t-butylhydroperoxide.[239] Several inhibitors of lysosomal proteases, such as the cysteine proteinase inhibitor t-trans-epoxysuccinyl-leucylamido(4-guandino)butane (E64), have been shown to protect against RPTC injury induced by cyclosporine A[240]; however, E64 was only slightly protective, as was an aspartic acid proteinase inhibitor. These results suggest that lysosomal cysteine and aspartic acid proteinases do not play a significant role in RPTC death produced by these nephrotoxicants.

Calpains

Calpains, a group of least 15 isoforms of Ca^{2+}-activated neutral cysteine proteinases, comprise two groups. Group 1 calpains, typical calpains, contain a Ca^{2+}-binding domain, and include calpains 1, 2, 3, 8, 9, 11, 12, and 14.[241,242] Group 2, atypical calpains, lack a Ca^{2+}-binding domain, and include calpains 5, 6, 7, 10, 13, and 15.[241,242] Calpains 1, 2, 5 7, 10, 13, and 15 are reported to be expressed ubiquitously in the cytosol, whereas calpains 3, 6, 8, 9, 11, and 12 have more select tissue expression.[243]

With regard to renal cell death, the most heavily studied calpains are 1, 2, and 10, which have been implicated in cell death induced by numerous toxicants including bromohydroquinone, antimycin A, tetrafluoroethyl-L-cysteine, and t-butylhydroperoxide.[216,218,244,245] Evidence for roles of calpain in cell death in many of these studies was derived from the use of calpain inhibitors such as calpeptin. Calpeptin also inhibited increases in calpain activity induced by hypoxia in RPTC, and protected against renal dysfunction in rats subjected to ischemia reperfusion.[246,247] These studies suggest that calpains mediate cell death by enhancing extracellular Ca^{2+} influx and/or by cleaving cytoskeletal proteins.[218,241,244] Thus, calpains may play a critical role in cell death produced by a wide range of nephrotoxicants and renal dysfunction; however, calpains are also suggested to play a role in the pathology of several other diseases, including Alzheimer, Duchenne muscular dystrophy, and diabetes.[243]

Historically, calpains were thought to be primarily cytosolic, but several membrane-associated calpains are known to exist, including calpain 10. Calpain 10 is expressed in the mitochondrial fraction of the kidney of multiple species, and it appears to mediate mitochondrial dysfunction induced by oxidants, Ca^{2+} overload, and thapsigargin.[248,249] Calpain 10 appears to induce mitochondrial dysfunction by cleaving complex I of the electron transport chain.[250] Recently, calpain 10 has been suggested to mediate renal cell viability and aging in the kidney in vivo,[251] and mitochondrial biogenesis in the kidney through regulation of the peroxisomal proliferator activator receptor γ coactivator 1-α (PGC-1α,[252] see later).

Calpains are key mediators of apoptosis and necrosis, inhibiting apoptosis and inducing necrosis by cleaving and deactivating caspases, including caspases-3, -7, -8, and 9.[253,254] Originally it was thought that calpains only inhibited apoptosis; however, it is now accepted that calpains can actually participate in apoptosis signaling cascades.[55,234] The role of calpains in necrosis or apoptosis appears to be cell type–dependent, and it is unclear whether calpains can also mediate apoptosis, or autophagy for that matter, in renal cells exposed to nephrotoxicants.

Caspases

Caspases are cysteine proteases. The role of caspase activation in renal cell apoptosis has been discussed previously, but it is important to note that caspase activity is not always

needed for apoptosis. For example, caspase-2-directed permeabilization of the mitochondrial membrane results in the release of the pro-apoptotic proteins cytochrome c and Omi, which occurs in the absence of any caspase-2 catalytic activity. Similar results are seen with caspase-2 mediated release of AIF.[234,255–257] Our work demonstrated that apoptosis induced by four diverse toxicants (cisplatin, vincristine, staurosporine, and A23187) proceeded in rabbit RPTC in the presence of caspase inhibitors and the absence of caspase-3, -8, and -9 activity.[258] The mechanisms involved in caspase-independent apoptosis in renal cells appear to involve the activation of DNAases such as Endo G and AIF.[69] This is supported by studies in renal cells showing that cisplatin-induced apoptosis is mediated by Endo G as opposed to caspases.[259,260]

Phospholipase A_2

Phospholipase A_2 (PLA_2) cleaves glycerophospholipids at the sn-2 ester bond, releasing fatty acids and lysophospholipids.[261] They are classified into five different families: secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), platelet-activation factor (PAF) acetylhydrolases, lysosomal PLA_2, and calcium-independent PLA_2 ($iPLA_2$).[261,262] These proteins have different substrate preferences, Ca^{2+}-dependencies, and biochemical characteristics. Isoforms representing $cPLA_2$, $sPLA_2$, and $iPLA_2$ are all expressed in human, rat, rabbit, and mouse kidneys.[263–269]

PAF acetylhydrolase is expressed in microsomes and cytosol in rat and human kidneys,[270,271] but information about its expression in renal epithelial cells is lacking. Lysosomal PLA_2 is Ca^{2+}-independent and is an acidic hydrolases identical to peroxiredoxin 6.[272,273] Few studies exists assessing lysosomal PLA_2 expression in the kidney, but studies of peroxiredoxin 6 indicate that it is expressed in the proximal and distal tubules of rat kidneys.[274] The roles of PAF acetylhydrolase and lysosomal PLA_2 in nephrotoxicant-induced cellular injury are not well understood and deserve further study.

PLA_2 contribute to the mechanisms of cell injury and death by metabolizing glycerophospholipids and releasing fatty acids. The high concentrations of polyunsaturated fatty acids in plasma and organelle membranes make them prime targets for oxidants. Increase in lipid peroxidation facilitates PLA_2 activation and the production of fatty acids and lysophospholipids. This further enhances PLA_2 activity, which enhances phospholipid degradation to the point that membrane integrity is lost and intracellular Ca^{2+} increases which facilitates cell swelling and the activation of $sPLA_2$.

Recently, roles for $cPLA_2$ and $iPLA_2$ in nephrotoxicity have received much attention. Data from these studies demonstrate that PLA_2 mediates nephrotoxicant-induced cell injury in a cell- and toxicant-dependent manner. $cPLA_2$ or $iPLA_2$ is reported to mediate nephrotoxicity induced by oxalate, cisplatin, and isoflurane, as well as oxidants such as H_2O_2, t-butylhydroperoxide, and menadione.[174,267,275,276] $cPLA_2$ and $iPLA_2$ also mediate cell death induced by Fas and TNF-α, hypoxia, anoxia, and ischemia/reperfusion.[72,67,277–280]

PLA_2 are both initiators and executioners of nephrotoxicant-induced cellular death. They can act as executioners when they are activated after initial increases in cytosolic Ca^{2+}. This is especially true for $sPLA_2$, which is activated by mM concentrations of Ca^{2+}, which typically occur after loss of membrane integrity.[281,282] $cPLA_2$ is not directly activated by Ca^{2+}, but μM increases in Ca^{2+} facilitate translocation of $cPLA_2$ to membranes, which increases its activity.[281,283] Increase in Ca^{2+} also induce signaling kinases, such as ERK, that activate $cPLA_2$ and $iPLA_2$.[284–286] The metabolites of PLA_2 (fatty acids and lysophospholipids) can induce cell death by causing inflammation, mitochondrial dysfunction, and lipid peroxidation.[174,267,277]

PLA_2 can also initiate nephrotoxicant cell injury independently of Ca^{2+}. This is more the case for $iPLA_2$ than $cPLA_2$ or $sPLA_2$. $iPLA_2$ mediates cisplatin- and oxidant-induced nephrotoxicity in primary cultures of RPTC and several other renal cell lines.[174,267,287] The mechanisms involved also include cleavage of phospholipids and release of lipid mediators, except Ca^{2+} is not involved in the initial phases of injury.[277,287]

Most of our knowledge about the role of $iPLA_2$ in nephrotoxicant-induced cell injury is derived from studies using pharmacologic inhibitors, such as bromoenol lactone.[262] Studies using small interfering RNA also demonstrate that inhibition of $iPLA_2$ isoforms (groups VIA and B, commonly called $iPLA_2\beta$ and γ) induce cytostasis, apoptosis, and mitochondrial dysfunction in renal cell lines and primary cultures of RPTC.[277,288,289] These pathologies have been directly linked to changes in phospholipid metabolism and release of fatty acids.[277,290] Genetically modified mice lacking specific forms of $iPLA_2$ do exist,[291,292] but few studies have used these models to address roles of $iPLA_2$ in nephrotoxicant-induced cell injury.

Similar to $iPLA_2$, much of the knowledge about the role of $cPLA_2$ in nephrotoxicant-induced cell injury is derived from studies using inhibitors such as arachidonyl trifluoromethyl ketone or methyl arachidonyl fluorophosphonate.[262] Alteration of $cPLA_2$ activity using arachidonyl trifluoromethyl ketone alters oxidant-induced renal cell necrosis in multiple renal cell models.[275,293] Genetically altered mice lacking specific forms of $cPLA_2$ (group IV PLA_2 or $cPLA_2\alpha$) do exist, and suggest that these mice experience some alteration in kidney function.[294,295] Few studies exist using knockout mouse models to study the role of $cPLA_2$ in nephrotoxicant-induced cell death.

Compared to $iPLA_2$ and $cPLA_2$, less is known about the role of $sPLA_2$ in nephrotoxicity. Activation of $sPLA_2$ increased cell death in renal carcinoma cells in correlation with membrane hydrolysis,[296] and mediated cell death induced by IL-1-stimulated release of fatty acids, such as arachidonic acid, in HEK293 cells.[297] Thus, $sPLA_2$ may mediate renal cell injury after inflammation.[298,299] One role for $sPLA_2$ in nephrotoxicity is regulation of other PLA_2. This hypothesis is supported by studies in LLC-PK1 cells exposed to the oxidants H_2O_2 and menadione.[293] These studies suggest that

PLA_2 cross-talk can mediate certain types of nephrotoxicant-induced renal cell injury.

MITOCHONDRIA, ENDOPLASMIC RETICULUM, LYSOSOMES, AND THE CELL MEMBRANE

Mitochondria, ER, lysosomes, and the cell membrane all play roles in nephrotoxic cell injury as well as play roles in necrosis, apoptosis, and autophagy. As mentioned previously, these types of cell death do not proceed through mutually exclusive pathways consisting of single-event sequences. The interplay among necrosis, apoptosis, and autophagy is often mediated by cellular organelles, and key biochemical mediators of switches among necrosis, apoptosis, and autophagy include Ca^{2+} and ATP. These mediators are often controlled by the function of specific organelles, such as the ER (Ca^{2+}), the cell membrane (Ca^{2+}), and the mitochondria (ATP). In addition, lysosomes are critical mediators of autophagy.

Because of the presence of multiple cell death pathways and multiple targets, inhibition of one pathway may not block nephrotoxicant-induced cell death, but rather switch it from one type to another. This is often accompanied by an alteration in organelle function. For example, if a treatment blocks oxidative stress associated with DCVC, pentachlorobutadienyl-L-cysteine, or tetrafluoroethyl-L-cysteine exposure to RPTC, the rate of cell death is diminished, but the cells eventually die due to mitochondrial dysfunction (Fig. 30.7).[300] Thus, a given chemical can cause cell death by interacting with numerous organelles, and blocking interaction at one organelle may not decrease cell death. Rather it

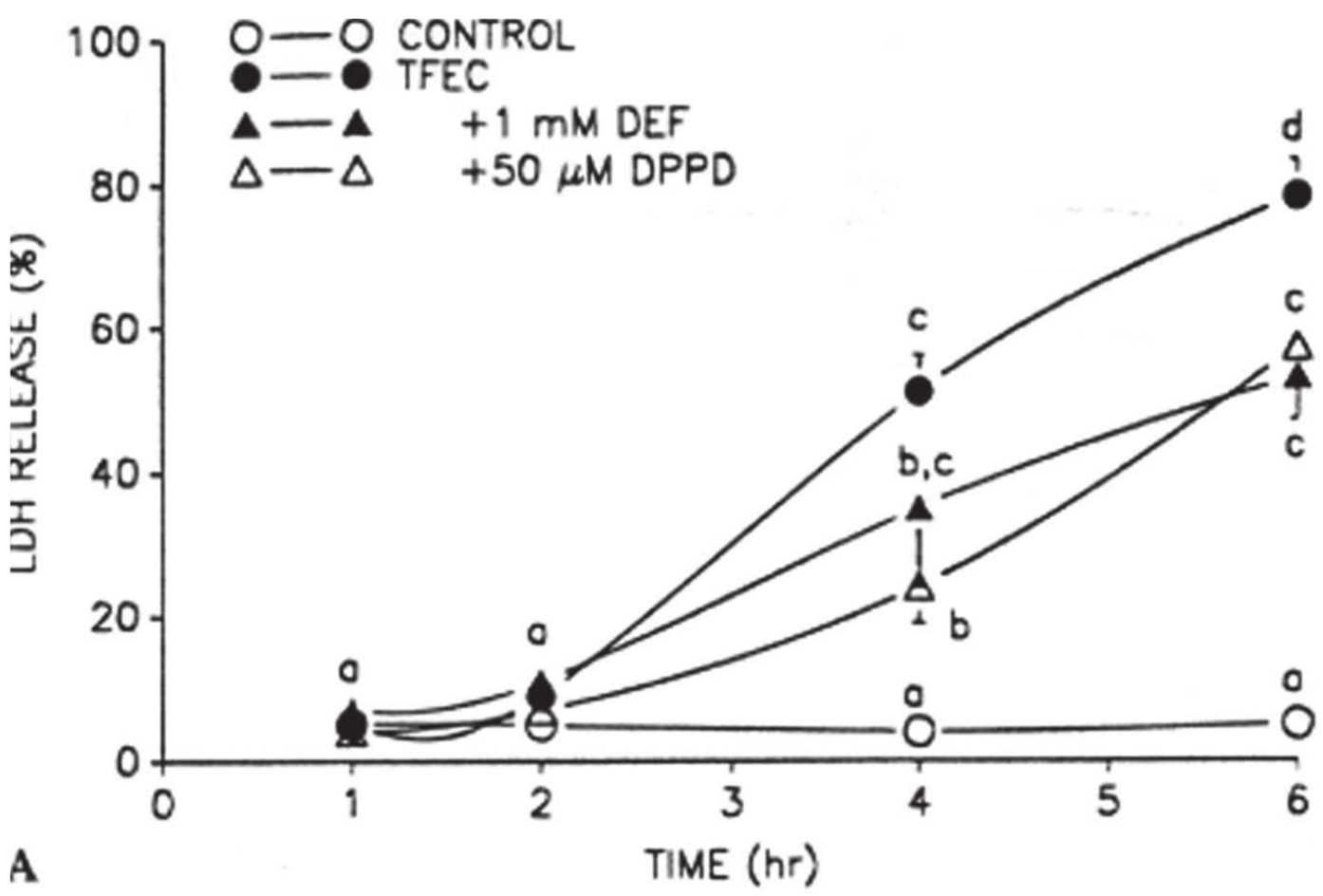

FIGURE 30.7 A: The time-dependent effects of deferoxamine (DEF) and N,N′-diphenyl-1,4-phenylenediamine (DPPD) on S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC)-induced cell death (lactate dehydrogenase [LDH] release) from rabbit renal proximal tubules. DEF and DPPD were added at the same time as TFEC (25 μM). Values are means ± SEM. Values at a given time point or within a given treatment with different superscripts are significantly different from one another ($P \leq 0.05$). **B:** The time-dependent effects of DEF and DPPD on TFEC-induced lipid peroxidation (malondialdehyde [MDA] formation) in rabbit renal proximal tubule suspensions. Values are means ± SEM. Only the TFEC alone is significantly different from controls ($P \leq 0.05$). (Adapted from Groves CE, Lock EA, Schnellmann RG. Role of lipid peroxidation in renal proximal tubule cell death induced by haloalkene cysteine conjugates. *Toxicol Appl Pharmacol.* 1991;107:54, with permission.)

may alter the organelle targeted, which can switch the mechanism of cell death.

Mitochondria

The renal tubular reabsorption of solutes and water requires a large expenditure of energy. Although ATP is generated by both oxidative phosphorylation and glycolysis, ~95% of renal ATP is formed by oxidative phosphorylation.[301] The amount of oxidative phosphorylation that occurs within a given cell varies along the nephron. Thus, toxicants that interfere with mitochondrial function and anoxia will produce cell injury and death, particularly in tubular cells that have limited glycolytic capabilities, such as the S_1 and S_2 segments of the proximal tubules.

Many nephrotoxicants cause mitochondrial dysfunction prior to cell death. For example, $HgCl_2$ altered mitochondrial function and mitochondria morphology in vivo in renal cortical mitochondria prior to proximal tubule necrosis.[302,303] When added to isolated rat renal cortical mitochondria, $HgCl_2$ produced similar changes in various respiratory parameters.[302,303] $HgCl_2$ also decreased mitochondrial function in rabbit RPTC prior to the onset of cell death.[304]

The mechanism of mitochondrial dysfunction induced by nephrotoxicants is toxicant-specific. Pentachlorobutadienyl-L-cysteine initially uncouples oxidative phosphorylation in RPTC cells by dissipating the proton gradient.[305–307] In contrast, tetrafluoroethyl-L-cysteine does not uncouple oxidative phosphorylation, but inhibits state-3 respiration by inhibiting sites I and II of the electron transport chain.[307] Other nephrotoxicants that have been shown to affect mitochondrial function include cisplatin,[308,309] citrinin,[310–313] ochratoxin A,[314,315] cephaloridine,[316,317] N-(3,5)-dichlorophenyl-succinimide,[318] DCVC,[319,320] and 2-bromohydroquinone.[321]

Mitochondria can act as primary or secondary mediators of necrosis, apoptosis, or autophagy.[55,322,323] When mitochondria are the primary target of nephrotoxicants, release of cytochrome c and other apoptotic inducing proteins (see previous) can occur early in the apoptotic process. If mitochondria are not a direct target of the nephrotoxicant, these proteins may still be released, but later in the apoptotic process. Central to the role of the mitochondrion in apoptosis is its ability to release pro-apoptotic proteins that activate caspases.[83] Mitochondria can also release caspase-independent DNAases such as Endo G and Omi (Fig. 30.4).

As previously described, a key difference in mitochondrial function during apoptosis and necrosis is the maintenance of ATP during apoptosis. Cellular ATP acts with the mitochondrial membrane potential as a switch that dictates whether a cell dies by apoptosis or necrosis.[82] If the mitochondrial membrane potential is lost quickly and cellular ATP is drastically decreased (below 20% to 10% of normal), then necrosis occurs. Events that result in the rapid loss of mitochondrial membrane potential include a rapid influx of Ca^{2+} into the mitochondria and the rupture of the inner and/or outer mitochondrial membranes.[82] In contrast, if the loss of membrane potential is slower and ATP is maintained through oxidative phosphorylation or glycolysis, then the cell is more likely to die through apoptosis. It should be noted that the majority of cells in culture derive their energy from glycolysis and can maintain ATP levels in the presence of mitochondrial dysfunction. Consequently, cultured cells are generally more susceptible to apoptosis than cells in vivo.

Mitochondria can mediate autophagy by mediating the activation of p53 and caspases.[55] As mentioned previously, cytosolic p53 can inhibit autophagy,[117] whereas caspases can cleave the pro-autophagic protein beclin-1.[55] Cleavage of beclin-1 by caspases is believed to be a primary signal by which cells switch from autophagy to apoptosis. However, more studies are needed to prove that such mechanism occurs during nephrotoxicant-induced renal cell death.

Studies are focusing on the exact protein targets within the mitochondria. For example, experiments seeking to determine the effect of hypoxia on mitochondrial electron transport chain constituents in rabbit RPTC reveal that complex I may be particularly sensitive.[324,325] Other studies have revealed that cisplatin-induced changes in oxidative phosphorylation, membrane potential, and ATP levels in rabbit RPTC are all preceded by inhibition of F(0)F(1)-ATPase (complex IV).[189]

Recent studies in renal cortical mitochondria suggest that an increase in Ca^{2+} influx activates mitochondrial calpain 10, which induces mitochondrial dysfunction by cleaving proteins in complex I of the electron transport chain (see previous text).[248,249,326] Such studies are critical to the understanding of the pathology of mitochondrial-mediated renal cell death and to identifying novel therapeutic targets for inhibition of renal cell death and possibly AKI.

The mechanisms by which toxicants induce release of mitochondrial cytochrome c have also received recent attention. Cytochrome c is normally bound to the inner mitochondrial membrane in association with cardiolipin, a mitochondrial-specific phospholipid. Oxidation of cardiolipin results in cytochrome c release,[55] and oxidant-induced release of cytochrome c is reported to be preceded by cardiolipin peroxidation.[327] These data may explain the protective effect of mitochondrial antioxidants[55] and may explain why inhibition of mitochondrial PLA_2 ($iPLA_2\gamma$)-induced apoptosis in renal cells, such as this enzyme, is suggested to aid in repair of phospholipid oxidation.[55,277] Once released from cardiolipin, cytochrome c may gain entry to the cytosol through a pore formed in the outer mitochondrial membrane by the pro-apoptotic proteins Bax, Bid, or Bak (see previous).

Endoplasmic Reticulum

The ER is the site of protein synthesis and processing as well as bioactivation and detoxification pathways, including those involving cytochrome P-450 and FMO. The ER is also a key regulator of cellular Ca^{2+} homeostasis. Under physiologic conditions, ER Ca^{2+} is typically released after receptor activation through the binding of inositol triphosphate (IP_3) to IP_3 receptors on the ER. Cytosolic free Ca^{2+} increases as a consequence of the ER Ca^{2+} release and is subsequently

decreased by ER uptake via the smooth ER Ca^{2+}-ATPases (SERCA) or extrusion via the plasma membrane Ca^{2+}-ATPase. Similar to the mitochondria, the ER can mediate necrosis, apoptosis, and autophagy.

Schnellmann and colleagues demonstrated that ER Ca^{2+} release is an important signaling pathway in RPTC necrosis.[214,216] Specifically, depletion of ER Ca^{2+} stores with the SERCA inhibitors thapsigargin or cyclopiazonic acid prior to antimycin A or hypoxia exposure inhibited necrosis.[214,216] Also, Ca^{2+} release from the ER activated calpains (calpain 1 and 2), which led to further disruption of ion homeostasis, cleavage of cytoskeleton proteins, and cell swelling, which ultimately resulted in necrosis.[214,218,219,244]

The cytoprotective effects of some stress proteins may be mediated through their ability to regulate ER Ca^{2+}. For example, iodoacetamide and DCVC can activate heat shock proteins (HSPs), calreticulin, and glucose related protein 78 (GRP78) in LLC-PK1 cells.[328] HSPs are typically ER localized proteins that are critical mediators of protein folding. Glucose-related protein 78 and calreticulin are Ca^{2+} binding proteins that aid in the sequestering of Ca^{2+} during toxic stress. Sequestering of Ca^{2+} by these proteins may protect renal cells by preventing cellular oxidative stress induced by Ca^{2+}-mediated mitochondrial injury.[329,330] The increased expression of Ca^{2+}-sequestering HSPs after injury is meant to condition the cell to withstand further necrotic injury.

The ER also mediates apoptosis induced by numerous nephrotoxicants, including acetaminophen, tunicamycin, Fas, and TNF-α.[109] A role for Ca^{2+} in calpain activation and subsequent activation of caspase has been described, as has a role for Ca^{2+} in induction of mitochondrial pore formation. It is suggested that the source of this Ca^{2+} is the ER.[55] ER Ca^{2+} release is also known to mediate the activation of the murine caspase-12 in mouse RPTC.[331] Mice in which caspase-12 had been genetically deleted were resistant to renal cell apoptosis induced by the ER stress agents tunicaymycin, brefeldin A, and thapsigargin, compared to wild-type animals. In contrast, kidneys from mice null for caspase-12 underwent similar degrees of apoptosis caused by the Fas antibody, TNF-α plus cycloheximide, or staurosporine—both agents that cause apoptosis by mechanisms other than ER stress. The key to the activation of caspases-12 in contrast to other caspases may be perturbations in the ER membrane and/or Ca^{2+} levels.

ER Ca^{2+} release is also hypothesized to mediate apoptosis by receptor-mediated mechanisms. Studies in nonrenal cells suggest that an ER Ca^{2+} receptor, type 3 inositol-1,4,5-trisphosphate (Ins[1,4,5]P_3), was upregulated in lymphocytes undergoing dexamethasone-induced apoptosis,[55,332] and that inhibition of Ins(1,4,5)P_3 receptors made T-lymphocytes resistant to apoptosis.[333] Interestingly, sensitivity to apoptosis in T-lymphocytes was restored after artificially increasing cytosolic Ca^{2+}. It is possible that Ca^{2+} released by the Ins(1,4,5) P3 receptor may induce apoptosis by mechanisms described previously (caspase activation, mitochondrial-mediated mechanisms, etc.). It is unknown whether Ins(1,4,5)P3 mediates nephrotoxicant-induced renal cell apoptosis.

Several studies have shown that autophagy is induced by ER stress and ER Ca^{2+} release,[56,115,116,334] and some of these studies have been performed in kidney tissue and renal cells.[56,116] For example, cyclosporine and thapsigargin, agents known to induce ER stress, increase autophagy in primary cultures of human renal cells and in rat kidneys after cyclosporine exposure in vivo.[116] Additionally, tunicamycin or brefeldin A induced autophagy in immortalized rat proximal tubular cells.[115] Mechanisms involved in autophagy induced by ER Ca^{2+} release have been discussed.[112]

Plasma and Organelle Membranes

Some nephrotoxicants can interact with the plasma membrane directly, increase ion permeability, and disrupt ion homeostasis. For example, amphotericin B is an antifungal polyene that binds to cholesterol in the plasma membrane and forms a pore that increases potassium and proton permeabilities.[118,335] Several heavy metals such as silver, gold, mercury, and copper can also react with the plasma membrane and increase potassium permeability.[336,337] It remains to be determined how changes in potassium and proton permeability ultimately lead to cell death.

Toxicants can disrupt cell volume and ion homeostasis by directly or indirectly inhibiting energy production. The loss of ATP inhibits the activity of membrane transporters that maintain differential ion gradients across the plasma membrane. The Na^+-K^+-ATPase is responsible for maintaining the normal Na^+ and K^+ gradients and the secondary ion transport processes. As ATP levels decrease, Na^+-K^+-ATPase activity decreases, resulting in K^+ efflux and Na^+ influx and a decrease in the normally negative membrane potential.[338] The decrease in the negative membrane potential allows Cl^-, as well as additional Na^+, to enter down a concentration gradient resulting in water influx and cellular swelling. Similar mechanisms can occur in kidney cells. For example, treatment of rabbit RPTC suspensions with the mitochondrial inhibitor antimycin A inhibits respiration within 1 minute, followed by ATP depletion, and the loss of the sodium and potassium gradients and transport over the next 5 to 10 minutes (Fig. 30.8).[219,339] Subsequent studies demonstrated that increases in Cl^- influx occurred between 15 and 30 minutes, during the late stages of cellular injury, followed by cellular rupture.[340] Decreasing extracellular NaCl concentrations by 50% with iso-osmotic substitution of mannitol decreased Cl^- influx, cellular swelling, and cellular rupture.[341] Furthermore, hyperosmotic incubation buffer decreased the cellular swelling and cellular lysis but not the increased Cl^- influx.[341] Thus, the delayed increase in Cl^- influx may be the trigger for the water influx and additional Na^+ influx that provides the osmotic force for cellular swelling and rupture.

Increased Cl^- influx occurs during the late stages of cell injury in RPTC and LLC-PK1 cells exposed to a variety of injury stimuli and toxicants, including $HgCl_2$, t-butylhydroperoxide, bromohydroquinone, tetrafluoroethyl-L-cysteine, and hypoxia.[341,342] The mechanism by

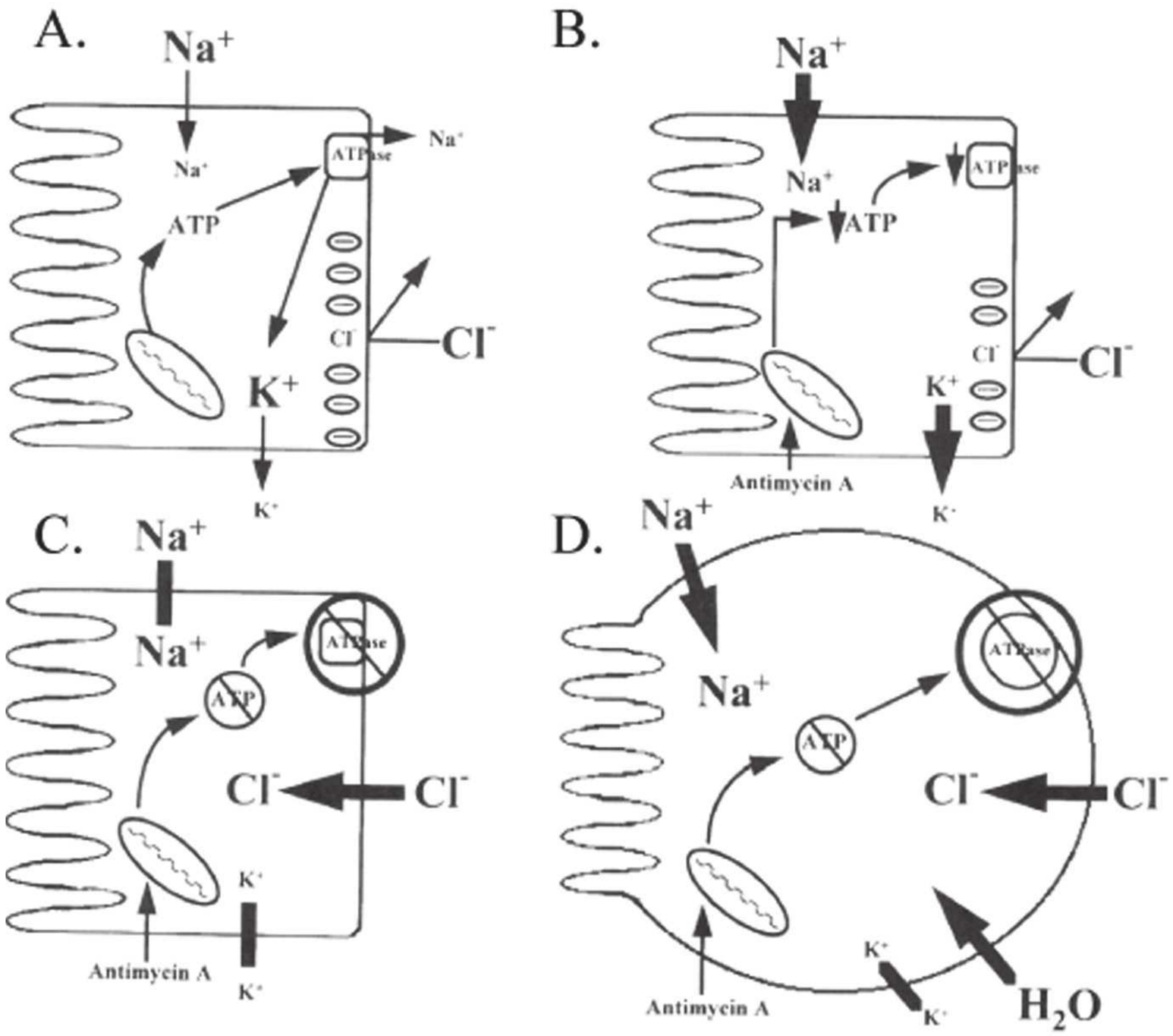

FIGURE 30.8 **A:** A schematic representation of a normally functioning renal cell. Note that the inside of the cell is negative with respect to the outside, which decreases the ability of Cl^- to enter the cell. **B:** The addition of a mitochondrial inhibitor such as antimycin A blocks cellular respiration, decreases ATP levels and Na^+-K^+-ATPase activity, increases Na^+ influx and K^+ efflux, and decreases the membrane potential. **C:** Subsequently, there is an increase in Cl^- influx (down the concentration gradient) by an unidentified pathway. **D:** The increase in Cl^- influx results in water influx, increased Na^+ influx, and cellular swelling. These processes provide the osmotic force that ultimately leads to cellular lysis.

which Cl^- influx occurs under these conditions is still under study, but was inhibited by blockers of Ca^{2+}-activated Cl^- channels (e.g., niflumic acid, indanyloxyacetic acid [IAA-94], 5-nitro-2-[3]-phenylpropylamino-benzoate [NPPB], and diphenylamine-2-carboxylate [DPC]).[150–153] The Cl^- influx was insensitive to the Cl^- channel blockers 4-acetamide-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) and diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) or to the Cl^- transport inhibitors bumetanide and hydrochlorothiazide.[341] Therefore, the Cl^- influx that occurs during the late phase of cell death may be through a Ca^{2+}-activated Cl^- channel.

The plasma membrane can also mediate apoptosis due to the presence of death receptors. These include receptors for TNF-α, Fas, and LPS, all of which mediate renal cell apoptosis.[343,344] The exact receptors involved depend on the toxicant and cell type, but typically activate the extrinsic caspase-mediated pathway (see previous text). Additionally, and as mentioned previously, plasma membrane Ca^{2+} receptors can directly mediate apoptosis[213]; however, this pathway has not received as much attention with regard to nephrotoxicant-induced renal cell injury.

Lysosomes

Lysosomes are membrane-bound vesicles that pinch off from the Golgi-apparatus and contain a variety of hydrolytic enzymes.[345] Lysosomes contain hydrolytic enzymes that function as intracellular digestive enzymes under normal conditions. Lysosomes play an important role in the nephrotoxicity of several compounds such as aminoglycoside antibiotics. Lysosomes can also mediate a specific pathology called α_{2u} nephropathy. α_{2u}-Nephropathy occurs in male rats when compounds such as unleaded gasoline, d-limonene, 1,4-dichlorobenzene, tetrachloroethylene, decalin, 2,2,4-trimethylpentane, and lindane bind to α_{2u}-globulin, which prevents its normal degradation in renal proximal tubular cells.[346,347] α_{2u}-Globulin is synthesized in the liver of male rats under androgen control. Serum α_{2u}-globulin (18.7 kDa) is freely filtered by the glomerulus with approximately half being reabsorbed via endocytosis in the S_2 segment of the proximal tubule. The binding of these agents to α_{2u}-globulin inhibits its normal degradation and results in the accumulation of α_{2u}-globulin in the proximal tubule. Over time, the size and number of lysosomes increase, and characteristic protein-droplet morphology is observed. Ultimately, this leads to single-cell necrosis, the formation of granular casts at the junction of the proximal tubule and the thin loop of Henle, and cellular regeneration.

Recent data show that a 73 kDa heat shock cognate protein mediates the binding of α_{2u}-globulin to a 96 kDa membrane glycoprotein in male rat kidney lysosomes.[348] This HSP also is involved in the degradation of other cellular proteins. Treatment of rats with 2,2,4-trimethylpentane increases the rate of transport of not only α_{2u}-globulin into the lysosome, but also increases the rate of lysosomal

transport of many proteins. Increased transport is a result of α_{2u}-globulin-mediated increases in the level of the receptor proteins in the lysosomal membrane. Thus, α_{2u}-globulin may induce lysosomal overload by increasing the rate of transport of cellular proteins to the lysosome. In this manner, chronic exposure to the preceding compounds may lead to a chronic nephropathy, and may increase the incidence of renal adenomas/carcinomas by nongenotoxic mechanisms.

α_{2u}-Globulin nephropathy is sex- and species-specific, occurring in particular strains of male rats but not in female rats, male or female mice, rabbits, or guinea pigs. This raises the question if humans are at risk for α_{2u}-globulin-induced nephropathy and renal tumors. The current evidence suggest no because: (1) humans do not synthesize α_{2u}-globulin; (2) humans secrete fewer proteins in general and, in particular, fewer low-molecular-weight proteins in the urine than the rat; (3) the low-molecular-weight proteins in human urine are either not related structurally to α_{2u}-globulin, do not bind to compounds that bind to α_{2u}-globulin, or are similar to proteins in female rats, male Black Reiter rats, rabbits, or guinea pigs that do not exhibit α_{2u}-globulin nephropathy; and (4) mice excrete a low-molecular-weight urinary protein that is 90% homologous to α_{2u}-globulin but do not exhibit α_{2u}-globulin nephropathy and renal tumors after exposure to α_{2u}-globulin nephropathy-inducing agents.[349]

Aminoglycoside antibiotics also induce lysosomal dysfunction and cause acute renal failure (ARF) (see Chapter 31).[120,350,351] In this case, the aminoglycosides are filtered, bound to anionic phospholipids in the brush border, reabsorbed by endocytosis in the S_1 and S_2 segments of the proximal tubule, and accumulated in the lysosomes. Over time, the size and number of lysosomes increase and electron-dense lamellar structures called myeloid bodies appear. The myeloid bodies contain undegraded phospholipids and are thought to occur through aminoglycoside-induced inhibition of lysosomal hydrolases such as sphingomyelinase and phospholipases. However, the steps between lysosomal phospholipid overload and tubule cell death are less clear.

Lysosomes play a central role in autophagy.[56,112,113,237] Basically, autophagosomes formed during autophagy fuse with the lysosomes, which leads to the degradation of cellular proteins.[112] Studies in renal cells and tissues demonstrate that autophagy occurs after nephrotoxicant exposure including that induced by cyclosporine, cisplatin, thapsigargin, and after ischemia reperfusion.[56,115,116,237,352] Because it is not clear if nephrotoxicant-induced renal cell autophagy mediates cell death or survival, it is not known if the role of the lysosome in this type of pathology is protective or damaging. Further studies are needed to address this gap in knowledge.

CELLULAR DEFENSES

Renal epithelial cells have numerous defenses against both reactive intermediates and ROS (Fig. 30.6). GSH is the primary cellular protectant and the most abundant cellular nonprotein thiol in cells. It is found in high concentrations in at least three subcellular compartments (cytosol, mitochondria, nucleus).[353] Normally, GSH detoxifies electrophiles by forming a glutathione conjugate either directly or with the aid of GST. This includes compounds containing a quinone nucleus such as bromohydroquinone, which results in formation of mono- and di-substituted glutathione conjugates in renal cells.[321]

GSH also acts in conjunction with glutathione peroxidase and glutathione reductase to neutralize ROS. This produces organic peroxide that is reduced to water and alcohol by glutathione peroxidase, forming glutathione disulfide (Fig. 30.6). Glutathione disulfides are reduced to glutathione by glutathione reductase in an NADPH-dependent reaction. Catalase and superoxide dismutase are two other enzymes that detoxify ROS. Superoxide dismutase converts the superoxide anion to hydrogen peroxide, and catalase converts the hydrogen peroxide to water.

Several studies show that the activity of glutathione-dependent enzymes differs among the nephron. Differences in the activity of these enzymes may account for differences in the susceptibility of different kidney regions to oxidative stress. Cummings and associates[354] reported that the levels of glutathione peroxidase and γ-glutamylcysteinyl synthetase are higher in rat proximal tubular cells than distal tubule cells. The activity of glutathione reductase and GST appeared to be equal between the two cell populations; however, the proximal tubular cells had a much higher concentration of glutathione than distal tubular cells (27 nmol per mg for proximal tubular cells versus 13 nmol per mg for distal tubular cells).[355]

In order to protect against nephrotoxicant-induced cellular injury, GSH must be able to cross plasma and organelle membranes. The dicarboxylate carrier is one protein responsible for transport of GSH into mitochondria, and overexpression of this protein protected normal rat kidney-52E cell lines from both oxidant (t-butylhydroperoxide) and DCVC-induced apoptosis.[356] Protection against injury correlated to increases in mitochondrial GSH concentration, as well as decreased mitochondrial dysfunction, the release of cytochrome c, and caspase activation.

Vitamin C (ascorbic acid) is a very effective reducing agent and free radical scavenger and functions in the recycling of the vitamin E radical back to vitamin E.[357] Like GSH, vitamin C can detoxify compounds containing a quinone nucleus such as bromohydroquinone, but in this case vitamin C reduces the bromoquinone and the bromoquinone radical back to bromohydroquinone.[321]

Vitamin C can also promote repair and regeneration after nephrotoxicant-induced injury. For example, pharmacologic levels of vitamin C improved recovery of rabbit RPTC after exposure to t-butylhydroperoxide and DCVC.[358–360] Increased recovery correlated to increased cell number and mitochondrial function.[358] Vitamin C may also improve recovery by promoting collagen deposition in the extracellular matrix.[358] The effect of vitamin C was not the result of its antioxidant function, because both t-butylhydroperoxide and

DCVC caused the same amount of damage in treated and untreated cultures, and vitamin C was added after injury and removal of the nephrotoxicants.

Vitamin E (α-tocopherol) is a lipid-soluble antioxidant found in cell membranes.[361] Vitamin E is a chain-breaking antioxidant because it contributes an electron to a peroxyl radical formed during lipid peroxidation and thereby prevents further lipid peroxidation. The vitamin E radical produced is unreactive and is recycled back to vitamin E. Vitamin E suppresses cyclosporin A-mediated toxicity in vivo in rat renal kidneys by inhibiting lipid peroxidation.[362] Vitamin E also protects against cephaloridine-induced toxicity in freshly isolated rat proximal tubule cells.[363] The protective effect of vitamin E on proximal tubule cell death correlated to decreases in lipid peroxidation.

Glycine

Glycine is cytoprotective in a number of models of renal injury.[364] In addition, several other small amino acids with similar structure to glycine, including D- and L-alanine, β-alanine, and 1-aminocylopropane-1-carboxylic acid, also protect against nephrotoxicity. This suggests that there is a structural requirement for cytoprotection with these compounds.

Glycine is cytoprotective against a diverse group of chemical insults such as anoxia, metabolic inhibitors, bromohydroquinone, halogenated alkene, and alkane cysteine conjugates and, to a lesser extent, t-butylhydroperoxide and $HgCl_2$.[215,365] The mechanism of glycine cytoprotection has remained elusive, but studies suggest that glycine acts during the terminal phase of cell injury.[365] Further, the mechanism of protection may be receptor-based as the neuronal glycine receptor antagonist strychnine protected against renal cell injury under a variety of conditions.[341,366] Strychnine binds to a low-affinity binding site on the basolateral membrane of the rabbit RPT cell in a saturable and reversible manner at the same concentrations that are cytoprotective.[245] Proteins corresponding to two of the three subunits of the neuronal strychnine-sensitive glycine receptor are expressed at the basolateral membrane as well, which may represent the glycine receptor β subunit.[366,367] The signal transduction pathway for the neuronal glycine receptor involves Cl^-. Collectively, these studies suggest that glycine and strychnine are cytoprotective by directly or indirectly altering Cl^- influx. These compounds may inhibit the ability of Cl^- to increase the osmotic force, which drives increases in cell swelling during injury.

Alternatively, Nichols and associates[368] proposed that glycine is cytoprotective in hepatocytes through its ability to inhibit calpains. However, Edelstein and coworkers[246] reported that glycine did not inhibit calpain activity in rat RPT exposed to hypoxia. Studies in rabbit RPT demonstrated that glycine and strychnine did not inhibit basal calpain activity, but did inhibit calpain activity observed during the late phase of cellular injury.[369] Later studies confirmed that glycine does not directly affect calpain activity, but rather inhibited toxicant-mediated extracellular Ca^{2+} influx, calpain translocation, and Cl^- influx.[369]

Acidosis

Acidosis is not a normal cellular defense mechanism per se; however, decreasing extracellular pH is cytoprotective in a variety of in vitro models of renal cell injury.[370,371] For example, reducing the extracellular pH to 6.8 to 7.0 protected against anoxia-induced cell death in isolated renal tubules.[371–373] In addition, Rodeheaver and Schnellmann[370] demonstrated that extracellular acidosis (pH 6.4) ameliorated renal proximal tubular cell death produced by a series of mitochondrial inhibitors (antimycin A, rotenone, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, oligomycin) and ion exchangers (nigericin, monensin, valinomycin), but potentiated cell death produced by the oxidants t-butylhydroperoxide, hydrogen peroxide, and ochratoxin A. Thus, the effect of extracellular acidosis on renal cell injury is toxicant-specific. Increased cell death in the presence of oxidants correlated to increases in oxidized GSH (GSSG), lipid peroxidation, and mitochondrial dysfunction. This suggests that extracellular acidosis during oxidant exposure decreases free radical detoxification.

It is unlikely that extracellular acidosis protects against nephrotoxic-induced renal cell injury by preserving mitochondrial function or ATP levels.[370–372] This hypothesis is supported by studies demonstrating that extracellular acidosis initiated at various times after toxicant exposure was still cytoprotective.[238,371] For example, extracellular acidosis initiated 15 minutes after antimycin A or carbonyl cyanide-p-trifluoromethoxy phenylhydrazone addition—a time point after the cessation of respiration, depletion of ATP, and increases in intracellular sodium and decreases in intracellular potassium—was completely cytoprotective at 45 and 105 minutes, respectively; however, cytoprotection did not prevent increases in Cl^- influx that occurs in the late stages of cell injury. Extracellular acidosis initiated 2 hours after tetrafluoroethyl-L-cysteine or t-butylhydroperoxide addition also was cytoprotective 2 hours later. These results demonstrate that the cytoprotective effect of extracellular acidosis occurs very late in the cell injury process distal to Cl^- influx.

Peroxisomes and Peroxisomal Proliferating Activated Receptors

Peroxisomes are membrane-bound vesicles that contain degradative enzymes for fatty acids and amino acids.[345] Peroxisomes also contain catalase, which converts H_2O_2 to oxygen and water. Thus, peroxisomes are a major site of antioxidant defense. In addition, peroxisomal proliferation has been linked to the preservation of mitochondrial function[374] and the reduction in renal cell death following injury induced by gallic acid,[375] cisplatin,[259,376] and ischemia/reperfusion-induced injury.

Peroxisomes also may protect against renal cell death via mechanisms linked to the activation of peroxisomal proliferator-linked receptors (PPAR). PPAR are members of a nuclear hormone-activated receptor and transactivation protein family.[377] At this time three different PPAR have been identified and cloned (PPAR-α, PAR-β/δ, and PPAR-γ,).[377] PPAR-β/δ are

detected in almost all tissues including the kidney cortex.[377–379] PPAR-γ is present in distal medullary collecting ducts, glomeruli, and the renal microvasculature.[379,380] PPAR-α is expressed in the proximal tubule, medullary thick ascending limbs, and the glomerular mesangial cells. It is hypothesized that differences in the distribution of PPAR isoforms may result in different mechanisms of protection between different cells.

Toxicants that activate PPAR are structurally diverse and include plasticizers (di(2-ethyhexl)phthalate),[381] herbicides,[382] hypolipidemic drugs (fenofibrate, clofibrate, and clofibric acid),[377] and antidiabetic drugs[377,383] (e.g., troglitazone and rosiglitazone).[383–385] Activators of PPAR increase the number of peroxisomes within the cell and increase the expression of enzymes for fatty acid β-oxidation including fatty acyl-CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme, and 3-ketoacyl-CoA thiolase.[381,382,386] Activators of PPAR isoforms also increase mitochondrial enzymes including carnitine palmitoyltransferase, medium chain acyl-CoA dehydrogenase, and pyruvate dehydrogenase complex.[376,379] The increase in these proteins is believed to be key in the protection against nephrotoxicants.[377,379,387]

Activation of PPAR, at least activation of PPAR-α, appears to protect against renal cell death. This hypothesis is supported by studies showing that the PPAR-α agonists clofibrate and WY14643 protect against ischemia/reperfusion-induced renal cell dysfunction in rat kidneys.[379,388] In addition, knocking out PPAR-α increased cisplatin-induced renal cell apoptosis in mouse kidneys, in correlation with the release of Endo G from the mitochondria.[259]

The mechanism of protection afforded by a PPAR-α agonist against nephrotoxicant-induced renal cell injury correlates to increases in mitochondrial function. This is demonstrated by in vivo studies demonstrating that PPAR-α induction correlated to increased mitochondrial medium-chain acyl-CoA dehydrogenase and pyruvate dehydrogenase complex activity, which correlated to decreases in cisplatin-induced proximal tubular necrosis.[376]

Schnellmann and colleagues demonstrated that overexpression of peroxisomal proliferator-activated receptor γ coactivator-1α (PGC-1α), the master regulator of mitochondrial biogenesis, induced mitochondrial biogenesis in renal proximal tubular cells.[252,389] Further, PGC-1α expression was increased following sublethal injury induced by t-butylhydroperoxide, and only returned to base values after mitochondrial function was restored. Interestingly, overexpression of PGC-1α following oxidant injury stimulated the recovery of mitochondrial and cellular function. These data suggest that PGC-1α is a therapeutic target for stimulating renal cell recovery and regeneration after nephrotoxicity.

SPECIFIC TOXICANTS

It is critical to identify the ultimate toxic species and the cell type targeted in order to understand the mechanism by which a chemical produces nephrotoxicity. For example, is the glomerulus, proximal convoluted tubule, proximal straight tubule, the thick ascending limb of Henle, or the distal convoluted tubule the target of the parent compound, a primary, or secondary metabolite? Thus, biotransformation, toxicokinetic, and morphologic studies are paramount in determining the sites of biotransformation, which metabolites reach the kidney, the quantity of metabolites in the kidney, the target cell type in the kidney, and ultimately the mechanism of nephrotoxicity. Other chapters in this book focus on specific toxicants such as analgesics (Chapter 32), antibiotics (Chapter 31), antineoplastics (Chapter 31), heavy metals (Chapter 34), immunosuppressives (Chapter 31), and radiocontrast media (Chapter 33).

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CHAPTER

31 Antibiotic- and Immunosuppression-Related Renal Failure

Marc E. De Broe

AMINOGLYCOSIDE ANTIBIOTICS

Nephrotoxic injury is a common complication of aminoglycoside antibiotic therapy. Studies that have used well-defined measures of nephrotoxicity indicate an incidence rate of 7% to 36%.[1–9] This variability reflects differences with respect to the nephrotoxicity potentials of aminoglycoside antibiotics in clinical use as well as differences among patients receiving these drugs. A survey of clinical studies published between 1975 and 1982 revealed that the average incidence of nephrotoxicity caused by specific aminoglycoside antibiotics was gentamicin, 14%; tobramycin, 12.9%; amikacin, 9.4%; and netilmicin, 8.9%.[10] In critically ill patients, the incidence of aminoglycoside nephrotoxicity may rise twofold.[11]

Clinical Aspects

The clinical expression of aminoglycoside nephrotoxicity has been well described.[12–16] Lopez-Novoa and colleagues wrote a comprehensive review on the mechanisms of aminoglycoside nephrotoxicity.[17] The earliest and most common expression of aminoglycoside renal tubular cell alterations is increased urinary excretion of low molecular weight proteins[18,19] and of lysosomal and brush-border membrane enzymes.[18–21] These changes may be detected within 24 hours of initiating drug therapy, and the frequency and magnitude of these changes increase as a function of dose and duration of therapy. Unfortunately, these changes do not predict which patients will progress to acute renal failure (ARF). This probably reflects the fact that several mechanisms underlie the expression of the enzymuria and proteinuria.[13] With repeated dosing, the amount of enzymes and low molecular weight proteins excreted in the urine may increase quite sharply, which may signify the onset of proximal tubular cell necrosis.[13]

Nonoliguric renal failure is a common expression of aminoglycoside nephrotoxicity[22] and may reflect a direct inhibitory effect on solute transport along the thick ascending limb of Henle's loop[23] or possibly tubulointerstitial cell injury,[24] which results in impaired ability to maintain a hypertonic medullary interstitium. Inhibition of adenylate cyclase may also contribute to the polyuria.[25] Neither mechanism, however, adequately explains the maintenance of normal to high urine output, even in the face of severe depression of whole kidney glomerular filtration rate (GFR). The slow evolution of ARF, which has been attributed to a variable susceptibility of renal proximal tubular cells to aminoglycoside toxicity,[12,26] may allow for the development of maximal compensatory adaptation by residual intact nephrons. In addition, micropuncture experiments[27] implicate a marked depression of solute and water transport along the proximal tubule such that the large increase in the fraction of filtrate escaping reabsorption along the proximal tubule may overwhelm the reabsorptive capacity of the distal nephron and contribute to the pattern of nonoliguric renal failure. When oliguria occurs, it usually signifies the influence of one or more complicating factors (e.g., ischemia or another nephrotoxin), especially if the oliguria appears early in the course of aminoglycoside administration. Studies in animals have shown that aminoglycoside therapy sensitizes the kidney to a subsequent ischemic or nephrotoxic insult,[28–37] such that the severity of the ARF is substantially greater than that predicted by the sum of the individual insults. Deterioration of other proximal tubular transport processes may occur during aminoglycoside toxicity and, in rare cases, may mimic a Fanconi-like syndrome.[38] Hypokalemia and hypomagnesemia secondary to renal potassium and magnesium wasting may also appear.[39,40]

Depression of GFR is a relatively late manifestation of aminoglycoside nephrotoxicity. In humans, depression of GFR typically does not occur before 5 to 7 days of therapy have been completed[15] unless there has been a major complicating factor such as renal ischemia. Studies in animal models of aminoglycoside nephrotoxicity have implicated activation of the renin–angiotensin system,[41] reduction in the size and density of glomerular endothelial fenestrae,[42–44] tubular obstruction,[45] tubular back leak,[27] and release of platelet activating factor from mesangial cells[46] as pathogenic factors causing depression of GFR.

The majority of patients with aminoglycoside nephrotoxicity recover renal function clinically, although in some cases the time to recovery may be prolonged.[16] Chronic renal failure is a distinctly uncommon complication of pure aminoglycoside

nephrotoxicity in humans, so that when it occurs, it usually signifies the contribution of some additional factor. Animal studies indicate, however, that incomplete regeneration with interstitial fibrosis does occur,[47] and the same may be true for humans.[48]

Morphologic Alterations

Aminoglycosides cause tubular cell necrosis that in animal models is largely confined to the proximal convoluted tubule and pars recta.[49–51] In humans, the renal tubular site of injury is less well established,[24,52] due in part to the fact that little human biopsy material has been available for study. Moreover, in human subjects, the development of ARF in conjunction with aminoglycoside administration typically occurs in association with other insults such as sepsis and renal ischemia,[24,53,54] and each of these insults has been shown to interact synergistically with aminoglycoside antibiotics to magnify the severity and sites of tubular cell injury.[31–37]

The earliest lesion seen by electron microscopy is an increase in the number and size of secondary lysosomes, also called cytosegrosomes or phagosomes.[49–51] Examples of this

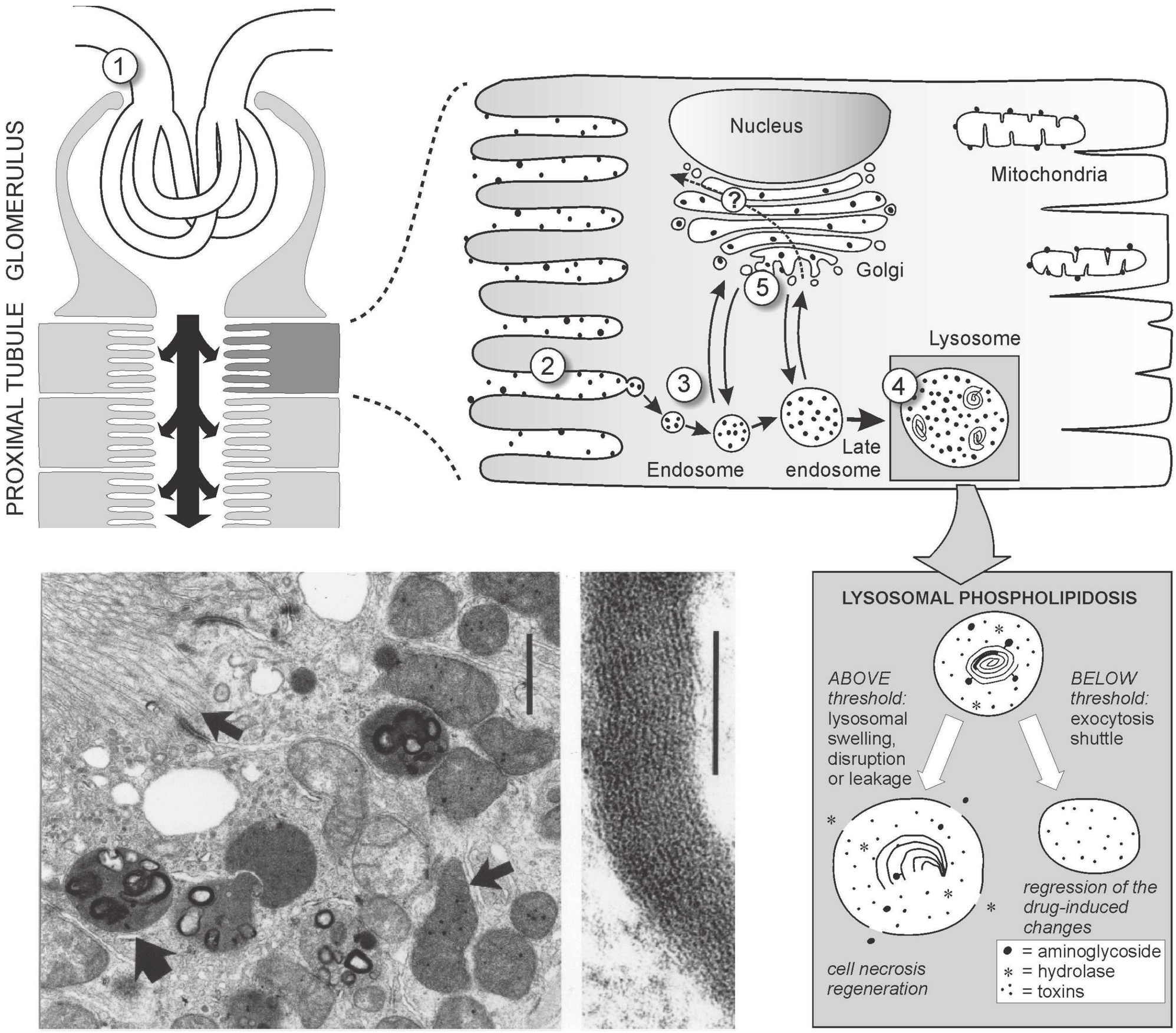

FIGURE 31.1 **Above**: Binding uptake and intracellular trafficking of gentamicin in renal proximal tubular cells. **A:** After glomerular filtration (*1*), gentamicin (•) is shown binding to the surface membrane (*2*) and being internalized by a receptor (megalin) mediated endocytic process (*3*). Gentamicin also enters the cell through fluid phase endocytosis. It moves through the endocytic system into late endosomes and from there into lysosomal structures (*4*). A small but quantifiable fraction (5%–10%) of gentamicin directly traffics from the surface membrane into the trans-Golgi network (*5*) and from there throughout the Golgi Apparatus. **Below left**: Ultrastructural appearance of proximal tubular cells after 4 days of gentamicin treatment, showing lysosomes containing dense lamellar and concentric structures (*large arrow*), while brush-border, mitochondria (*small arrow*), and peroxisomes are unaltered. Upon higher magnification the structures in lysosomes show a periodic pattern. Bar left = 1 μm, middle = 0.1 μm. **Below right**: Internalization and lysosomal sequestration of gentamicin. (Adapted from Verpooten GA, Tulkens PM, Molitoris BA. Aminoglycosides and vancomycin. In: De Broe ME, Porter GA, Bennett VM, Verpooten GA, eds. *Clinical Nephrotoxins – Renal Injury from Drugs and Chemicals,* 2nd ed. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2003:151–170.)

lesion are shown in Figure 31.1. Secondary lysosomes are primary lysosomes that have coalesced with endocytic or autophagic vacuoles. Many of these lysosomes contain myeloid bodies, electron-dense lamellar structures of concentrically arranged and densely packed membranes. These lysosomal alterations probably represent autophagic vacuoles arising from sequestration of fragments of membranes and organelles damaged in the early phase of toxicity and are undergoing lysosomal processing. In experimental animals receiving single parenteral drug doses or continuous drug infusion, these changes have been observed as early as 6 to 12 hours posttreatment.[56] Both the number and size of lysosomal myeloid bodies increase as a function of dose and duration of drug therapy and are accompanied by progressive expansion of the volume of the cell occupied by engorged lysosomes.[50,56,57] These morphologic alterations also have been convincingly demonstrated in human kidney material.[58,59] Studies in experimental animals and in cultured cells have demonstrated that the myeloid bodies are composed of membranes rich in phospholipids[60,61] and form as a consequence of the lysosomal accumulation in high concentration of aminoglycosides. This lysosomal accumulation of aminoglycosides inhibits lysosomal phospholipases[62,63] and possibly other lysosomal enzymes and impairs the degradation of cell membrane.[61,64] Similar alterations have been induced by a variety of compounds that accumulate within the lysosomal compartment and interfere with the activity of lysosomal enzymes.[65–67]

Following lysosomal alterations, the following occurs: a decrease in the density and height of brush-border microvilli, dilation of the cisternae of rough endoplasmic reticulum, and the appearance of cytoplasmic vacuolization in tubular epithelial cells.[51,56] As injury progresses, brush-border membrane fragments and extruded myeloid bodies, membrane vesicles, and cytoplasmic debris begin to be seen within tubular lumina.[51,68] Later in the course of nephrotoxicity, mitochondrial swelling becomes evident, and patchy but extensive tubular epithelial cell necrosis and desquamation occur. Many tubules, both proximal and distal, are filled with eosinophilic, granular material that, by electron microscopy, is composed of cytoplasmic debris, membrane fragments, and myeloid bodies. Transmission electron microscopy of the urine reveals the presence of myeloid bodies and fragments of brush-border membranes.[68–70]

Proximal tubular cells manifest an apparent variable susceptibility to aminoglycoside toxicity evident by the appearance of cell regeneration simultaneously with ongoing cell necrosis.[26,47,50,57,71] In several animal studies virtually complete recovery of renal structure and function has been observed during continued aminoglycoside administration.[72,73] One explanation for these observations is that the renal tubular epithelium had acquired resistance to the nephrotoxic effects of the aminoglycoside antibiotic. Sundin and colleagues[74] report that the "acquired resistance" reflects selective inhibition of aminoglycoside uptake by renal proximal tubular cells, the mechanism of which does not involve a reduction in the membrane content of phosphatidylinositol or megalin. In animal models, cell regeneration can be detected by [^{3}H]thymidine incorporation into DNA after only 4 days of low-dose aminoglycoside administration and before cell necrosis is evident by light microscopy.[57,74] The magnitude of DNA labeling correlates with the dose and duration of drug administration.[75] Of particular interest is the observation that quantitatively similar labeling is observed in renal cortical interstitial cells as in tubular epithelial cells.[57,75,76] This finding raises the question of the role of these interstitial cells in the pathogenesis of aminoglycoside toxicity. Eventually most areas of the affected kidney regain normal architecture and function, but residual scarring containing collections of collapsed, atrophic tubules may occur focally in the cortex.[47,48,51] In animal models of aminoglycoside nephrotoxicity, the degree of tubular cell necrosis correlates reasonably well with the decline in renal excretory function. A similar correlation is lacking in human material.[24,52,58]

Pathogenesis

The pathogenesis of aminoglycoside nephrotoxicity is intimately linked to the renal pharmacology of these drugs.[77–80] Aminoglycoside antibiotics are organic polycations with a net cationic charge that, at pH 7.4, ranges from +4.47 in the case of neomycin to +2.39 for amikacin. Because these compounds are highly hydrophilic, they are poorly absorbed across the intestinal tract and therefore must be given parenterally. They are distributed in a volume slightly greater than extracellular volume and are eliminated from the body without metabolic transformation. The route of elimination is almost exclusively by the kidneys, and the principal mechanism of excretion is glomerular filtration. Of toxicologic significance is the fact that small amounts of aminoglycoside antibiotics are selectively transported into proximal tubular cells by adsorptive endocytosis,[81–83] which has been shown to occur across the basolateral as well as the apical membrane.[83] Several lines of evidence have implicated anionic phosphatidylinositol as a membrane binding site for aminoglycosides.[84,85] More recent studies also suggest a role for megalin, an endocytic receptor for cationic ligands, in the uptake of aminoglycoside antibiotics across the brush-border membrane of renal proximal tubular cells.[86] Indeed, by using the specific antagonist receptor-associated protein, blocking the activity of megalin in perfused rat proximal tubules, a reduction of 20% in gentamicin clearance ensued. Nagai demonstrated similar results in rats treated with maleate, impairing the receptor-mediated uptake of megalin ligands.[87] Megalin knockout mice are protected against aminoglycoside nephrotoxicity.[88]

Following endocytosis, the aminoglycosides are translocated into the lysosomal compartment, where they accumulate in millimolar concentrations and reside with a half-life measured in days.[78] As noted, the lysosomal compartment is the site of myeloid body formation consequent to aminoglycoside-induced inhibition of lysosomal enzymes such as phospholipase, sphyngomyelase, etc. When the concentration of drug and/or the amount of lysosomal phospholipid reaches a critical threshold, an injury cascade is triggered that eventuates in irreversible cell injury with

progression to necrosis.[56] However, neither the sequence nor the specific mechanisms involved in the progression to cell death have been clearly established. Sandoval and colleagues report that within 15 minutes of endocytosis gentamicin traffics to the Golgi complex as well as to the lysosomal compartment of LLC-PK1 cells[89,90] and rat renal proximal tubular cells.[91] These observations raise the possibility that the Golgi complex may provide a pathway for the redistribution of aminoglycoside antibiotics to other intracellular compartments and thereby broaden the potential for these drugs to disrupt a variety of organellar functions. For example, the depression of protein synthesis observed early in the course of gentamicin administration may signify retrograde transport of gentamicin to the endoplasmic reticulum.[91] The reason gentamicin and presumably other aminoglycoside antibiotics are transported from the endosomal compartment to the Golgi complex is not known; but, it may reflect an effect of these agents to perturb endosomal fusion[92] possibly as a consequence of binding to megalin[92] or to membrane-acidic phospholipids.[93,94]

A growing body of evidence supports the view that the pathogenesis of aminoglycoside toxicity is causally related to the capacity of these cationic drugs to bind to and perturb the function and structure of biologic membranes. Aminoglycosides have been shown to bind to anionic[62,84,95–102] but not to neutral phospholipids.[62,84,96,98] Among the anionic phospholipids, aminoglycosides bind most avidly to phosphatidylinositol 4,5-bisphosphate (PIP_2).[84,97,103–105] Several approaches have been used to gain insight into the molecular interaction between aminoglycosides and anionic phospholipids.[84,95,98,101,102,106–108] All models indicate an electrostatic interaction between a protonated amino group and the anionic phosphate group. Ramsammy and Kaloyanides[107] propose a model that, in addition to an electrostatic interaction between a protonated amino group and the phosphate group, also involves formation of hydrogen bonds between an amino group of gentamicin and the carbonyl groups of glycerol. This model explains aminoglycoside-induced changes in the biophysical properties of artificial membranes (i.e., an increase in the transition temperature and a decrease in glycerol permeability of phosphatidylinositol [PI]-containing liposomes).[100] Both changes signify that gentamicin induces a decrease in membrane fluidity, and this finding has been confirmed in brush-border membranes as assessed by changes in the fluorescence polarization of membrane probes[96] and by electron spin resonance spectroscopy.[109] Aminoglycosides also have been shown to promote membrane aggregation,[106,110] a process that requires neutralization of surface charge. In a comparative study of aminoglycoside-induced aggregation of PI-containing liposomes,[106] it was observed that the rank order with respect to efficacy in neutralizing membrane surface charge was neomycin > gentamicin = tobramycin = netilmicin = spermine. The rank order for inducing aggregation of liposomes was neomycin > gentamicin > tobramycin > netilmicin = spermine and was identical to the rank order of these agents with respect to depressing glycerol permeability.[106] This rank order also coincides precisely with the established clinical nephrotoxicity potentials of these drugs. Because depression of glycerol permeability was shown to be dependent on hydrogen bonding between one or more amino groups of the drug and carbonyl groups of the glycerol backbone,[107] these data suggest that the membrane toxicity of aminoglycosides is closely linked to their potentials to engage in hydrogen bonding. Importantly, the rank order in terms of nephrotoxicity potentials does not coincide with the net cationic charge of these agents.[106] This observation emphasizes that spatial orientation of charge rather than net charge is a critical determinant of toxicity.

Schacht and colleagues[97,99,104,111] utilize a variety of methods to assess aminoglycoside-induced perturbations of PIP_2-containing membranes as a measure of the ototoxicity potentials of these antibiotics. Increased fluorescence of 1-anilino-8-naphthalenesulfonate,[99] increased permeability to carboxy fluorescein,[104] and increased surface tension of monomolecular film of phosphatidylcholine (PC)/PIP_2[111] were shown to correlate precisely with the ototoxicity potentials of aminoglycoside antibiotics. These studies have led to the hypothesis that the ototoxicity of aminoglycosides is causally related to their binding to PIP_2 and disruption of this signaling mechanism.[112]

The studies cited here provide the foundation for the hypothesis that the toxicity of aminoglycoside antibiotics is causally related to their capacity to interact electrostatically and by hydrogen bonding to membrane anionic phospholipids and, thereby, to perturb the biophysical properties and function of cell membranes. It is well established that these drugs interact with and perturb the function of plasma membranes,[13,113–116] lysosomes,[13,56,57–64,117–122] mitochondria,[51,123–126] and microsomes.[127–129] It remains unclear, however, whether toxicity results from disruption of a single critical membrane function or multiple membrane functions. It is possible that the injury cascade is triggered by the rupture of lysosomes engorged with aminoglycoside antibiotic and with myeloid bodies. The resultant release of potent acid hydrolases and high concentrations of drug into the cytoplasm might cause disruption of a number of critical intracellular processes including mitochondrial respiration,[51,123–126] microsomal protein synthesis,[127–129] intracellular signaling via the PI cascade[130–133] as well as generation of hydroxyl radicals[134–136]—all of which have been observed in experimental models of aminoglycoside toxicity. However, the observation that gentamicin is transported to the Golgi complex shortly after endocytic uptake[89,90] provides an alternate mechanism by which these drugs gain access to other organelles. Recently, proteomic analysis following gentamicin administration indicates energy production impairment and a mitochondrial dysfunction occurring in parallel to the onset of nephrotoxicity.[137]

Further insight into the pathogenesis of aminoglycoside nephrotoxicity has been gleaned from studies of interventions that modify the severity of this disorder in

experimental animals. Williams and colleagues[138–140] first reported that polyasparagine and polyaspartic acid (PAA) inhibited binding of gentamicin to rat renal brush-border membrane in vitro and when injected in vivo conferred protection against the development of aminoglycoside nephrotoxicity without inhibiting the renal cortical accumulation of drug. These findings have been confirmed and extended by three groups of investigators.[141–149] The mechanism by which PAA protects against aminoglycoside nephrotoxicity was shown to be related to the ability of PAA, a polyanion, to form electrostatic complexes with the polycationic aminoglycoside antibiotics[146,150,151] presumably within the endocytic compartment,[148] thereby preventing aminoglycosides from binding to anionic phospholipids, from inhibiting lysosomal phospholipase degradation of phospholipid, from forming lysosomal myeloid bodies, and from disrupting the PI cascade.[150] Additional support for this theory is provided by the observation that PAA prevented gentamicin from depressing glycerol permeability or aggregating PI-containing liposomes,[150] effects previously shown to be dependent on gentamicin binding electrostatically and by hydrogen bonding to PI.[100,107] Subsequently, other compounds capable of forming electrostatic complexes with aminoglycosides have been reported to protect against nephrotoxicity.[152–155]

An analog of pentoxifylline, HWA-448, was shown to protect against gentamicin toxicity in a cell culture model.[156] Similar to PAA, HWA-448 did not depress the membrane binding or cellular uptake of gentamicin. It remains unknown whether HWA-448 forms a complex with gentamicin within the endosomal compartment.

Recently, it was demonstrated that glibenclamide (a sulfonylurea) has protective effects against gentamicin-induced nephrotoxicity in rats.[157] Morales et al. suggest that the pleiotropic effects of metformin can decrease gentamicin nephrotoxicity by improving mitochondrial homeostasis.[158]

31.1 Risk Factors for Aminoglycoside Nephrotoxicity

Patient factors
- Older patients[a]
- Preexisting renal disease
- Magnesium potassium, calcium deficiency[a]
- Intravascular volume depletion,[a] hypotension[a]
- Hepatic syndrome
- Sepsis syndrome[a]
Aminoglycoside factors
- Recent aminoglycoside therapy
- Larger doses[a]
- Treatment of three or more days[a]
- Drug choice: gentamicin,[a] amikacin[a]
- Frequent dosing interval[a]
Concomitant drugs
- Amphotericin B
- Cephalosporines
- Cisplatin
- Clindamycin
- Cyclosporine
- Foscarnet
- Furosemide
- Intravenous radiocontrast agents
- Piperacillin
- Vancomycin

[a]Concurrent with experimental nephrotoxicity data.
Adapted from Verpooten GA, Tulkens PM, Molitoris BA. Aminoglycosides and vancomycin. In: De Broe ME, Porter GA, Bennett VM, Verpooten GA, eds. Clinical Nephrotoxins – Renal Injury from Drugs and Chemicals, 2nd ed. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2003:151–170.

Treatment and Prevention of Aminoglycoside Nephrotoxicity

The efficacy of PAA and other anionic compounds in preventing nephrotoxicity in humans has yet to be established. Therefore, the primary focus of treatment is prevention, and this can be accomplished by understanding and modifying, when possible, the risk factors (Table 31.1) for this complication.[159–161] Risk factors may be categorized into those that are determined by the individual patient and not easily influenced, if at all, and those that are determined by the clinician and potentially controllable (Table 31.1).

Prominent among the risk factors peculiar to the patient and not modifiable is advanced age.[159] The mechanism is probably multifactorial and includes age-related decline of renal function that, if not appreciated and corrected for, results in excessive dosing.[162] Animal studies suggest that aging is associated with altered renal pharmacokinetics accompanied by increased renal cortical accumulation of drug.[163] Increased susceptibility of the aging kidney to aminoglycoside toxicity has also been suggested,[164] possibly on the basis of an age-related impaired capacity for cellular repair and regeneration. Male gender has been shown to carry increased risk for aminoglycoside nephrotoxicity in the rat,[165] whereas female gender has been identified as a risk factor in humans.[159] The reason for this difference has not been established.

Obesity carries increased risk for aminoglycoside nephrotoxicity that is unexplained by differences in the volume of distribution or renal clearance of drug.[166] The increased risk associated with chronic liver disease[159] may be related to the alterations in extracellular volume, hemodynamics, and electrolyte balance commonly observed in this disorder, all of which are known to promote renal cortical accumulation of drug.[78] Preexisting chronic renal insufficiency is associated with increased risk primarily due to failure to adjust appropriately the dose of aminoglycoside for the level of impaired kidney function.[167] Renal hypoperfusion from any

cause carries an increased risk of aminoglycoside nephrotoxicity whether the renal ischemic insult occurs before,[85] during,[34] or after drug administration.[32] The latter observation is particularly worthy of note because it implies that the increased risk of nephrotoxicity persists even after the drug has been discontinued. The prolonged half-life of aminoglycosides in renal cortex[78] may contribute to this risk. Three components of the septic state—renal hypoperfusion, endotoxemia, and hyperthermia—have been identified as factors contributing to the heightened risk of nephrotoxicity during aminoglycoside therapy.[35–37] Renal hypoperfusion[35,85] and endotoxemia[33,168] are associated with increased accumulation of drug in renal cortex; however, this factor alone does not explain the increased risk.

Of those risk factors that are potentially modifiable by the clinician, the most important are daily drug dose, interval of dosing, and the duration of therapy. A direct relationship between total dose (daily dose plus duration of therapy) and nephrotoxicity has been consistently found in experimental animals[26,47,51,56,71] and in humans.[9,15,159,160,167] Animal studies have shown that the same dose of a drug administered in two or three divided doses leads to greater renal accumulation of the drug and greater nephrotoxicity than if it was given as a single dose.[169,170] Two trials in humans found that the dosage schedule had a critical effect on the renal uptake of gentamicin, netilmicin,[171] amikacin, and tobramycin.[172] The study was carried out in patients with normal renal function (serum creatinine between 0.9 and 1.2 mg per dL, proteinuria lower than 300 mg per day) who had renal cancer and submitted to nephrectomy. Before surgery patients received gentamicin (4.5 mg/kg/day), netilmicin (5 mg/kg/day), amikacin (15 mg/kg/day), or tobramycin (4.5 mg/kg/day), as a single injection or as a continuous intravenous infusion over 24 hours. The single-injection schedule resulted in a 30% to 50% lower cortical drug concentration of netilmicin, gentamicin, and amikacin compared with administration by continuous infusion (Figs. 31.2 and 31.3). For tobramycin, in humans as well as in rats, no difference in renal accumulation could be found, indicating the linear cortical uptake of this particular aminoglycoside. Administration of drug by continuous intravenous (IV) infusion carries the highest risk of nephrotoxicity with respect to gentamicin, tobramycin, and netilmicin but not amikacin.[80,170,173] These observations have stimulated studies in humans to assess the antimicrobial efficacy of once per day dosing with an aminoglycoside administered alone or in combination with a β-lactam antibiotic.[174–177]

Several meta-analyses pooled the data of individual randomized controlled trials (RCTs) (Table 31.2),[178–187] including a meta-analysis specifically of the studies in immunocompromised patients.[187] It is apparent that only the meta-analyses that combine the results of the individual RCT by means of a fixed-effects model yielded significant results in favor of less nephrotoxicity in the single daily dose regimens. However, given the inhomogeneity of the study designs and the different aminoglycoside used, it seems

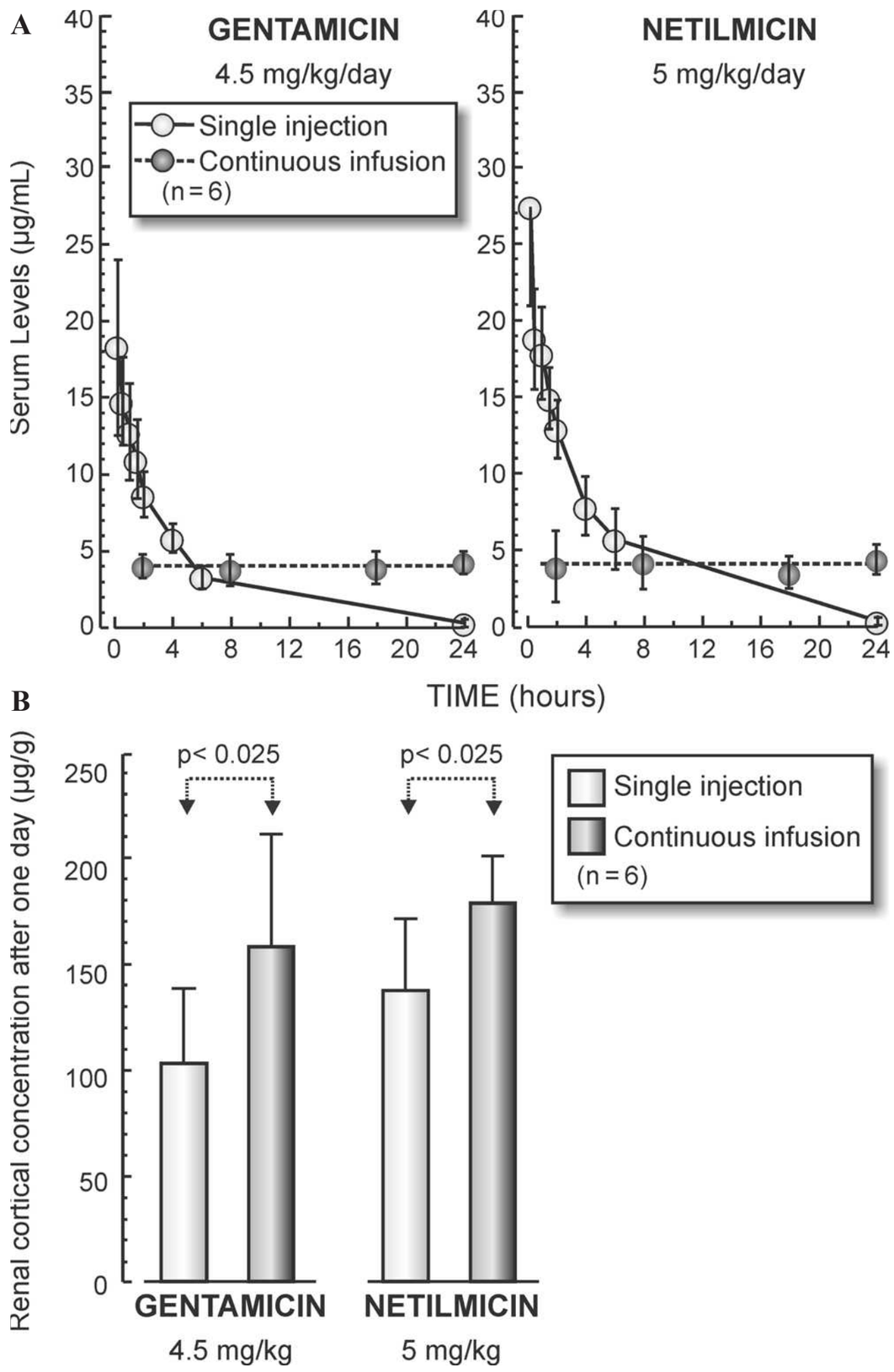

FIGURE 31.2 **A:** Course of serum concentrations of gentamicin and netilmicin after administration of the dose by a 30-minute intravenous injection or by continuous infusion of 24 hours. **B:** Cortical concentration of gentamicin and netilmicin after administration by the previously mentioned administration schedules. (From Verpooten GA, et al. Once-daily dosing decreases renal accumulation of gentamicin and netilmicin. *Clin Pharmacol Ther.* 1989;45:22, with permission.)

prudent to use the random effects model to combine the individual studies. The meta-analyses that used this technique did not show a significant difference in the two dosing regimens. Nevertheless, in all analyses the single daily dose regimen was associated with a decrease in nephrotoxicity. Even the most recent prospective study[188] evaluating the efficacy and nephrotoxicity of once daily administration of gentamicin versus multiple daily administration in 52 children could not show a difference in incidence of nephrotoxicity in both groups. Although a decrease in nephrotoxicity rates in once daily dose regimens has not been established, extended interval dosing strategies have never been associated with an increased risk of nephrotoxicity. The main reason why the majority of acute care hospitals[189] have adopted this strategy

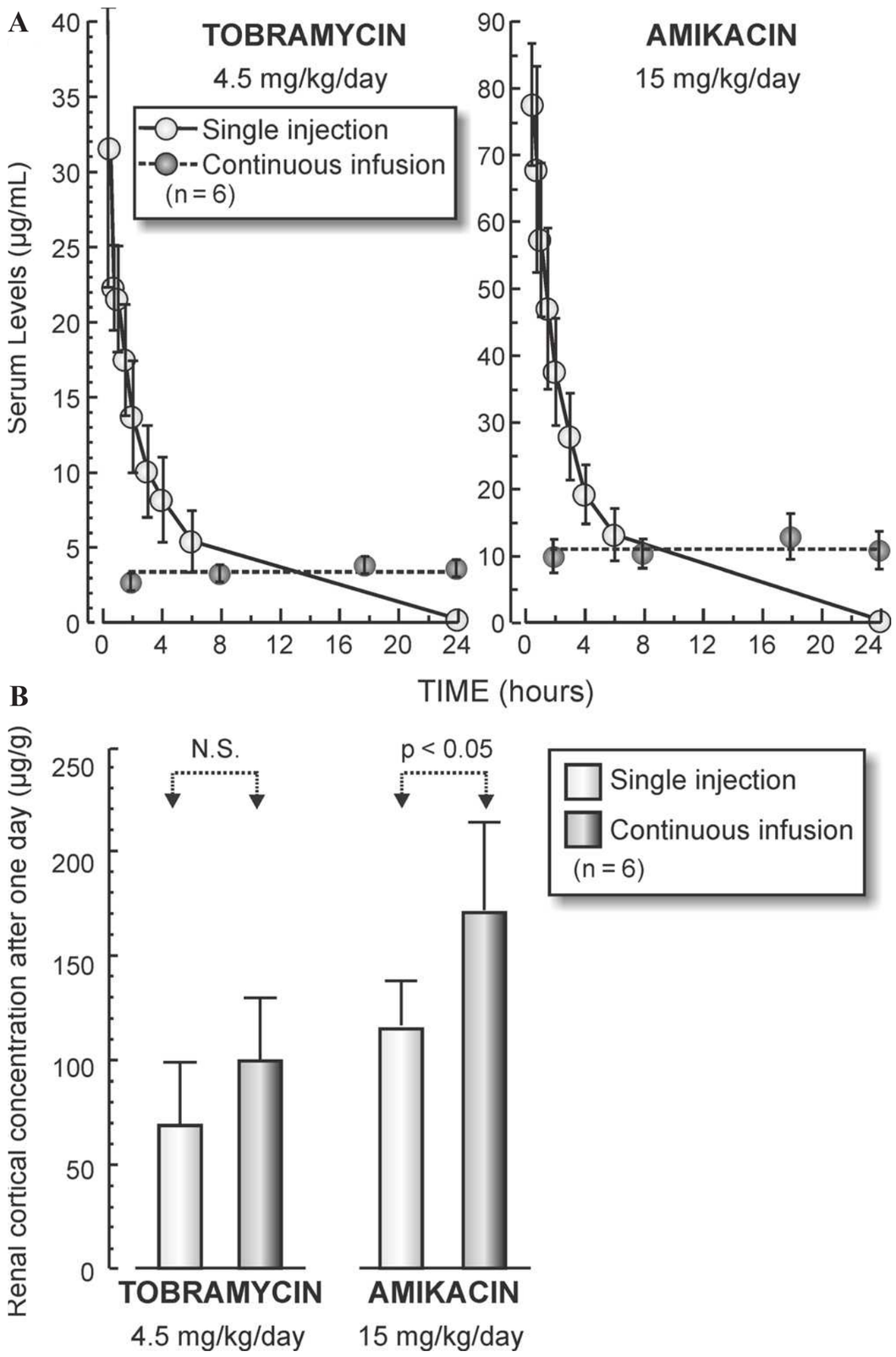

FIGURE 31.3 **A:** Course of serum concentrations of tobramycin and amikacin after administration of the dose by a 30-minute intravenous injection or by continuous infusion of 24 hours. **B:** Cortical concentration of tobramycin and amikacin after administration by the previously mentioned administration schedules. (From De Broe ME, Giuliano RA, Verpooten GA. Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. *J Antimicrob Chemother.* 1991;27[Suppl C]:41, with permission.)

is that once daily dosing provides a cost-effective method for administration of aminoglycosides by reducing work load among service personnel and by reducing or even eliminating the need for therapeutic drug monitoring.[190–192]

Volume depletion,[193] hypokalemia,[194] hypomagnesemia,[195] and metabolic acidosis[196] all carry increased risk for nephrotoxicity. In the case of volume depletion and hypokalemia, the increased risk appears to be related to increased accumulation of drug in renal cortex.[78] The mechanism underlying the increased risk associated with hypomagnesemia has not been definitely established but may relate to the competition between divalent cations and the cationic aminoglycoside antibiotics for critical membrane binding sites.[197] In the case of metabolic acidosis, the reduced pH promotes increased protonation of aminoglycoside antibiotics and augments the reactivity of these organic polycations with membrane anionic phospholipids.[64,101,119]

In rats it was shown that uric acid worsens gentamicin-induced nephrotoxicity. The mechanism is likely to implicate downregulation of MMP9.[198]

Finally, the risk of nephrotoxicity has been shown to be augmented when aminoglycoside antibiotics are administered in conjunction with certain drugs and pharmaceutical agents, some of which have intrinsic nephrotoxicity potential. These include amphotericin B,[177] cephalothin but not third generation cephalosporins,[199] vancomycin,[200,201] cisplatin,[202] furosemide,[203] calcium channel blockers,[204] radiocontrast agents,[205] and nonsteroidal anti-inflammatory drugs.[206] Many of these synergistic interactions have been identified in animal studies so that the relevance of these observations to humans remains to be established. Nevertheless, prudence dictates that potentially nephrotoxic drugs should be avoided if possible in patients who are receiving or have recently completed therapy with aminoglycoside antibiotics.

The prevention of aminoglycoside nephrotoxicity requires that these drugs be used only for well-defined indications and that they be prescribed in the appropriate dose and for the appropriate duration to achieve the therapeutic goal. Optimization of therapy for aminoglycosides requires understanding the relationship between exposure and response as well as that between exposure and toxicity. Furthermore, daily administration is much preferred, and stopping therapy as quickly as possible (a week or less may be optimal) will contribute to the ability to optimize therapy.[207] Dosing based on individualized drug pharmacokinetics derived from measurements of serum drug concentration would appear to be a rational approach. Unfortunately, prospective studies have failed to demonstrate that dosing based on drug pharmacokinetics reduces the incidence of nephrotoxicity.[208] Indeed, eight prospective, RCTs specifically designed to investigate the effect of pharmacokinetic dosing[209] on aminoglycoside expression of nephrotoxicity could be identified from the literature.[210–217] These individual studies have been unable to detect any change in the incidence of this adverse event. Nevertheless, close monitoring of serum drug concentration is still warranted, especially in high risk patients to ensure that therapeutic concentrations are achieved. Even when those factors known to influence risk are absent or have been minimized or eliminated, aminoglycoside nephrotoxicity will still occur in a certain percentage of appropriately dosed patients. These patients exhibit excessive renal accumulation of drug or increased sensitivity to a given level of drug accumulation.[218] The clinician must be constantly alert to the possibility of aminoglycoside nephrotoxicity and monitor all patients on aminoglycoside therapy for this potential complication. The intensity of monitoring is dictated in part by the relative risk factors present. At a minimum, frequent measurements of serum creatinine concentration,

31.2 Meta-analysis of the Incidence of Nephrotoxicity in Single Daily Dosing versus Multiple Dosing of Aminoglycosides

Author	No. of RCT	Method	Results (95% CI)
Blaser and König, 1995[172]	24	Summation	RR 0.82
Galloe et al., 1995[173]	16	Not given	RR 1.00 (0.98–1.02)
Barza et al. 1996[174]	21	Random effects model	RR 0.78 (0.57–1.07)
Munckhof et al., 1996[175]	15	Random effects model	RD −1.3% (−5%–3.1%)
Ferriols-Lisart and Alos-Aliminanan, 1996[176]	18	Fixed effects model	OR 0.60 (0.40–0.86)
Freeman and Strayer, 1996[177]	15	Fixed effects Peto	OR 0.70 (0.51–0.94)
Hatala et al., 1996[178]	13	Random effects model	RR 0.87 (0.60–1.26)
Ali and Goetz, 1997[179]	26	Random effects model	RD −0.18% (−0.99%–3.75%)
Bailey et al., 1997[180]	22	Random effects model	RD −0.6% (−2.4%–1.1%)
Hatala et al., 1997[181]	4	Random effects model	RR 0.78 (0.31–1.94)

CI, confidence interval; OR, odds ratio; RR, risk ratio; RD, risk difference; RCT, randomized controlled trials.
Adapted from Verpooten GA, Tulkens PM, Molitoris BA. Aminoglycosides and vancomycin. In: De Broe ME, Porter GA, Bennett VM, Verpooten GA, eds. Clinical Nephrotoxins – Renal Injury from Drugs and Chemicals, 2nd ed. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2003:151–170.

generally every 2 to 3 days, should be performed. In high risk patients, daily creatinine clearances and urinalysis may be required to detect early signs of toxicity before a rise in serum creatinine concentration or serum trough level of drug becomes evident. Hoffmann et al. showed in rats that a number of recently developed urinary markers (among them Kim-1) had an increased sensitivity of aminoglycoside nephrotoxicity in rats.[219,220] How far this can be extrapolated to the human situation has not been studied thouroughly. If renal injury occurs, then the drug should be stopped if possible or the dosage should be reduced to prevent the accumulation of drug in serum and further toxic injury related thereto. Careful attention must be paid to maintaining fluid and electrolyte balance and avoiding potential insults to the kidney related to renal hypoperfusion or exposure to other potential nephrotoxins. Even when nephrotoxicity is recognized early and the drug is discontinued, renal failure may progress over the next 5 to 10 days, with the serum creatinine and blood urea nitrogen (BUN) rising to disturbingly high levels, where they may remain for a number of days before renal function slowly begins to improve. No specific therapy for hastening recovery has been identified to be effective in humans. In an animal model, epidermal growth factor was shown to accelerate recovery.[221] The prognosis for recovery of renal function is generally good except in those cases where the underlying disease exposes the kidney to persisting or recurrent insults related to sepsis, hypotension, and hypoperfusion.

Nagai and Takano reviewed the possibility of coadministration of agents which may inhibit the binding of nephrotoxicity drugs (particularly aminoglyclosides) to receptor(s) responsible for the endocytic processes in renal proximal tubular cells which might reduce the incidence of nephrotoxicity.[222]

Trimetazidine is an anti-ischemic metabolic agent improving cardiac glucose utilization through inhibition of fatty acid. Gentamicin nephrotoxicity is attenuated by the cytoprotective effect of trimetazidine. It may be inferred that trimetazidine inhibits also the reabsorption and consequently the accumulation of gentamicin in the proximal tubular cell.[223]

β-LACTAM ANTIBIOTICS

The β-lactam antibiotics comprise the penicillins, cephalosporins, and carbapenems. ARF has been observed with this class of antibiotics as a result of acute proximal tubular cell necrosis or allergic interstitial nephritis. Studies in animals have established the relative nephrotoxicity potentials of β-lactam antibiotics as cephaloglycin > cephaloridine >> cefaclor > cefazolin > cephalothin >>> cephalexin, ceftazidime, and penicillins, which do not exhibit clinical

nephrotoxicity.[224] The selective toxic potential of β-lactam antibiotics toward renal proximal tubular cells appears to be causally linked to their concentrative uptake by the organic anion transport system and their intrinsic reactivity toward sensitive intracellular target proteins.[224,225] The importance of the organic anion transport system to the nephrotoxic potential of these agents is supported by the observations that (1) toxicity is restricted to β-lactams that are secreted by this transport system, (2) toxicity can be prevented by inhibition of organic anion transport, and (3) maneuvers that increase the intracellular uptake of drug augment toxicity.[224,225] The product of intracellular drug concentration and time, defined as the area under the curve (AUC), is an important determinant of toxicity. Among the cephalosporins, the greatest AUC is observed with cephaloridine.[224,225] This agent is readily transported into proximal tubular cells across the basolateral membrane; however, its egress across the apical membrane is retarded due to the fact that cephaloridine is a zwitterion and the cationic moiety impedes its permeation across the luminal membrane.[226] Therefore, at equivalent doses, the AUC for cephaloridine is significantly higher than that of other cephalosporins. Cephaloglycin, the most nephrotoxic of the cephalosporins released for clinical use, has a renal cortical AUC only one fifth that of cephaloridine.[227] The greater nephrotoxicity of cephaloglycin reflects the fact that it is far more reactive than cephaloridine toward sensitive intracellular target proteins.[224,228] Three molecular mechanisms have been implicated in the pathogenesis of cephaloridine nephrotoxicity: (1) lipid peroxidation,[229] (2) competitive inhibition of mitochondrial carnitine transport and fatty acid oxidation,[230,231] and (3) inhibition of mitochondrial respiration consequent to inactivation by acylation of mitochondrial anionic substrate transporters.[232,233]

In the case of the other nephrotoxic β-lactam antibiotics, the pathogenesis of toxicity appears to be linked primarily to depression of mitochondrial respiration. This conclusion is supported by the following observations from in vivo animal studies.[224,225,227,232,233]

1. The nephrotoxic potential of β-lactams correlates with the magnitude of inhibition of mitochondrial respiration.
2. Irreversible inhibition of mitochondrial respiration occurs within 1 hour after administration of a nephrotoxic dose.
3. Inhibition of respiration precedes the appearance of ultrastructural mitochondrial damage that resembles ischemic and cyanide injury.

Although only a limited number of β-lactam antibiotics cause toxic injury after in vivo exposure, many of these agents exhibit the capacity to inhibit in vitro mitochondrial respiration, especially that component supported by succinate.[234] Inhibition of mitochondrial respiration is observed within 5 minutes of in vitro drug exposure. Increasing the concentration of succinate reverses the inhibition, presumably as a consequence of competitive displacement of drug from the mitochondrial membrane anionic carrier. However, as the exposure of mitochondria to drug is augmented by raising the product of drug concentration and time, inhibition of mitochondrial respiration becomes progressively irreversible, which has been attributed to drug-induced acylation and inactivation of the transporter.[224,225] The rank order of cephalosporins with respect to their potential to acylate target proteins in vitro is ceftazidime > cefaclor > cephaloglycin > cephalothin > cephaloridine > cefazolin >> cephalexin, and several penicillins.[224,225] This order is at variance with their in vivo nephrotoxicity potential, which is cephaloglycin > cephaloridine >> cefaclor > cefazolin > cephalothin >>> cephalexin, ceftazidime, and the penicillins. The explanation for the differences between the in vitro and in vivo toxicity potentials of these drugs resides in the important role of concentrative uptake of these drugs into intact proximal tubular cells by the organic anion transport system. Although ceftazidime and cefaclor exhibit high acylation activity in vitro, the AUC of these agents is low (only 7% that of cephaloridine and only 37% that of cephaloglycin), and this severely restricts their interaction with the mitochondrial anion transporter.[224,225] Mitochondrial injury also has been implicated as the major mechanism of nephrotoxicity caused by imipenem.[235,236] This drug is marketed in combination with cilastin, which inhibits the enzymatic breakdown of imipenem by cytoplasmic and brush-border dihydropeptidase and also inhibits its nephrotoxicity.

The therapeutic–nephrotoxic ratio of these agents is much more favorable than that of aminoglycoside antibiotics. The incidence of serum creatinine elevations is difficult to say with certainty, but severe nephrotoxic ARF is uncommon.[237,238] Similar to other antibiotics, high doses and prolonged therapy elevate the risk of nephrotoxicity. In animal studies, the incidence and severity of toxicity associated with β-lactam antibiotics were augmented by combined therapy with aminoglycoside antibiotics,[239] by renal ischemia,[240] and by endotoxemia.[241] In three prospective studies in human subjects, the combination of an aminoglycoside antibiotic with cephalothin was associated with a significantly higher incidence of nephrotoxicity.[242–244] Early reports suggested a possible interaction between several second generation cephalosporins and aminoglycoside antibiotics.[245] In contrast, a recent prospective study provides no evidence that combination therapy with third generation cephalosporins and an aminoglycoside antibiotic potentiates the risk of nephrotoxicity.[177]

The diagnosis of nephrotoxic ARF secondary to β-lactam antibiotics is suggested by the appropriate clinical setting in combination with a urine sediment and urinary indices typical of acute tubular cell necrosis. Establishing the precise diagnosis may be difficult in the presence of septicemia, hypotension, or other nephrotoxic drugs. It should be kept in mind that β-lactam antibiotics also cause ARF secondary to allergic interstitial nephritis.[246] The pattern of the rise in the BUN and serum creatinine may be indistinguishable from that seen with acute tubular cell necrosis.

The presence of large numbers of red and white blood cells in the urinary sediment, especially if associated with eosinophiluria and systemic signs of hypersensitivity (rash, fever, and eosinophilia), strongly suggests the diagnosis of allergic interstitial nephritis. However, in many patients, these clues are equivocal so that it may be necessary to perform a kidney biopsy to establish the correct diagnosis.

VANCOMYCIN

Vancomycin use in clinical medicine has increased significantly in recent years as a consequence of the rise in the incidence of methicillin-resistant staphylococcal infections. Because this antibiotic is poorly absorbed from the gastrointestinal tract, it is usually administered intravenously for the treatment of systemic infections. Vancomycin is not appreciably metabolized, and it is excreted essentially (80%–90%) entirely by the kidneys, primarily by glomerular filtration, as there is no evidence that the drug undergoes tubular absorption or secretion.[247] Therefore, drug dosing must be modified in subjects with renal failure.[248] Animal studies demonstrated that vancomycin had nephrotoxic and ototoxic potential.[249] The present data[250] suggest that oxidative stress and oxidative phosphorylation play an important role in vancomycin-induced nephrotoxicity. Erythropoietin seems to act as an antioxidant, diminishing the toxic oxidative effects of vancomycin on renal tissue. Early clinical experience in human subjects revealed a significant incidence of nephrotoxicity, which in retrospect may have been due to impurities generated during the initial manufacturing process.[251] More recent reports indicate that the incidence of nephrotoxicity associated with vancomycin ranges between 0% and 7% when given as sole therapy.[252] Animal studies initially suggested that vancomycin and aminoglycoside antibiotics interacted synergistically to cause ARF.[253] Recent reports indicate that a similar interaction occurs in humans.[201,202] Indeed, in a meta-analysis the incidence of nephrotoxicity associated with combination therapy was 13.3% greater than therapy with vancomycin alone. In a prospective study, comparing continuous versus intermittent infusion of vancomycin in severely ill patients, Wysocki et al.[254] found a significant rise in serum creatinine during treatment only in those patients who received vancomycin with other antibiotics including aminoglycosides. Monitoring vancomycin serum concentrations is not cost-effective in preventing vancomycin-induced nephrotoxicity in patients with normal renal function because the correlation between serum levels and antibacterial efficacy or toxicity remains controversial.[255] It should be noted that vancomycin has been reported to cause allergic interstitial nephritis[256]; however, this appears to be an uncommon complication. Teicoplanin, a glycopeptide antibiotic similar to vancomycin, is devoid of nephrotoxicity.

Recent data suggest higher rates of nephrotoxicity with recently recommended doses aiming to achieve the currently recommended trough level of 15 to 20 μg per mL.[257–260] These studies show an incremental risk of nephrotoxicity associated with higher vancomycin doses, ranging from 12% to 42.7% of patients. The risk increases with higher vancomycin maximum trough levels, longer duration of vancomycin use, concomitant use of other nephrotoxic agents, and in paitents who are crtically ill or have a previously compromised renal function.

Vancomycin has been a cornerstone antibiotic for the treatment of severe gram-positive infections in dialysis patients for decades. Whereas subtherapeutic vancomycin levels convey a risk of treatment failure and the further emergence of resistance in staphylococci, supratherapeutic vancomycin levels are associated with a dose-related incremental risk for nephrotoxicity and ototoxicity. Consequently, a narrow therapeutic range with a trough-level target between 15 and 20 μg per mL is recommended. Vancomycin dosing in hemodialysis patients is mainly influenced by the timing of administration (during or after dialysis), the type of filter used, and the duration of dialysis. Actual body weight, the interdialytic interval, and residual renal function are also considerations. As in patients with normal kidney function, a weight-based loading dose of 20 to 25 mg per kg should be used in dialysis patients. Although most fixed-dose maintenance regimens fail to reach target levels in the majority of hemodialysis patients, straightforward evidence on optimal maintenance dosing is lacking.[261]

Studies on the optimal dosing strategy for vancomycin in chronic kidney disease (CKD) patients and those on dialysis are needed.

SULFONAMIDE ANTIBIOTICS

The sulfonamide antibiotics and their metabolites are excreted primarily by the kidneys by a process involving glomerular filtration, tubular absorption, and tubular secretion.[262] The high incidence of nephrotoxic ARF observed with the first generation sulfonamides was due to their low solubility and the resultant precipitation of drug in the form of crystals that caused intratubular obstruction.[263] Sulfadiazine, a poorly soluble sulfonamide, continues to be used today in combination with pyrimethamine for the treatment of Toxoplasma encephalitis; nephrotoxicity manifested as hematuria, crystalluria, renal colic, and ARF may complicate therapy in 5% of cases.[264,265] These abnormalities usually subside with hydration and alkalinization of the urine.

Trimethoprim-sulfamethoxazole is administered intravenously in high concentration as therapy for Pneumocystis jiroveci pneumonia. Although the solubility of sulfamethoxazole is high, ARF secondary to crystal deposition of the parent drug or a metabolite has been reported.[266,267] More commonly, the elevation of serum creatinine observed in patients treated with this combination drug reflects inhibition of tubular secretion of creatinine by trimethoprim.[268,269] This effect is more pronounced in subjects with baseline elevation

of the serum creatinine secondary to underlying chronic renal insufficiency. Failure of the BUN to rise in proportion to the rise in serum creatinine should call attention to the correct diagnosis.

Sulfonamides including sulfamethoxazole also have been implicated in causing acute hypersensitivity reactions and ARF secondary to allergic interstitial nephritis.[246]

ANTIFUNGAL AGENTS

Amphotericin B is widely used as the drug of choice for the therapy of systemic fungal infections, especially in immunocompromised patients.[270,271] Unfortunately, the clinical application of this drug is accompanied by a number of dose-dependent toxic side effects, the most serious of which is ARF.[272,273] Amphotericin B is a polyene that consists of a large lactone ring with seven conjugated double bonds, seven hydroxyl groups, and a sugar moiety. It exhibits the propensity to bind to membrane sterols and form membrane pores, which in mammalian cells are estimated to be composed of eight molecules of cholesterol alternating with eight molecules of drug.[274] The resultant increase in membrane permeability to small electrolytes is thought to be a dominant factor in the toxicity of the drug. Amphotericin B binds preferentially to ergosterol, the major sterol of fungi, and this presumably explains the selective toxicity of this and similar drugs for fungi.[275]

The reason amphotericin B causes nephrotoxicity in humans and experimental animals is not apparent from its pharmacokinetics.[276,277] Because amphotericin B is poorly transported across the gastrointestinal tract, it must be administered intravenously. Its volume of distribution is about 4 L per kg. Up to 95% of drug in serum is bound, primarily to β-lipoproteins. The major depot site for amphotericin B is the liver, where up to 41% of administered drug can be recovered compared to 6% in the lung and 2% in the kidney. The elimination of amphotericin B from serum can be described by a triexponential curve, the half-lives of which are 24 hours, 48 hours, and 15 days, respectively. Less than 10% of administered drug is recovered in the urine, and there are no known metabolites.

Although the kidney is not a major route of amphotericin B elimination, it is the major site of toxicity, the incidence of which is influenced by daily drug dose, duration of therapy, and the presence of potentiating factors.[278,279] The clinical expression of amphotericin B nephrotoxicity is dominated by the appearance of azotemia and creatininemia, which may occur early in the course of drug therapy[279–281] and reflects depression of renal blood flow and GFR secondary initially to a reversible rise in renal vascular resistance. With prolonged therapy, depression of renal function may persist as a consequence of injury to tubular epithelium[282] and possibly the renal vasculature.[283] A variety of abnormalities of tubular function may be seen as well. These include incomplete distal renal tubular acidosis,[284] hypokalemia and hypomagnesemia secondary to renal tubular wasting of these cations,[285,286] and loss of urine concentrating capacity.[287] The urinary sediment frequently contains evidence of microscopic hematuria, pyuria, and cylinduria. Although most of these abnormalities are reversible after the drug is discontinued, full recovery may be delayed for a number of months. Chronic renal insufficiency may occur with prolonged or multiple courses of therapy.

Insight into the pathogenesis of amphotericin B nephrotoxicity has been gleaned from studies in experimental animals.[288] It has been shown that intravenous administration of amphotericin B elicits an acute depression of renal blood flow and GFR in association with an increase in renal vascular resistance that is not mediated by the renal nerves, by angiotensin II, by endothelium-dependent factors, or by tubular glomerular feedback.[289–292] These hemodynamic alterations have been shown to be modifiable by a variety of interventions including administration of calcium channel blockers,[293] a selective dopamine-1 receptor agonist,[294] saline loading,[295,296] atrial natriuretic peptide,[292,297] and theophylline suggesting its direct vasoconstrictive effect.[292] Depolarization of vascular smooth muscle consequent to the formation of membrane pores was postulated as the basic mechanism by which amphotericin B augmented renal vascular resistance.[288,292] Amphotericin B also induces tubular dysfunction in the rat that mimics alterations observed in humans.[297] The dominant site of tubular injury in the rat is the inner stripe of the outer medulla,[298] a zone that functions on the verge of hypoxia even under physiologic conditions. Investigators have postulated that hypoxic injury to this zone results from the demand for increased oxygen to support increased sodium transport stimulated by the heightened influx of sodium across the apical membrane made permeable by amphotericin B at a time when the supply of oxygen is reduced as a consequence of amphotericin B-induced reduction in renal blood flow.[298,299]

A contributory factor to the toxicity of amphotericin B is deoxycholate, the vehicle in which the drug is suspended. Deoxycholate was shown to be cytotoxic to renal tubular cells in vitro.[300] Various alternate vehicles and formulations for suspending amphotericin have been investigated in an attempt to reduce toxicity. Administration of amphotericin B in liposomes[276,301] or with other lipid preparations[302] has been reported to reduce the nephrotoxicity of this agent without compromising its therapeutic efficacy.

Lipid preparations of amphotericin B, commonly used to treat fungal infections, have been demonstrated to have reduced nephrotoxicity compared to conventional amphotericin B. However, a comprehensive comparison of nephrotoxicity induced by different lipid preparations of amphotericin B has not been performed. A meta-analysis was conducted to evaluate nephrotoxicity associated with amphotericin B lipid complex (ABLC) and liposomal amphotericin B (L-AmB).[303] Eleven studies reported between 1995 and 2008 were identified comparing nephrotoxicity resulting from the use of these agents. Eight of the 11 studies

were included in the meta-analysis. The Cochran-Mantel-Haenszel test was used to determine odds ratio (OR) and relative risk (RR), and the Breslow-Day test was used to analyze homogeneity of ORs across different studies. Analysis of all 8 studies (n = 1160) included in the meta-analysis showed an increased probability of nephrotoxicity in patients treated with ABLC versus L-AmB (OR, 1.75; RR, 1.55), but there was a significant lack of homogeneity across these studies ($P < 0.001$). After excluding the study by Wingard et al.,[304] the probability of experiencing nephrotoxicity was more similar between the two AmB lipid preparations (OR, 1.31; RR, 1.24; n = 916), particularly when the analysis included only the salvage patient population reported by Hachem et al.[305] (OR, 1.12; RR, 1.09; n = 839); the seven remaining studies were more homogeneous by Breslow-Day test ($P = 0.054$). Their results suggest that nephrotoxicity is generally similar for ABLC and L-AmB in patients receiving antifungal therapy and prophylaxis.

In a recent retrospective study conducted in 100 consecutive patients receiving L-AmB atdoses of 1, 3, and 5 mg per kg, hepatotoxicity was defined as an increase of bilirubin greater than 1.5 mg per dL or AST and ALT greater than three times the normal range. Nephrotoxicity was defined as an increase in serum creatinine of 0.5 mg per dL or an increase of 50% from baseline. Overall nephrotoxicity with L-AmB was common and often multifactorial. Lipid amphotericin B products are associated with lower rates of nephrotoxicity than conventional amphotericin; however, in this analysis, L-AmB was associated with a high incidence of nephrotoxicity.[306]

A recent study aimed at comparing the available evidence on the efficacy and safety of deoxycholate and lipid amphotericin B formulations (AMBF) in the treatment of invasive fungal disease in neonates.[307] The reviewed reports show that both amphotericin B deoxycholate (DAMB) and lipid formulations appear to have equal efficacy in treating invasive fungal disease (IFD) in neonates. The adverse effects of DAMB in neonates are considerably less than those in older children and adults. There is a trend of more nephrotoxicity reported with DAMB than with lipid formulations; however, the range reported is very wide (0%–70%). Neonates with normal baseline renal function appeared to tolerate DAMB relatively well. DAMB is inexpensive and effective in treating neonatal IFD. It appears to be safe for use as first-line therapy if the underlying risk for nephrotoxicity is low. Renal function and potassium have to be monitored closely. A sodium intake of 4 mEq/kg/day may significantly reduce DAMB nephrotoxicity.

A number of factors have been identified as potentiating the risk of amphotericin B nephrotoxicity (Table 31.3), and the physician should strive to eliminate or minimize these risk factors whenever possible. Fisher and coworkers[278] observed a 1.8-fold increase in risk of nephrotoxicity for each 0.1 mg per kg increment in the daily dose of amphotericin B. The risk of nephrotoxicity was increased 15.4-fold in patients who had an elevated serum creatinine prior to the start of amphotericin B therapy and 12.5-fold in patients who received diuretics during the course of amphotericin B therapy. The latter observation may reflect the powerful influence of sodium depletion on this complication. Sodium loading has been shown to minimize amphotericin B nephrotoxicity[279] so that special attention should be paid to ensure that the patient is optimally volume-repleted prior to the initiation of therapy with this agent (Fig. 31.4).

31.3 Risk Factors for Amphotericin B Nephrotoxicity

Daily drug dose
Duration of therapy
Chronic renal insufficiency
Sodium depletion
Renal hypoperfusion
Concomitant drug therapy/exposure
Diuretics
Aminoglycosides
Cisplatin
Radiocontrast agents
Cyclosporine

ANTIVIRAL AGENTS

Acyclovir is a potent antiviral agent effective in the treatment of infections caused by herpes simplex viruses.[309] Its major route of excretion is the kidney, which accounts for approximately 80% of total body clearance.[310] Given the fact that the renal clearance of acyclovir exceeds the creatinine clearance by severalfold, it follows that a substantial fraction of drug must be eliminated by tubular secretion, which promotes the attainment of tubular fluid concentrations in excess of the drug's estimated solubility of 1.3 mg per L.[310] The objective of the study by Gunness P et al.[311] was to determine whether acyclovir is a substrate for human BCRP. Transfected human embryonic kidney (HEK293) cells (containing the wild-type ABCG2 gene) were exposed to [8-(14) C]acyclovir (1 μmol per L) in the presence or absence of the BCRP inhibitor fumitremorgin C (FTC). Intracellular acyclovir accumulation was assessed using a liquid scintillation counter. Coexposure to FTC resulted in a significant (five-fold) increase in the intracellular accumulation of acyclovir.

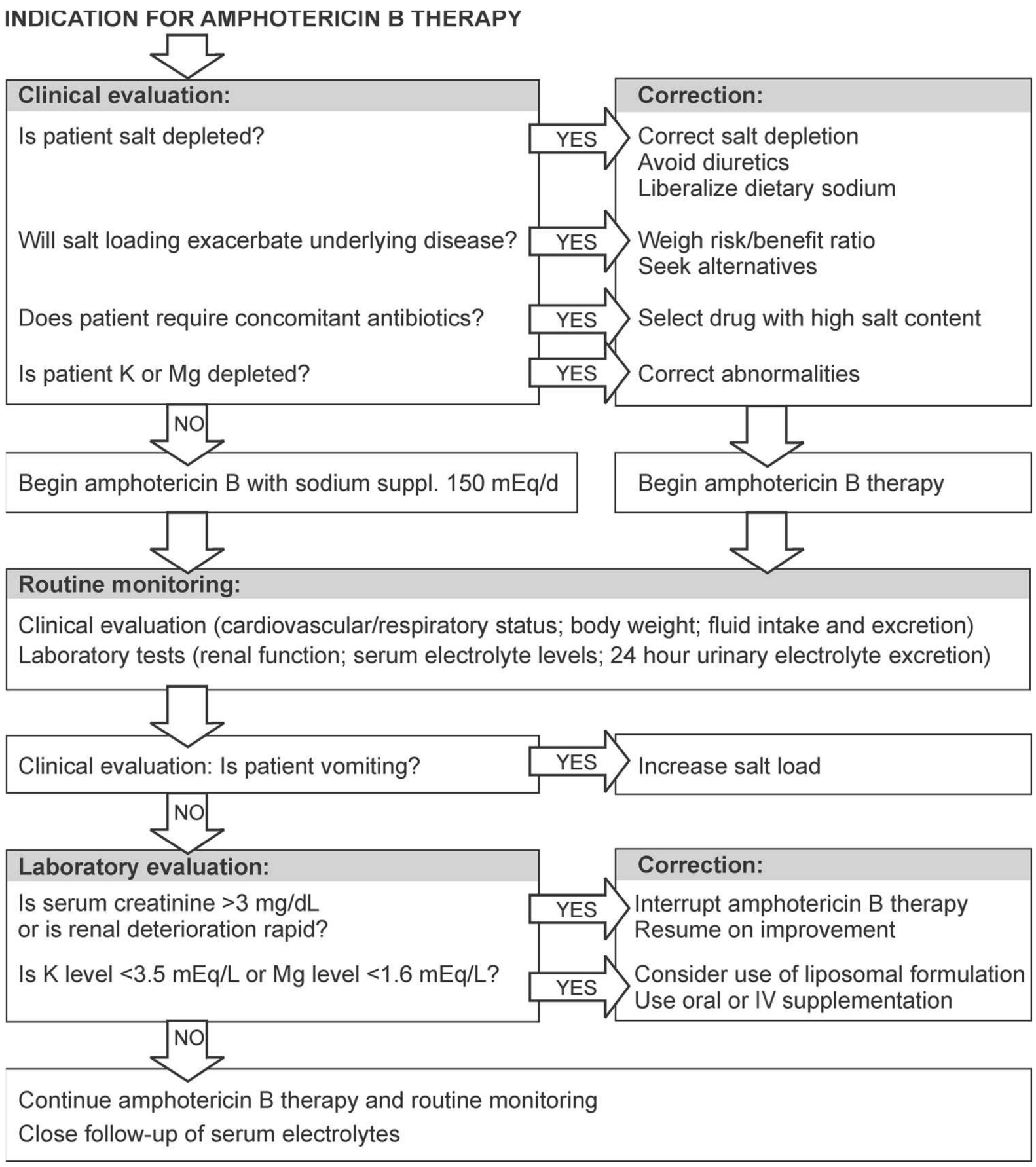

FIGURE 31.4 Proposed approach for management of amphotericin B therapy. (From Bernardo JF, Sabra R, Vyas SJ, Branch RA. Amphotericin B. In: De Broe ME, Porter GA, Bennett VM, Verpooten GA, eds. *Clinical Nephrotoxins – Renal Injury from Drugs and Chemicals,* 2nd ed. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2003:199–222.)

They found that acyclovir is a substrate for human BCRP/ABCG2. The study provides direct evidence for the role of human BCRP in acyclovir transport and its potential significance with respect to renal tubular transport of acyclovir and the direct renal tubular insult (nephrotoxicity) induced by the drug. Approximately 85% of the drug recovered in the urine is unchanged; the remainder is recovered as the principal metabolite, 9-carboxymethoxymethylguanine.[310] In several large series, acyclovir has been reported to cause elevation of the BUN and serum creatinine in 10% to 15% of cases.[312,313] In one series of 23 patients, an incidence of acute renal insufficiency of 48% was reported.[314] The clinical expression of nephrotoxicity may range from asymptomatic azotemia to renal colic with nausea and vomiting. Examination of the urinary sediment may reveal microscopic hematuria, pyuria, and birefringent crystals. The pathogenesis of acyclovir-induced ARF has been attributed to intratubular obstruction caused by precipitation of drug[313] as well as direct tubular cell toxicity.[315,316] High drug dose, rapid drug infusion, and low urine volume predispose to the development of ARF. In about half the cases, the onset of azotemia occurs during the first few days of therapy; it is usually transient and frequently resolves in response to increased fluid intake even when drug therapy is continued. Severe renal failure has been reported, however, even in patients who were prehydrated.[317] Fortunately, even in these cases, renal function usually recovers. In the rat infusion of acyclovir caused a decrease in whole kidney and single nephron GFR and renal plasma flow in association with an increase in renal vascular resistance.[318]

A retrospective review was conducted by Schreiber et al.[319] on all children (mean age 81 months; n = 126 [74 boys])

who were treated with aciclovir in a tertiary center between July 2005 and January 2006 and who met our inclusion criteria. GFR was calculated on the first day of treatment and at the peak measured creatinine level while on therapy, using Schwartz's method. Aciclovir therapy was associated with a significant increase in serum creatinine levels and a parallel decrease in GFR (n = 93; both P ≤.0001). Children with immunosuppression who received a variety of other nephrotoxic drugs exhibited more severe nephrotoxicity than those not receiving nephrotoxic drugs. In multiple regression analysis, the use of nephrotoxic drugs (P = .02) and impaired GFR at baseline (P = .04) were predictive for nephrotoxicity. Within the recommended age-dependent dosage schedule of aciclovir there was no effect of dose per kilogram, age, or sex on nephrotoxicity. The predictors of aciclovir nephrotoxicity were the concomitant use of nephrotoxic drugs and impaired GFR at baseline.

Foscarnet is an antiviral agent that is being used with increasing frequency for the treatment of cytomegalovirus infections and acyclovir-resistant herpes virus infections, particularly in immunocompromised individuals.[320,321] This agent is excreted unchanged in the urine by glomerular filtration and tubular secretion.[322] Major complications of therapy include ARF, often severe and of uncertain pathogenesis,[323,324] and electrolyte abnormalities that include hypercalcemia, hypocalcemia, hypophosphatemia, hypomagnesemia, and hypokalemia.[265,325] ARF secondary to crystal deposition has been described as well.[326] Volume expansion by infusing saline has been reported to greatly reduce the incidence and severity of ARF.[324,327]

Cidofovir is an antiviral nucleotide analog indicated for the treatment of cytomegalovirus retinitis in patients with AIDS.[328] The drug is eliminated primarily by the kidneys by glomerular filtration and tubular secretion via the organic acid transport system.[329] The major complication of therapy with this agent is nephrotoxic injury to proximal tubular cells but this complication can be significantly reduced by the coadministration of probenecid which presumably blocks the renal tubular uptake of cidofovir and decreases the renal elimination of the agent.[330]

Atazanavir belongs to the protease inhibitor class and is used in combination with other antiretroviral drugs. Recently stone formation and less common crystal nephropathy was decribed with this drug.[331,332] Atazanavir is metabolized in the liver and only 6% is excreted unchanged by the kidneys and insoluble at acid pH. Consequently risk factors to develop renal complications are volume depletion, alkaline urine, and liver dysfunction resulting in a decrease of metabolism of the drug.[333]

Tenofovir, another antiretroviral drug, has gained widespread use on the basis of its efficacy, tolerability, and patient-friendly dosing schedule.[334] Herlitz and colleagues[335] demonstrated that tenofovir is a proximal tubular mitochondrial toxin in humans. Renal histology in 10 HIV patients with tenofovir associated clinical nephrotoxicity demonstrated that proximal tubular injury with tenofovir was associated with nephrotoxicity and varying degrees of chronic tubulointerstitial scarring. Prominent eosinophilic inclusions within proximal tubular cell cytoplasm, which represented giant, abnormal mitochondria, were noted on light microscopy. These inclusions are easily identifiable, as they stain brightly with hemaotxylin and eosin stain or fuchsinophilic with trichrome stain. On electron microscopy, mitochondria varied widely in shape and size; some were small and rounded, whereas others were swollen with irregular contours. Loss and disorientation of cristae were observed in enlarged mitochondria, whereas the overall number of mitochondria was significantly decreased in some tubular cells.

These drugs act primarily by decreasing mitochondrial DNA (mtDNA) replication by inhibiting mitochondrial DNA polymerase-γ, which is the only enzme capable of replicating mtDNA. As a result, mtDNA and a number of the mtDNA-encoded enzymes involved in electron transport chain function and oxidative phosphrylation are depleted resulting in disturbed mitochondrial function. This ultimately causes, among other effects, a deficit in adenosine triphosphate production, impaired cell function, and cell injury and/or death.[336,337]

Renal handling of tenofovir consists in a combination of glomerular filtration and proximal tubular secretion, which in part explains the proximal tubular toxicity of tenofovir.[338] Tenofovir is transported via organic anion transporter-1 (OAT-1) from the basolateral into proximal tubular cells, where it is translocated into the urine through apical efflux transporters such as multidrug resistance protein-2 (MRP-2) and MRP-4. Using kidney tissue from OAT-1, MRP-4 knockout mice and wild type mice, Kohler et al. demonstrated that both OAT1 and MRP4 have a direct role in transport and efflux of tenofovir, regulating levels of tenofovir in proximal tubules. Disruption of OAT1 activity prevents tenofovir toxicity but loss of MRP4 can lead to increased renal proximal tubular toxicity. These data help to explain mechanisms of human TDF renal toxicity.[339] Impaired MRP-driven efflux activity can reduce tenofovir secretion and increase intracellular concedntrations. A single-nucleotide polymorphism in the MRP-2 efflux transporter gene (ABCC2) has been documented in HIV-positive patients who developed tenofovir-induced nephrotoxicity, supporting this hypothesis.[340] Endogenous anions and other drugs may compete with tenofovir for these efflux transport pathways. The excretory pathway defects can lead to increased tenofovir trafficking through and/or increased concentrations within proximal tubular cells, enhancing risk for mtDNA depletion and mitochondrial dysfunction. Genetic factor testing (for the single-nucleotide polymorphism in ABCC2) to identify high-risk patients and targeted interventions reduces OAT-1 transport of tenofovir into tubular cells and may allow HIV-positive patients to be protected from nephrotoxicity of the drug. Out of these series of observations, one may conclude that tenofovir may cause toxic tubular damage (mitochondrial toxin) in exposed HIV patients. The clinical expression

of this form of nephrotoxicity can develop at any time point during treatment with this drug. Patients may not recover from the injury and develop CKD. The renal handling of tenofovir can explain the small subset of HIV patients developing this form of nephrotoxicity.

PENTAMIDINE

Pentamidine has been used for the treatment of P. jiroveci pneumonia since the 1950s. In the pre-AIDS era, pentamidine therapy was complicated by ARF in about 25% of cases.[341] The incidence of ARF in patients with AIDS treated with pentamidine appears to be substantially higher than this figure, and it is unexplained by greater drug dose, longer duration of therapy, or concomitant therapy with other potentially nephrotoxic agents.[342] The mechanism of pentamidine-induced ARF has not been established. Although pentamidine is concentrated in the kidney,[343–345] pharmacokinetic studies utilizing a high-performance liquid chromatography assay indicate that <5% of the drug is excreted in the urine each day.[344,346] The mechanism of renal elimination is not known.

Pentamidine nephrotoxicity presents as nonoliguric ARF beginning 7 to 10 days after the start of therapy. Urinalysis reveals mild proteinuria, microscopic hematuria, pyuria, and cylinduria. Most patients experience mild to moderate ARF, but occasionally severe renal failure necessitating dialysis therapy occurs. In one series, azotemia was accompanied by hyperkalemia in association with a picture of hyperchloremic metabolic acidosis.[342] Renal magnesium wasting has been observed in several cases.[347] Recovery of renal function usually begins within a week after stopping drug therapy and in most cases returns to baseline within several weeks.

Chronic renal insufficiency, volume depletion, cumulative dose, and concurrent use of other nephrotoxic drugs heighten the risk of pentamidine nephrotoxicity in humans.[269,348] In the rat, pentamidine nephrotoxicity was potentiated by amphotericin B, tobramycin, and cyclosporine, whereas it was ameliorated by fosfomycin, D-glucaro-1,5-lactam, verapamil, and enalapril.[349]

NEPHROTOXICITY OF CYCLOSPORINE

Since its clinical use as an immunosuppressant drug in the early 1980s, cyclosporin A (CsA) has tremendously improved the outcome of solid organ (kidney, heart, liver, lung, and pancreas) and bone marrow transplants.[350,351] In more recent years, the immunosuppressive properties of CsA have also been used in the treatment of autoimmune diseases (psoriasis, uveitis, and severe rheumatoid arthritis) as well as steroid-resistant nephrotic syndrome.

The major side effect of CsA is its renal toxicity. Although, in preclinical animal studies, renal side effects were not observed,[352–354] early reports from clinical practice revealed the nephrotoxicity of CsA.[355–357] Since that time, numerous observations have added to the overwhelming evidence of three different forms of cyclosporine nephrotoxicity.[358–366] This toxicity is not restricted to only the field of kidney transplantation but has also unequivocally been documented in heart,[367,368] bone marrow,[368] liver,[369,370] and pancreas transplantation,[371] as well as in a variety of autoimmune diseases,[372–375] in which a priori rejection of the kidney graft is absent.

Based on experimental data and clinical experience, this chapter intends to summarize the present knowledge about the three different forms of cyclosporine nephrotoxicity: ARF (with sometimes protracted course evolving to chronicity); the hemolytic–uremic-like syndrome; and chronic irreversible nephrotoxicity.

Clinical Pharmacology of Cyclosporin A

The selective immunosuppressive effects of CsA were described for the first time in 1976.[376] CsA is a lipophilic fungal peptide with a molecular weight of 1.203 daltons, consisting of 11 amino acids (Fig. 31.5). As a consequence of its high hydrophobicity, CsA interacts easily with phospholipid bilayer membranes, whereas some CsA amino acids form a hydrophilic active immunosuppressive site.[377]

CsA is available for clinical use in three formulations: one stabilized in castor oil (Cremophor) for IV injection, the second as a microemulsion formulation (Neoral), and a third formulation as soft gelatin capsules. The pharmacokinetic profile of the conventional CsA formulation (Sandimmune) exhibits a high degree of interpatient and intrapatient variability.[378–380] Pharmacokinetic studies in healthy subjects and renal transplant recipients have shown that the more recent microemulsion formulation of CsA possesses superior pharmacokinetic characteristics, with more complete and predictable absorption of the drug from the gastrointestinal tract, resulting in less pharmacokinetic variability.[381,382] In clinical trials, the microemulsion formulation of CsA increased drug exposure and reduced the incidence of acute rejections, without incremental toxicity.[383–385]

In the circulation, CsA is mainly bound to high, low, or very low density lipoproteins and to chylomicrons.[386] Only a small fraction of CsA circulates unbound. The volume of distribution ranges from 4 to 8 L per kg of body weight.[387] Due to its hydrophobicity, CsA dissolves extensively in cell membranes and tissue lipids.[388] CsA accumulates in lymphocytes, liver, kidney, heart, lung, and neural and muscle cells.[389]

CsA has a median half-life of 6.4 to 8.7 hours and is predominantly eliminated by hepatic metabolism through specific isoenzymes of the cytochrome P-450 superfamily.[390] More than 90% of the parent compound and the metabolites are excreted in the bile, whereas only 6% is eliminated by the kidneys.[388] Significant individual differences in CsA clearance rates,[390,391] with a median value of 12 mL/min/kg, can be explained by wide genetic differences among individuals in the content of cytochrome P-450 isoenzymes, as well as a variety of other factors such as patient age,[392] the functional status of the liver,[393] and interactions with other

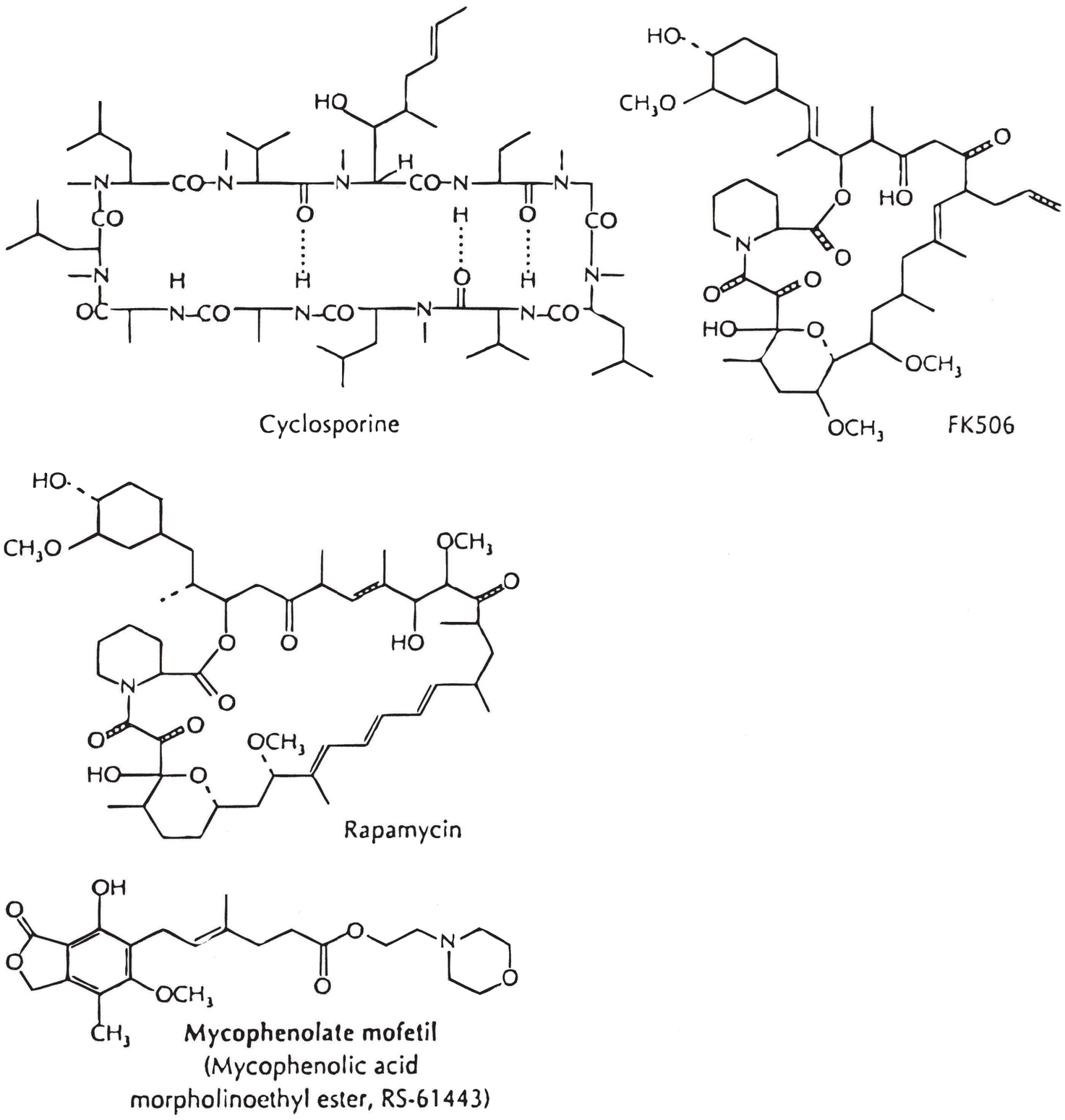

FIGURE 31.5 Structure of new immunosuppressive drugs.

drugs.[394] Independent of the intrinsic nephrotoxicity of CsA, its complex clinical pharmacokinetic profile entails the potential hazard of incorrect dosing, ultimately resulting in irreversible renal damage or acute rejection of the graft.

To optimize CsA therapy, monitoring of CsA trough levels in serum, plasma, or whole blood by means of radioimmunoassays or high-performance liquid chromatography is common clinical practice.[395] Monitoring CsA trough levels has limited value, however, for the assessment of adequate immunosuppression or predicting protection from nephrotoxicity.[396,397] The AUC is more informative and a better indicator of drug exposure[398] but is expensive and time consuming. Large-scale clinical trials using Neoral C_2 monitoring in renal and liver transplant recipients have demonstrated low acute rejection rates and good tolerability with a low adverse event profile to at least one year posttransplant.[399–403] Neoral C_2 monitoring provided a more accurate assessment of delayed and/or low absorbers of CsA in these studies. Neoral C_2 monitoring in maintenance renal transplant recipients showed that 26% to 49% of the patients, managed by monitoring of cyclosporine trough levels, were treated with excessive doses of CsA, adversely affecting graft function.[404–406] Dose reduction to optimal C_2 levels, between 600 and 800 ng per mL, in these patients, resulted in improvement of graft function, without increased risk for rejection.[404] These data provide evidence that monitoring of C_2 levels may result in more adequate dosing of cyclosporine.

Immunosuppressive Mechanism of Cyclosporin A

CsA blocks the activation of T cells, mainly through inhibition of transcription of lymphokines, most notably interleukin-2 (IL-2), the main growth factor for T cells.[407] By inhibiting IL-2 expression in T cells, CsA prevents helper T cells from orchestrating a response to foreign antigens.

The immunosuppressive effect of FK506 (tacrolimus) is similar to that of CsA,[408] as a logical consequence of a similar molecular mechanism of action of both drugs (Fig. 31.6).[409] CsA and FK506 bind with high affinity to intracellular target proteins, called immunophilins, which possess cis–trans isomerase activity. These immunophilins have been identified respectively as cyclophilins in the case of CsA[410] and FK-binding proteins in the case of FK506 and rapamycin.[411] The binding of CsA or FK506 is a prerequisite of their immunosuppressive potential because it has been demonstrated that the CsA- or FK506-immunophilin complex competitively binds directly to the serine–threonine phosphatase

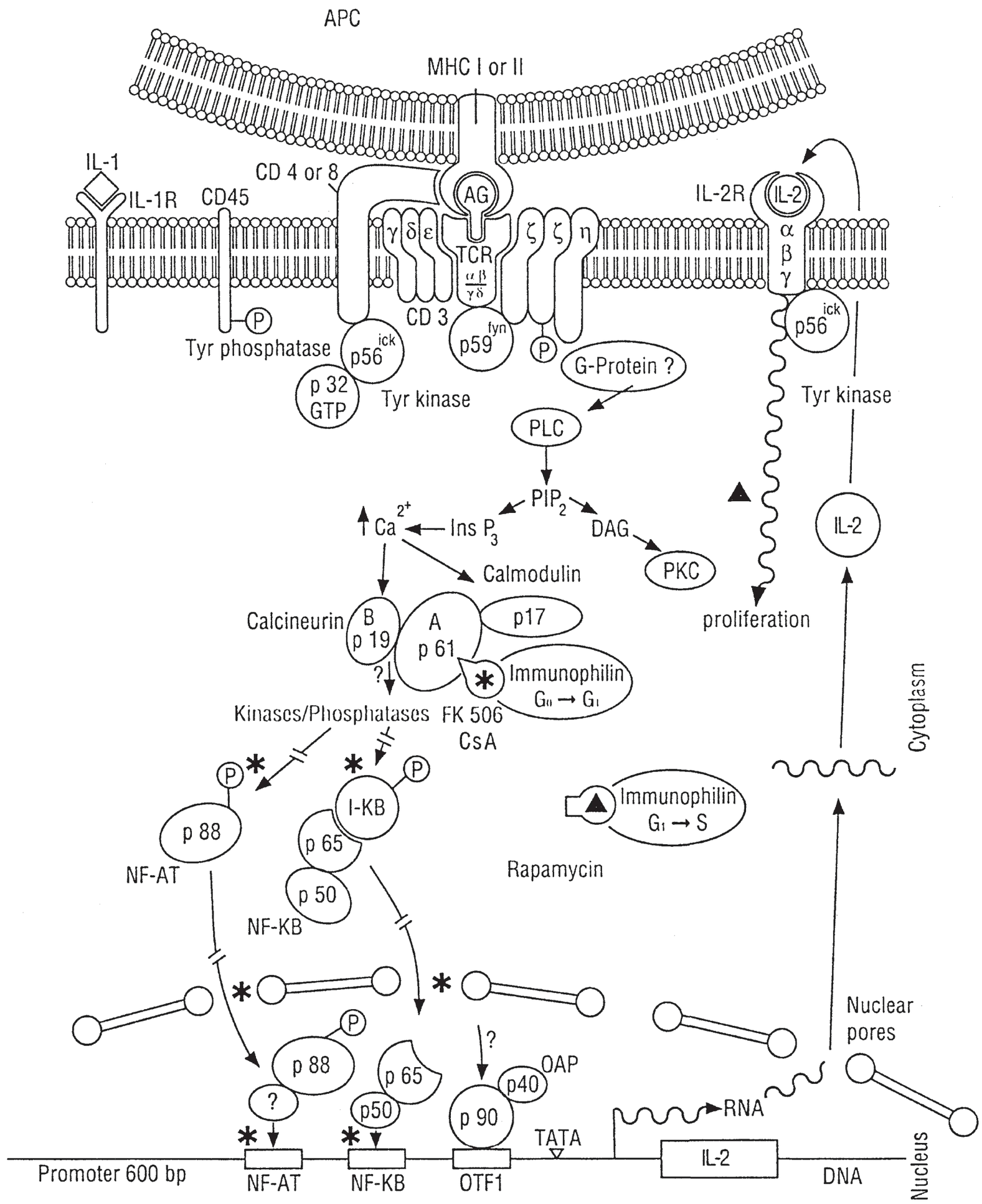

FIGURE 31.6 Cyclosporine (CsA) and FK506 both interfere, by binding to their respective immunophilins, with the function of intracellular molecules that transmit calcium-associated signals between the cell receptor (TCR) and the activation of lymphokine genes (interleukin-2) in the nucleus. Transcriptional regulation of interleukin-2 (IL-2) gene expression is modulated by the combination of transcription factors (e.g., NF-AT, NF-κ B, OTF-1) interacting with their corresponding recognition sites at the IL-2 promoter. These DNA/protein complexes, together with RNA polymerase II (RNA pol II), result in the antigen-inducible transcription of IL-2. Potential intervention sites for the pentameric complex (Calcineurin A [p61], B [p19], calmodulin [p17], immunophilin, drug), involving (e.g., modification and translocation) antigen-inducible transcription factors (NF-AT [p88], NF-κ B [p50, p65]), are indicated by *asterisks*. CyA and FK506 interfere with the G_0 to G_1 transition of the cell cycle, whereas rapamycin interferes with the G_1 to S transition (indicated by a *triangle*). *AG,* antigen; *APC,* antigen presenting cell; *DAG,* diacylglycerol; *Ins P_3,* inositoltriphosphate; *MHC,* major histocompatibility class; *PiP_2,* phosphatidylinositolbiphosphate; *PKC,* protein kinase C; *PLC,* phospholipase C. (From Baumann G. Molecular mechanism of immunosuppressive agents. *Transplant Proc.* 1992;24[Suppl 2]:4, with permission.)

calcineurin[412,413] in the presence of Ca^{2+}. The inhibition of calcineurin by the drug–immunophilin complex results in an altered modification pattern of the cytoplasmic components of transcription factors, thereby disturbing their nuclear translocation.[409]

Potential substrates for calcineurin are the nuclear factor of activated T cells (NF-AT) and the nuclear factor of immunoglobulin (light chain in B cells) (NF-κB), which were both reported as being affected in their IL-2 promoter binding activity by CsA and FK506, but not by rapamycin. This could explain the similarity of the immunosuppressive effect of CsA and FK506, in contrast to rapamycin, although rapamycin shares the same affinity as FK506 for the FK-binding protein.[414,415]

Experimental Nephrotoxicity of Cyclosporin A

Much of our knowledge of cyclosporine nephrotoxicity derives from experimental studies in animal models. The understanding of the pathogenesis of chronic cyclosporine nephrotoxicity has long been hampered, however, by the lack of an experimental model until a number of investigators developed a suitable animal model of chronic cyclosporine nephrotoxicity.[416–420]

Functional Alterations Induced by Cyclosporin A

It has been unequivocally established that CsA profoundly alters renal and glomerular hemodynamics. Acute and/or chronic CsA administration induces a decline in GFR, with a concomitant reduction in renal blood flow and an increase in renal vascular resistance as a consequence of vasoconstriction,[421–424] preferentially at the level of afferent arterioles.[425] These hemodynamic effects are mainly responsible for the acute cyclosporine nephrotoxicity and probably contribute also to the pathogenesis of chronic nephrotoxicity by inducing chronic renal ischemia. The possible underlying mechanisms of this vasoconstrictor effect of CsA are discussed in "Pathophysiologic Studies of Cyclosporine Nephrotoxicity."

Although early pathologic studies suggested that CsA is a primary tubular toxin,[425] the results of clearance experiments conflict with that premise.[426] CsA reduces the end proximal tubular flow rate[427] and increases proximal fractional reabsorption.[428] The latter is due to an inadequate adaptive reduction in the absolute rate of proximal reabsorption.[429] CsA causes a resetting of the tubuloglomerular feedback with onset at lower tubular flow rates and greater maximum response.[430]

CsA-treated rats show a reduction of sodium chloride reabsorption in the distal nephron, including Henle's loop,[427,428] most likely as a secondary response to the decreased proximal tubular fluid delivery. This reduced sodium delivery to and reabsorption from the distal nephron results in reduced distal acidification and potassium secretory capacity through a decreased generation of a negative transmembrane potential. This explains the observed hyperkalemic metabolic acidosis in CsA-treated rats[431] as well as in CsA-treated kidney transplant recipients.[432]

In summary, CsA reduces renal blood flow and GFR predominantly through vasoconstriction of the afferent arterioles. Effects of CsA on tubular function consist of increased proximal tubular reabsorption and decreased distal sodium delivery, which induce hyperkalemic metabolic acidosis.

Morphologic Alterations Induced by Cyclosporin A

The renal pathology induced by CsA is largely dose-related and time-related. For clarity, we focus on two distinct pathologic patterns: the acute (potentially reversible) toxic injury and the chronic (essentially irreversible) nephrotoxicity induced by CsA.

At supratherapeutic levels (100 mg/kg/day by mouth [PO]), CsA induces mainly tubular pathology consisting of isometric vacuolization of tubular cells, accumulation of eosinophilic bodies often representing giant mitochondria, and microcalcifications in proximal tubules by 21 days.[425,433–435] The effects on the proximal tubule tend to be most prominent in the S3 segment[436] and become more widespread at very high doses. These pathologic alterations are reversible after dose reduction or withdrawal of CsA. In contrast to the acute toxic injury, chronic administration of CsA (12.5 mg/kg/day) for 3 to 10 weeks induces striking morphologic alterations in the medullary rays of the cortex of the rat.[416] These changes progress in time and result in areas of focal or striped interstitial fibrosis with foci of atrophic proximal tubules, which are most prominent in the subcapsular cortex.[437,438] The severity of the lesion progresses with treatment and is exacerbated by salt depletion.[420] Withdrawal of CsA does not reverse the observed structural changes.[437]

Besides the striped interstitial fibrosis, mild glomerular endothelial damage, glomerular hypercellularity, and mesangial matrix expansion[417] are observed after long-term CsA administration. Morphometric analysis with three-dimensional reconstruction of individual glomeruli[439] shows subsets of glomeruli with small volume with significant reduction in GFR,[440] alternating with hypertrophic glomeruli.

At the vascular level, scanning electron microscopy shows focal narrowing of the afferent arteriolar diameter that progresses with time of CsA treatment and parallels the decrease in inulin clearance.[362] CsA nephropathy is associated with degenerative hyaline changes in the walls of afferent arteriolar-sized blood vessels,[441] which can disappear after discontinuation of CsA.[442,443]

Pathophysiologic Studies of Cyclosporine Nephrotoxicity

Numerous studies have focused on different pathophysiologic mechanisms of cyclosporine nephrotoxicity. We sequentially discuss mechanisms of renal vasoconstriction, cellular and molecular mechanisms, mechanisms of matrix protein accumulation, and studies on lipid peroxidation.

Mechanisms of Cyclosporin A-Mediated Renal Vasoconstriction. As mentioned previously, administration of CsA induces a marked afferent arteriolar vasoconstriction resulting in decreased renal blood flow and GFR. The renal sympathetic nervous system has been implicated in the renal functional effects of CsA because the α-adrenergic antagonists phenoxybenzamine[421] and prazosin[444] prevent a CsA-induced fall in renal blood flow and GFR. Moreover, a significant increase in renal afferent and efferent nerve activity has been demonstrated in CsA-treated rats.[445] The relevance of the activated sympathetic nervous system to the pathophysiology of cyclosporine nephrotoxicity in kidney transplantation is questionable, however, because the renal allograft is denervated. Nevertheless, increased sensitivity of the denervated organ to circulating catecholamines[445] or significant reinnervation of renal allograft after transplantation[446] is a possible explanation.

Rodent models of cyclosporine nephrotoxicity consistently show activation of the renin–angiotensin–aldosterone axis, in contrast to results in humans. Besides increased plasma renin activity in CsA-treated rats,[434,447] hyperplasia of the juxtaglomerular apparatus,[419,448,449] as well as elevated renin synthesis and release in juxtaglomerular cells,[450,451] has been documented in experimental animals during CsA therapy. However, angiotensin-converting enzyme (ACE) inhibitors show conflicting effects on renal blood flow in CsA-treated rodents, with improvement in some studies[421,452] but not in others.[422,453] More recent experimental studies suggest that CsA-related chronic interstitial injury is mediated by angiotensin II, because renin–angiotensin blockade prevents CsA-induced tubulointerstitial fibrosis.[454,455] However, in human cardiac and renal allograft recipients treated with CsA, plasma renin is suppressed,[367,456] suggesting that the renin–angiotensin system is not of primary importance in human cyclosporine nephrotoxicity.

Hypovolemia could contribute to renal vasoconstriction with CsA therapy because CsA-treated rats have reduced plasma volume and saline expansion reverses the deficits in renal blood flow and GFR.[457] Studies with furosemide,[458] mannitol,[459] and chronic sodium depletion[416] have demonstrated that hypovolemia potentiates cyclosporine nephrotoxicity. Sodium depletion enhanced fibrosis and the expression of TGF-β1 and matrix proteins in experimental CsA nephropathy.[460] However, there is evidence implicating hypovolemia and sodium-depletion as an exacerbating rather than as a causative factor of human cyclosporine nephrotoxicity.

Much attention has been paid to the potential role of an altered eicosanoid metabolism in cyclosporine nephrotoxicity. In animal models, CsA consistently increases the generation of thromboxane A_2 (TxA_2), a potent renal vasoconstrictor,[461–463] whereas its effects on vasodilatory prostaglandins are controversial.[457,464,465] Pharmacologic manipulation of thromboxane metabolism with a specific TxA_2 receptor antagonist[466,467] or a TxA_2 synthase inhibitor[468] partially prevented the CsA-induced acute decline in GFR and renal blood flow in normal rats. The TxA_2 receptor antagonist also attenuated chronic cyclosporine nephrotoxicity in rats with renal isograft.[469] The relevance of these data to human cyclosporine nephrotoxicity, however, is controversial.[470,471]

Another potential mediator of CsA-induced vasoconstriction is the platelet activating factor (PAF) because it has been demonstrated that rat mesangial cells release increased quantities of PAF when incubated with CsA, and CsA-stimulated cell contraction is abolished by the PAF antagonists BN52021 and alprazolam.[472] Chronic cyclosporine nephrotoxicity is also attenuated in rats treated with the PAF antagonist BN52063.[473]

More recently, the role of endothelin, the most potent vasoconstrictor yet identified, has been advocated in CsA-induced vasoconstriction. CsA treatment has been shown to stimulate endothelin production[474,475] and promote glomerular endothelin binding in vivo.[476] Endothelin appears to mediate CsA-induced renal vasoconstriction in the rat.[477] The resulting reduced single-nephron GFR and glomerular plasma flow rate, as well as the decreased glomerular capillary pressure, were attenuated by an antiendothelin antibody.[478] Similarly, the endothelin receptor antagonist BQ123 has the potential to prevent hypoperfusion and hypofiltration induced by CsA.[479] Recent work additionally demonstrated that CsA selectively modulates renal mRNA expression for endothelin peptide and one of its receptor subtypes in a site-specific way.[478] In humans, the endothelin receptor antagonist bosentan markedly blunted the renal hypoperfusion effect of CsA.[480]

Experimental data indicate that an enhanced 5-HT2 (serotonin)-mediated vasoconstriction plays an important role in the suppression of renal blood flow (RBF) autoregulation induced by CsA, because the administration of ritanserin, a pure 5-HT2 antagonist, restored the RBF autoregulation.[481] In vivo studies in humans demonstrated reduced basal and stimulated NO production from the endothelium of forearm resistance vessels in cyclosporine-treated renal transplant recipients.[482] This suggests endothelial dysfunction and may provide a potential mechanism to explain cyclosporine-induced hypertension.

Elevated cytosolic calcium is yet another attractive candidate to explain the CsA-induced vasoconstriction. This has been demonstrated in cultured rat mesangial cells[483] as well as in vascular smooth muscle cells.[484] The augmented transmembrane Ca^{2+} influx and intracellular Ca^{2+} mobilization could account for the protective effects of calcium channel antagonists in acute[485–487] as well as chronic[488,489] cyclosporine nephrotoxicity.

In summary, the acute or subacute effects of CsA on renal hemodynamics are likely mediated by a number of vasoactive substances such as endothelin, serotonin, impaired NO production, TxA_2, and PAF. At the cellular level, CsA induces increased intracellular Ca^{2+}, resulting in contraction of vascular smooth muscle cells as well as mesangial cells. Calcium channel blockers are able to protect against these effects.

Cellular and Molecular Mechanisms of Cyclosporine Nephrotoxicity. CsA modulates mitochondrial calcium fluxes, resulting in reduced mitochondrial swelling, respiration, and calcium discharge.[490,491] Additionally, CsA modulates cytosolic calcium regulation in mesangial cells.[483,492]

In T lymphocytes, CsA only affects calcium-dependent pathways on T-lymphocyte activation. As stated previously in "Immunosuppressive Mechanism of Cyclosporin A," the immunophilin-ligand complex inhibits the Ca^{2+}-dependent phosphatase calcineurin, which is an important step in signal transmission pathways. The analogy of immunosuppressive effect of FK506, as well as its nephrotoxicity, has led to an attractive hypothesis stating that cyclosporine nephrotoxicity could be inherent to its immunosuppressive effect[493]; a similar hypothesis was formulated with regard to FK506.[494]

The mechanisms underlying the linkage of nephrotoxic effects to immunosuppressive effects of CsA or FK506 are still unknown. However, the blocking of T cell activation by CsA or FK506 is an attractive explanation because it has been shown that a mononuclear cell infiltrate is part of cyclosporine nephrotoxicity.[495] Alteration of the repair function of these cells could therefore be a possible mechanism inducing interstitial fibrosis.

Administration of CsA in vivo to rats causes a marked impairment of microsomal protein synthesis.[496] Additional studies have shown a dose-dependent and time-dependent translational alteration of intracellular protein synthesis produced by cyclosporine.[497]

The role of this decreased renal microsomal protein synthesis induced by CsA is speculative but could, if persistent with long-term cyclosporine treatment, alter renal cell matrix and interstitial cell interactions favoring fibrosis.[497]

Recent in vitro studies showed that CsA induces apoptosis in tubular epithelial cells in a dose-dependent and time-dependent manner.[498–501] This effect was mediated by the induction of iNOS,[498] caspases,[499] or Fas.[500]

Mechanisms of Matrix Protein Accumulation. Interstitial fibrosis, the end point of chronic cyclosporine nephrotoxicity, results from an excessive extracellular matrix accumulation, which represents an imbalance between rates of extracellular matrix production and degradation. Cyclosporine has been shown to enhance the production of specific extracellular matrix components in mouse and rat kidney,[502,503] as well as in renal cells in culture.[504] In the presence of CsA, angiotensin II is known to induce interstitial collagen formation.[505] Blockade of the renin–angiotensin system with angiotensin II receptor antagonists or ACE inhibitors markedly abrogated the tubulointerstitial fibrosis without improving renal hemodynamics.[454,455,506] The location of the angiotensin receptor type 1 mRNA in the outer medulla and medullary rays might explain the peculiar striped pattern of fibrosis noted in an experimental model of chronic cyclosporine nephrotoxicity.[507] All together, these data strongly suggest that CsA-induced interstitial fibrosis could be mediated by angiotensin II, independent of its hemodynamic effects.

Several recent studies also implicated transforming growth factor-β1, a potent immunosuppressive and fibrogenic cytokine, as a potential mediator of CsA-induced interstitial fibrosis.[508–514] Enhanced intragraft expression of TGF-β was associated with interstitial fibrosis in patients treated with CsA.[508] In animals, CsA induced an increased expression of TGF-β1, both at the mRNA and the protein level, again associated with tubulointerstitial fibrosis.[509–511,514] Similarly, CsA stimulated expression of TGF-β1 in renal cells.[512,513] The fibrogenic effects, induced by CsA, were abrogated by a neutralizing anti-TGF-β1 antibody.[513,514]

In contrast to the already mentioned enhanced collagen formation induced by CsA, recent work demonstrates an increased expression at both the transcriptional (mRNA) level and protein level of tissue inhibitor of metalloproteinases (TIMP-1) in a rat model of chronic cyclosporine nephrotoxicity.[515,516] Moreover, Duymelinck and associates show that cholesterol feeding accentuates the cyclosporine-induced elevation of renal plasminogen activator inhibitor type 1 (PAI-1).[517] This increased expression and production of TIMP-1 and PAI-1, induced by CsA, could result in a decreased degradation of extracellular matrix, which would in turn lead to progressive extracellular matrix accumulation and interstitial fibrosis.

In summary, CsA-induced interstitial fibrosis results from a combination of increased synthesis of matrix components, as well as decreased degradation of extracellular matrix. Angiotensin II and transforming growth factor-β1 may play a role in the process of increased collagen formation induced by CsA, whereas the TIMP-1 and the PAI-1 likely mediate the decreased degradation of extracellular matrix induced by CsA.

Studies on Lipid Peroxidation. It has been shown that in vitro incubation of rat renal microsomes or human liver microsomes with CsA induces dose-related lipid peroxidation.[518,519] Lipid peroxidation seems to be the main mechanism of free-radical toxicity.[520–522] Reactive oxygen species through a peroxidative process may increase the availability of arachidonate metabolites and enhance prostanoid production.[523–525] Recent in vivo studies in the rat indicated that cyclosporine nephrotoxicity is accompanied by dose-related systemic and renal lipid peroxidation,[526] preceding the fall in GFR.[527] Concurrent treatment with antioxidants (i.e., vitamin E,[526] melatonin,[528] or N-acetylcysteine[529]), suppressed CsA-induced lipid peroxidation and reduced functional and structural damage. The mechanism by which CsA-induced lipid peroxidation could contribute to cyclosporine nephrotoxicity is putative, including direct cellular toxicity,[530] thromboxane-mediated ischemia,[531] or peroxidation-linked excess extracellular matrix production.[532] Several reports suggest that calcineurin inhibitors, CsA and tacrolimus, have pro-oxidant activity and they increase the susceptibility of low density lipoprotein to oxidation in humans.[533–535]

Clinical Nephrotoxicity of Cyclosporin A

CsA can cause a wide spectrum of renal functional and morphologic impairments, including a marked and rapidly reversible decrease in GFR and renal plasma flow[359] and a chronic form of renal damage in patients treated for more than 6 months with a potential evolution to end-stage renal disease.[364,367] Thrombotic microangiopathy is another relatively uncommon but serious adverse effect of cyclosporine.[362,536]

Acute Renal Failure Induced by Cyclosporin A

Acute cyclosporine renal dysfunction is not infrequent in clinical practice and occurs not only in patients with kidney transplantation[355] but also in heart,[537] liver,[358] and bone marrow[356] transplant recipients. This acute form of nephrotoxicity may occur within weeks following initiation of CsA therapy and can also be observed after years of drug therapy.[538] The incidence of this acute renal injury can be enhanced by extended graft preservation,[361] preexistent renal histologic lesions,[539] donor hypotension, and perioperative complications.[540]

Acute cyclosporine nephrotoxicity has clinical features similar to those of acute renal allograft rejection, including an abrupt fall in GFR, impaired urinary concentrating capacity, and sodium retention.[541] Hypertension is observed in up to 50% of patients, whereas metabolic acidosis, hyperkalemia, and hyperuricemia are less frequent.[542] Characteristic of this syndrome of acute reversible renal dysfunction induced by CsA is the rapid recovery of renal function on reduction of the CsA dose.[359,543]

Delayed kidney graft function is a less frequent severe form of protracted ARF with oliguria induced by CsA.[541] Its incidence varies largely between centers,[544,545] presumably reflecting different strategies of immunosuppressive treatment or variations in time of ischemia of the kidney before transplantation.[546,547]

Although nephrotoxicity due to cyclosporine alone is rarely observed with CsA trough blood levels below 200 ng per mL,[380,548] blood level monitoring has proved unreliable in the differential diagnosis between acute cyclosporine nephrotoxicity and acute rejection of kidney allografts.[380]

The difficulty in differentiating acute rejection from cyclosporine nephrotoxicity in the setting of kidney transplantation often compels performance of a kidney biopsy.[549,550] On a histologic basis, cyclosporine nephrotoxicity is often a diagnosis of exclusion with the absence of definite signs of acute rejection, such as intimal arteritis[551,552] or intratubular lymphocytes.[553] Histologic features of cyclosporine nephrotoxicity are nonspecific and include arteriolar hyalinosis,[554,555] as well as isometric vacuolization of proximal tubular cells.[556]

Analogous to experimental data obtained in animal models, CsA causes a dose-related and time-related fall in GFR and renal plasma flow in humans induced by renal vasoconstriction.[557,558] In two studies, the intrarenal blood flow was significantly reduced after oral cyclosporine intake, but hypoperfusion could not be elicited by tacrolimus.[559,560] The beneficial effects of different calcium channel blockers on this CsA-induced renal hypoperfusion[561–566] suggest this vasoconstriction is mainly affected at the afferent arteriolar level because it has been demonstrated that calcium antagonists preferentially reduce glomerular afferent arteriolar tone.[567]

In contrast, coadministration of indomethacin unmasks CsA-induced renal vasoconstriction and potentiates cyclosporine nephrotoxicity by reducing the intrarenal prostaglandins.[568] This suggests a role for the eicosanoids in the CsA-induced vasoconstriction. Further arguments in favor of this possibility are the partial beneficial effects observed with a specific TxA_2 synthase inhibitor[471] and with dietary regimens with omega-3 polyunsaturated fatty acids.[569]

Although a role for increased vascular renin activity in cyclosporine-induced renal and peripheral vasoconstriction has been suggested,[570,571] investigators have never detected any significant preventive effect of ACE inhibition on the decline in renal blood flow and the increase in renal vascular resistance induced by CsA.

Unlike in animal models, prazosin did not significantly affect GFR, renal plasma flow, or renal vascular resistance in patients who had undergone transplant and were treated with CsA,[572] thus questioning the role of the sympathetic nervous system in cyclosporine nephrotoxicity.

Endothelin has been implicated as a causative agent in CsA-induced vasoconstriction (see "Experimental Nephrotoxicity of Cyclosporin A"). Although intrarenal injections of antiendothelin antibodies protected against the effects of cyclosporine,[474] administration of specific endothelin receptor antagonists has shown conflicting results.[479,573]

Chronic Cyclosporine Nephrotoxicity

The main clinical issue associated with CsA treatment is, however, the chronic nephrotoxicity[367] that is clinically defined by progressive renal dysfunction with hypertension. Histologic lesions can already appear after 6 months of CsA therapy,[364,574] with progression over time, even after CsA dose reduction.[371] As mentioned previously, chronic cyclosporine nephrotoxicity has been documented in other clinical settings besides kidney transplantation.[367–375] Chronic cyclosporine nephrotoxicity is related to the cumulative CsA dose[371,575] and may be irreversible even after CsA discontinuation.[576]

The clinical features of chronic cyclosporine nephrotoxicity are nonspecific, including a slowly progressive decline of renal function over months or years, severe arterial hypertension, mild proteinuria, and tubular dysfunction.[541] In renal allografts, differential diagnosis with chronic rejection is often impossible on clinical grounds alone, thus necessitating the performance of a kidney biopsy.[577]

The histopathologic lesions of chronic cyclosporine nephrotoxicity have been extensively studied and are now well known.[365,366,578,579] Histopathologic findings in 2-year protocol biopsies from a randomized study showed

comparable lesions in renal allografts under cyclosporine and tacrolimus treatment.[580] They include renal arteriolar damage (the so-called CsA-associated arteriolopathy), tubular atrophy, and (striped) interstitial fibrosis, as well as glomerular sclerosis. These lesions are nonspecific, however, except for the CsA-associated arteriolopathy.

The vascular lesions are located almost exclusively in the arterioles and arteries, with up to two layers of smooth muscle cells, and usually consist of circular nodular protein deposits or mucoid thickening of the intima, which contributes to narrowing or occlusion of the lumen.[365] CsA-associated arteriolopathy affects a limited number of arterioles in a dose-related manner.[579]

Tubulointerstitial changes may be nearly diffuse, but usually there are narrow stripes of atrophy and fibrosis, apparently corresponding to areas of cortex with afferent arteriolar lesions.[365] This interstitial fibrosis progresses over time.[371] Tubular atrophy is nearly always found in areas with interstitial fibrosis[581] and likewise progresses with time.[371] CsA-induced glomerulopathy consists of global or focal and segmental sclerosis.[366,582] Again, the number of affected glomeruli increase with time.[371]

Although the histologic features of chronic cyclosporine nephrotoxicity have been well characterized, the differential diagnosis with chronic rejection of the renal allograft in kidney transplantation still often remains difficult.[583]

A great matter of debate is whether prolonged therapy with CsA can result in progressive, irreversible renal damage, ultimately leading to end-stage renal disease. This was advocated by some authors[584,585] but denied by others.[572,586] Multicenter studies in renal transplant patients showed reduced but stable renal function after up to 3 to 5 years of CsA treatment.[587,588] Conversion from CsA to azathioprine in kidney transplant recipients after 3 months significantly improved the creatinine clearance at 5 years' posttransplantation.[589] In patients who have undergone pancreas transplant, a sequential functional and morphologic study has unequivocally shown the progressive character of the histologic lesions due to cyclosporine nephrotoxicity.[371] This was strongly correlated with CsA blood levels, CsA dose, and magnitude of the decline in creatinine clearance during the first posttransplant year.[371] Analysis of sequential protocol biopsies of renal allografts over a period of 10 years, in a prospective study of 120 kidney-pancreas transplant recipients, confirmed this progressive character of renal histologic lesions, induced by calcineurin inhibitors.[590] In this study, severe histologic damage was present in 58.4% of the renal allografts by 10 years.

Altogether, these data point out that chronic cyclosporine nephrotoxicity has a progressive and irreversible character once the histologic lesions have arisen. Assessment of the renal function, be it by means of serum creatinine or creatinine clearance, underestimates the magnitude of the problem due to the relatively low sensitivity of those methods and to the slow progression of the renal damage induced by cyclosporine.

The pathophysiology of chronic cyclosporine nephrotoxicity in humans is a matter of extensive investigation, mainly through experimental models (see "Pathophysiologic Studies of Cyclosporine Nephrotoxicity").

Hemolytic–Uremic-like Syndrome Induced by Cyclosporin A

Thrombotic microangiopathy is a relatively uncommon but serious adverse effect of cyclosporine in renal[362] and nonrenal[536] transplant recipients, with an overall 43% graft survival rate.[591,592] The most striking morphologic changes are an extensive thrombotic process in the renal microcirculation, with several glomerular capillaries occluded by thrombi extending from the afferent arterioles and containing platelet aggregates.[365] Laboratory anomalies include thrombocytopenia, hemolytic anemia, and deterioration of the renal function.[362] In the setting of kidney transplantation, the differential diagnosis of hemolytic–uremic syndrome and vascular rejection is not obvious.[591] According to a retrospective study of 29 patients with calcineurin-inhibitor induced thrombotic microangiopathy, repeated plasma-exchange induced a recovery of the renal allograft function in 80% of the patients.[593]

This hemolytic–uremic-like syndrome induced by CsA reinforces the concept that the vascular endothelium is the main target in this form of CsA toxicity. That CsA can damage vascular endothelium is confirmed by the high plasma concentration of factor VIII-related antigen, found in recipients of renal allograft given CsA and having clinical signs of nephrotoxicity.[594] Recent work shows significantly higher plasminogen-activator inhibitor levels in patients treated with CsA who underwent renal transplant, compared to patients who were not treated with CsA, suggesting a decreased fibrinolytic activity in the former patients.[595] This could account for the increased risk of hemolytic–uremic syndrome induced by CsA.

Pharmacogenetics of Calcineurin Inhibitors

With nephrotoxicity continuing to be a major factor in late kidney damage, it is important to understand better the mechanisms of drug-induced nephrotoxicity to develop therapeutic strategies and identify early biomarkers.[596] However, from a clinical point of view, none of these biologic pathways identified in experimental studies brought a clinical benefit for the patients, either in terms of early markers or in terms of therapeutic alternatives, including endothelin inhibitors or angiotensin receptor blockers.

Variability in calcineurin inhibitor (CNI) intestinal absorption and metabolism is attributed, at least in part, to the variability in expression and function of CYP3A enzymes (mainly 3A4 and 3A5) and the ATP dependent multidrug efflux transmembrane transporter P-glycoprotein (Pgp), a product of ABCB1 gene (previously MDR-1).

Pgp, a product of ABCB1 gene (previously MDR1), is a membrane protein that functions as an ATP-dependent

exporter of intracellular xenobiotics. In the kidney, Pgp is constitutively expressed on the brush border of proximal tubular cells and on the distal tubule. It has been suggested that Pgp is instrumental in CsA nephrotoxicity.[597] CsA is a substrate of Pgp, and variations in expression and/or function of Pgp could lead to accumulation of CsA, along with other cytotoxic agents, within the tubular cell.[598]

Already in 1998, Napoli et al. demonstrated a pharmacokinetic interaction between sirolimus CsA increasing the concentration of both immunosuppressive drugs in the renal tissue.[599] In clinical studies, it was noticed that sirolimus had no nephrotoxic effect; however, when used in combination with CsA, the nephrotoxic effect of CsA was potentiated.[600]

Anglicheau et al.[601] studied in vitro the role of P-glycoprotein (Pgp) in CsA cytotoxicity and the CsA-sirolimus interaction. Cyclosporine and sirolimus are Pgp substrates. The authors hypothesized that the Pgp activity level may affect cyclosporine cytotoxicity by interfering with the ability of Pgp to remove cyclosporine from within tubular cells, and that an interaction between cyclosporine and sirolimus on Pgp function may explain the enhancement of cyclosporine nephrotoxicity by sirolimus. Cyclosporine cytotoxicity was evaluated in primary cultures of normal human renal epithelial cells (HRECs) by cell viability and cytotoxicity assays. Verapamil, quinine, PSC833, and PGP-4008 were used as Pgp inhibitors. Rhodamine-123 (R-123), a fluorescent substrate of Pgp, was used to assess Pgp-mediated transport. Cyclosporine exerted a concentration-dependent cytotoxic effect on HRECs that was significantly increased by inhibition of Pgp activity. Sirolimus exerted an inhibitory effect on R-123 efflux in HRECs and increased cellular cyclosporine concentrations in a dose-dependent manner. These data demonstrate that Pgp plays a critical role in protecting renal epithelial cells from cyclosporine toxicity. The inhibitory effect of sirolimus on Pgp-mediated efflux and the cellular concentration of cyclosporine could explain the exacerbation of cyclosporine nephrotoxicity observed clinically.

Transcriptomic analyses of in vitro models of CNI nephrotoxicity have been used to identify two new molecular mechanisms that may play a role in early CNI-induced nephrotoxicity: EMT and endoplasmic reticulum (ER) stress. In vitro evidence that CsA induces EMT in proximal tubular epithelial cells has been provided. Potential mediators and downstream effectors of CsA-induced changes were identified by large-scale expression analysis using Affymetrix microarrays. PKC-β has been identified as a potentially important mediator, which may be responsible for CsA-induced TGF-β1 upregulation. In addition, the E2A transcription factors E12/E47 may play a key role in the altered expression profile of CsA-treated cells and, thus, cell phenotype.[602] These in vitro findings regarding EMT have been translated in vivo. De novo vimentin expression has been used as a marker of tubular mesenchymal transition in rats treated with CsA. Whereas tubular vimentin, a nonspecific marker of regeneration, is virtually absent from vehicle-treated rat kidneys, de novo expression is markedly increased in CsA-treated rat kidneys, together with a CsA-induced increase in collagen 3 and fibronectin mRNA expression, suggesting that CsA induces tubular expression of mesenchymal markers in vivo.[603] Whether CsA EMT is important to the increasingly recognized role of EMT in renal fibrosis needs further investigation using more specific tools indicating EMT.

Endoplasmic reticulum stress results from the accumulation of misfolded proteins within the ER. The relevance of the induction of ER stress by CsA was boosted by the finding that CsA exposure in vivo results in the upregulation of the ER stress marker BiP mRNA in renal allograft biopsies. In a rat model of CsA nephrotoxicity, Han et al. reported that a short-term treatment of CsA for 7 days activated the unfolded protein response, exemplified by the induction of BiP, and a proapoptotic response characterized by the upregulation of capsase 12 and CHOP.[604]

Recent progress in molecular biology and functional genetics like transcriptomics and whole genome studies will lead to the discovery of genes associated with kidney injury and the characterization of stress response pathways.

Summary

CsA is a potent immunosuppressive drug with nephrotoxic side effects. Independent of the intrinsic nephrotoxic properties of CsA, its complex clinical pharmacokinetic profile could cause incorrect dosing, ultimately resulting in irreversible renal damage.

The clinical nephrotoxicity of CsA consists of three entities with different expressions of renal damage induced by CsA (i.e., ARF, hemolytic–uremic-like syndrome, and chronic cyclosporine nephrotoxicity). The ARF is essentially reversible and mainly hemodynamically mediated through afferent arteriolar vasoconstriction. Dosage reduction of CsA reverses the nephrotoxic effects. The hemolytic–uremic-like syndrome consists of an extensive thrombotic process at the level of the glomerular capillaries, causing loss of kidney function in more than half of the cases. Chronic cyclosporine nephrotoxicity is an irreversible renal damage characterized by a specific arteriolopathy and striped interstitial fibrosis, resulting in slow progressive decline of renal function.

TACROLIMUS (FK506)

Tacrolimus (FK506) is a fungal product, a new macrolide immunosuppressant agent, which has shown important potential in transplantation and in the treatment of autoimmune diseases.[605–607] Although it is many times more potent than cyclosporine, allowing the use of lower doses, both drugs have similar nephrotoxic properties.[608,609]

Molecular Action

Cyclosporine and FK506 have dissimilar chemical structures (Fig. 31.5)—nevertheless, both agents bind to a similar class of ubiquitous intracellular receptors: immunophilins,

molecules that are cis–trans prolyl isomerases. These intracellular binding proteins are well conserved through evolution and change the confirmation of cyclosporine and FK506. The cytosolic receptor for FK506 (FKBP) has been well characterized.[610] This drug-immunophilin complex must bind to calcineurin, a calcium-dependent protein phosphatase, to allow the immunosuppressant actions of the drugs in lymphocytes (Fig. 31.6).[611,612] Similar calcineurin-mediated dephosphorylation of cyclosporine and FK506 may lead to inhibition of signal transduction in other cell types and organs, which mediates both the desirable immunosuppressant effects and the possibly toxic effects.

Cyclosporine and FK506 are powerful immunosuppressive drugs that inhibit the calcium–calmodulin-dependent phosphatase calcineurin in T cells, thereby preventing the activation of T cell-specific transcription factors such as NF-AT involved in lymphokine gene expression (Fig. 31.6). Although this may, at least in part, explain the mechanism of cyclosporine and FK506 immunosuppression, additional mechanisms have to be invoked to explain the pharmacologic properties and toxic effect of these drugs such as nephrotoxicity and neurotoxicity. Schwaninger and coworkers[613] studied the effect of cyclosporine and FK506 on calcineurin phosphatase activity and gene transcription mediated by the cyclic adenosine monophosphate-responsive element (CRE), a binding site of the ubiquitous transcription factor CREB. An imported gene was placed under the transcriptional control of the CRE of the rat glucagon gene and transiently transfected into the glucagon expressing cell line αTC2. Cyclosporine and FK506 inhibited depolarization-induced gene transcription in a concentration-dependent manner. Both cyclosporine and FK506 inhibited calcineurin phosphatase activity at the drug concentrations that inhibited gene transcription. The FK506 analog rapamycin had no effect on calcineurin activity and gene transcription, but excess concentrations of rapamycin prevented the effect of FK506 on both calcineurin activity and gene transcription. These results further support the notion that the interaction of drug-immunophilin complexes with calcineurin may be the molecular basis of cyclosporine- and FK506-induced inhibition of CREB/CRE-mediated gene transcription. The ability to interfere with CREB/CRE-mediated gene transcription represents a new mechanism of cyclosporine and FK506 action that may underlie pharmacologic effects and toxic manifestations of these potent immunosuppressive drugs.[613]

A recent report demonstrated that in vivo FK506 treatment eliminated antigen-stimulated T cells through DNA fragmentation (apoptosis), representing one of the mechanisms of immunologic tolerance.[614]

Experimental Studies

Cell Culture

McCauley and colleagues[615] demonstrated a cyclosporine- and FK506-mediated, dose-dependent inhibition of renal cell proliferation using LLC-PK1 cells (an established cell line derived from the pig proximal tubule) in culture. Although FK506 inhibited renal cell proliferation to a greater degree than cyclosporine at the same concentration, when clinically relevant concentrations were compared, FK506 was significantly less inhibitory than cyclosporine. Moutabarrik and associates[616] observed similar effects in the same cell line but could not make a clear distinction between the FK506 and the cyclosporine effects on release of 3H thymidine from prelabeled cells, N-acetyl-β-D-glucosaminidase release, and cell detachment. Ultrastructural changes such as vacuolization, swelling, and mitochondrial enlargement and inhibition of the growth of the cultured tubular cells were also observed at high concentrations of FK506 and cyclosporine. Low concentrations of FK506 and cyclosporine were not cytotoxic and induced only a minimal inhibitory effect on the growth of tubular cells in vitro. Cyclosporine and FK506 also induced a time-dependent stimulation of the secretion of endothelin by cultured tubular cells. The concentration of cyclosporine that induced these effects was 10 to 100 times higher than that required for FK506. The concentrations of FK506 and cyclosporine inducing endothelin secretion were not cytolytic for tubular cells in vitro. Yatscoff and coworkers[617] compare the effect of rapamycin and FK506 on the release of prostacyclin and endothelin in vitro using cultured rabbit mesangial and endothelial cells. The effects of both rapamycin and FK506 on the basal or stimulated release of prostacyclin or endothelin from mesangial cells and endothelial cells are similar with the following exceptions: Rapamycin results in a significant increase in the release of prostacyclin, whereas FK506 results in a significant decrease in the release of prostacyclin from the endothelial cells. Benigni and colleagues[618] review the vascular effects of FK506 as compared to cyclosporine in endothelial cell culture and intact organ. FK506, unlike cyclosporine, is without significant effect on thromboxane B_2, 6-ketoprostaglandin $F_1\alpha$, or endothelin release in bovine aortic endothelial cells grown in culture and does not alter the renal vascular resistance in vivo. These findings suggest that FK506 causes much less pronounced endothelial cell injury, at least in vitro.

Atcherson and Trifillis[619] examine in vitro cytotoxicity of FK506 on normal human proximal tubule cells. They find that FK506 is reversibly and mildly toxic to monolayers of human renal proximal tubule cells.

Edkins and associates[620] compare the effect of FK506 (2.5 mg per kg for 7 days) and cyclosporine (50 mg/kg/day) on renal and hepatic brain and cochlear-reduced glutathione content. Both cyclosporine and FK506 increase glutathione levels in kidney to approximately equivalent levels after 5 days of treatment. Only FK506 increases glutathione levels in liver, and neither drug changes levels in other tissues.

Shah and coworkers[621] show that FK506 exhibits a broad, powerful inhibitory effect on human hepatic microsomal cytochrome P-450-dependent drug metabolism. However, the full potential for drug interactions can only be determined by investigating its effects on other P-450 families using both in vivo and in vitro studies. On the other

hand, Yoshimura and coworkers[622] recently report that, in rats, both FK506 and rapamycin are without significant effects in contrast to cyclosporine on renal microsomal P-450- dependent drug metabolism.

Yoshimura and coworkers[623] review the effect of FK506 and rapamycin on renal P-450 systems in rat models. They find that although cyclosporine has a strong effect on renal P-450 systems and induces such a system in kidney cortex (microsomal P-450), FK506 and rapamycin have no substantial effect on the induction of renal P-450.

The role of intracellular calcium in the pathogenesis of cyclosporine nephrotoxicity has received great attention[624,625] and has resulted in therapeutic implications to prevent nephrotoxic effects of the drug. The effect of cyclosporine and FK506 on microsomes and mitochondria of rabbit renal cortex tissue has been studied by Prasad and associates.[626] Both drugs decrease calcium uptake and A23187-induced calcium release from microsomes and mitochondria in a dose-dependent manner (0.5 to 10.0 μg per mL). The effect of FK506 is significantly less at equivalent concentrations, and microsomal calcium-stimulated ATPase is not changed by either drug.

The potential role of the FK506 binding protein (FKBP12) in cellular calcium homeostasis has been suggested. Indeed, Jayaraman and colleagues[627] find that a 12-kd protein tightly bound to the calcium release channel in skeletal muscles of rabbit is FKBP12. Obviously, if this observation can be confirmed in vascular smooth muscle, it may explain the mechanism of FK506-induced vasoconstriction in renal vasculature. This process also is probably calcineurin-drug complex mediated. A further role of calcineurin in α-adrenergic stimulation of Na^+-K^+-ATPase activity in renal tubular cells is illustrated by Aperia and coworkers.[628] They demonstrate that FK506 inhibited Na^+-K^+-ATPase activity induced by oxymetazoline, an α-adrenergic agonist. This study may suggest a role for FK506-mediated renal nerve changes in sodium and potassium homeostasis. In this context, Palevsky and colleagues[629] report a resistance to the effect of aldosterone on renal cells in cultures exposed to FK506.

Animal Studies

Animal studies have shown both acute and chronic nephrotoxicity produced by FK506.[630] Somewhat different than cyclosporine, FK506 produces toxicity at blood levels that are clinically relevant; however, the doses necessary to achieve these blood levels on a weight basis are at least 10-fold larger than those used clinically. This contrasts with cyclosporine, with which acute and chronic nephrotoxicity can be produced with doses on a weight basis that are very close to those clinically used, particularly in the salt-depleted rat model; however, the blood levels achieved with these doses are at least three to four times those achieved clinically.[631]

Preclinical animal studies gave few hints of nephrotoxicity.[632] However, a troubling series of side effects soon appeared including vasculitis, myocardial necrosis, and severe weight loss. Fortunately, most of these side effects turned out to be species-specific. Nephrotoxicity of FK506 became apparent from the initial series of rescue patients treated with the drug.[633]

Several studies have documented reduction in effective renal plasma flow and GFR in animal models. Ueda and colleagues[634] have measured renal cortical blood flow, using a hydrogen ion clearance method, serum creatinine, and juxtaglomerular cell cross-sectional area in mice treated with FK506, 3 mg/kg/day given subcutaneously as compared with saline-treated control animals. Cortical blood flow is significantly reduced in FK506-treated animals as compared with control animals, as is juxtaglomerular cell area. Kumano and coworkers[635] also note a reduction in GFR and effective renal plasma flow using inulin and p-aminohippuric acid in a heminephrectomized rat model in response to an acute infusion of FK506 and after 21 days of treatment. Proximal tubular vacuolization typical of cyclosporine nephrotoxicity is noted, and diltiazem improves both the functional and morphologic changes caused by FK506. Lieberman and associates[613] note a significant volume reduction in both cyclosporine-treated and FK506-treated glomeruli that are inhibited by verapamil. Mitamura and colleagues[637] review the FK506-induced nephrotoxicity in spontaneous hypertensive rats. These results indicate that the acute nephrotoxicity of FK506 is derived from impaired glomerular function associated with renal arteriolar constriction brought about by the drug. All of these renal disorders induced by FK506 recover completely or partially when the drug is withdrawn for 2 or 4 weeks. Thus, the acute nephrotoxicity of FK506 in spontaneous hypertensive rats is reversible.

Ryffel and colleagues[638] explore the nephrotoxicity of immunosuppressants in rats. Specifically, they compare the nephrotoxic effects of FK506 and rapamycin with that of cyclosporine in male Wistar rats. FK506 causes proximal tubular epithelial changes consisting of atrophy, vacuolization, inclusion bodies, microcalcification, and focal mononuclear interstitial infiltrate as described for cyclosporine. The most striking alteration is hypertrophy of the juxtaglomerular apparatus. The percentage of renin-containing juxtaglomerular apparatus and the extent of renin immunoreactivity along afferent vessels are significantly increased in FK506-treated and CsA-treated rats. By contrast, no renal morphologic lesions are found in rapamycin-treated animals. Renal cortical extracts contain abundant cyclophilin and FK506-binding protein, the main intracytoplasmic receptors for cyclosporine and FK506, respectively. The authors hypothesize that both the immunosuppressive and toxic effects of FK506 and cyclosporine, but not of rapamycin, are mediated through an immunophilin–drug–calcineurin complex. The renal substrate of calcineurin, which mediates renal vasoconstriction, is yet to be identified.

Andoh and associates[639] also compare the acute rapamycin nephrotoxicity with cyclosporine and tacrolimus. They find that cyclosporine and FK506 strikingly decrease

urinary excretion of nitric oxide, renal blood flow, and GFR, whereas rapamycin does not. In contrast, all three of these drugs cause significant hypomagnesemia associated with inappropriately high fractional excretion of magnesium, suggesting renal magnesium wasting. In addition, with all three drugs there are lesions in the rat kidneys consisting of tubular collapse, vacuolization, and nephrocalcinosis. These researchers show that only the calcineurin inhibitors produce glomerular dysfunction in an acute experimental model of nephrotoxicity.

Of interest is the experiment of Hara and colleagues[640] that shows that FK506 is effective in the prevention of the development of rapid glomerular injury in rats with accelerated nephrotoxic serum glomerulonephritis.

Abnormalities in mineral metabolism are common complications of organ transplantation. The role of immunosuppressive agents in alteration of mineral metabolism is not clear. An animal study was conducted to investigate the effects of cyclosporine A (CsA), tacrolimus, and sirolimus on renal calcium, magnesium, and vitamin D metabolism.[641] CsA and tacrolimus induced a two- to threefold and 1.6- to 1.8-fold increase in urinary calcium and magnesium excretion, respectively, whereas rapamycin had no effects on calcium, but doubled the urinary magnesium excretion. CsA and tacrolimus, but not rapamycin, elevated serum 1,25(OH)(2) vitamin D without affecting the parathyroid hormone level. CsA and tacrolimus reduced mRNA abundance in TRPV5 (CsA: 64 ± 3% of control; tacrolimus: 50 ± 3%) calbindin-D28k (CsA: 62 ± 4%; tacrolimus: 43 ± 3%), and vitamin D receptor (CsA: 52 ± 3%; tacrolimus: 58 ± 2%, all $P < .05$). Rapamycin did not affect gene expression in any of studied proteins. The immunofluorescence staining study demonstrated a 50% reduction of TRPV5 and calbindin-D28k by CsA and tacrolimus. The suppression of VDR by calcineurin inhibitors is probably the underlying mechanism of renal calcium wasting. In spite of an increased 1,25(OH)(2) vitamin D level, the kidney is not able to reserve calcium, suggesting a role of vitamin D resistance that may be related to bone loss.

Clinical Studies

Since the initial reports on the use of tacrolimus in clinical transplantation by Starzl and Shapiro,[642,643] numerous large scale trials compared the efficacy and safety of tacrolimus and cyclosporine mainly in liver[644,645] and kidney[646–648] transplantation. According to five out of six of these large trials, renal function and the incidence of renal impairment were comparable in both treatment arms at 1 year posttransplantation. Similar results were reported in a long-term comparison of nephrotoxicity between tacrolimus and cyclosporine in pediatric heart transplant recipients.[649] In contrast, Ashan and coworkers reported a significantly better renal allograft function at 2 and 3 years under tacrolimus compared to cyclosporine, both in combination with steroids and mycophenolate mofetil.[648,650] However, graft survival at 2 and 3 years was comparable in both groups. In an intention-to-treat analysis, graft survival at 5 years was comparable between patients, initially randomized to tacrolimus or cyclosporine in the large U.S. multicenter trial.[646,651]

In a recent study, once daily administration of tacrolimus may be a good option, considering its nephrotoxic effects for kidney transplant patients. Indeed, the trough levels of tacrolimus showed a slight significant reduction after the conversion from the twice daily to the once daily extended release tacrolimus formulations. Serum creatinine and glomerular filtration rate showed a significant improvement without an association with the tacrolimus trough levels.[652]

Ekberg et al.[653] asked the question if in view of the most common immunosuppressive treatment in de novo renal transplantation being a triple regimen that includes tacrolimus, mycophenolate mofetil (MMF), and corticosteroids, and that may also include antibody induction. Whether nephrotoxicity is an issue with tacrolimus at the currently used dosages remains an open question. Data from three large, randomized, de novo renal transplantation studies (Symphony, Fixed Dose Concentration Controlled [FDCC], and OptiCept) that used variations of the triple regimen with respect to tacrolimus target levels, MMF dosing, and antibody induction were pooled. The analysis population consisted of 998 patients. On average, tacrolimus levels were in a range considered low (mean ± standard deviation 7.2 ± 2.54 ng per mL), and MMF dose was 1.5 ± 0.61 g per day. Lower tacrolimus levels and higher MMF doses were associated with significantly better renal function. There were other variables associated with renal function, most notably acute rejection, donor age, and delayed graft function. Subanalyses in each of the three studies gave a consistent picture. There was no overt difference in the effect sizes when patients with stage II (estimated glomerular filtration rate 60–89 mL per min) or stage III (30–59 mL per min) chronic kidney disease were assessed separately. Tacrolimus seems to have a moderate but consistent nephrotoxic effect even in modern efficient immunosuppressive regimens where it is used at lower doses than in previous years.

In all these large trials, the incidence of acute rejection was significantly lower in the tacrolimus treated patients, compared to the cyclosporine-treated patients. Similarly there was a different profile of adverse effects with higher incidence of posttransplant diabetes mellitus and neurotoxicity under tacrolimus, compared to a higher incidence of hypertension, hyperlipidemia, hirsutism, and gum hyperplasia in cyclosporine treated patients.

According to three studies, the intrarenal hemodynamics are less affected by tacrolimus compared to cyclosporine, with better preservation of the renal plasma flow.[559,560,654]

The histopathologic changes, induced by tacrolimus, in the (transplanted) kidney are entirely comparable to those induced by cyclosporine (i.e., arteriolar hyalinosis and striped interstitial fibrosis).[580,655–658] The intrarenal expression of TGF-β, collagen, fibronectin, MMP-2, TIMP-1, and osteopontin was assessed by RT-PCR, and proved to be similar in kidneys treated with either tacrolimus or cyclosporine.[659]

A recent study in children with liver transplantation investigated the influence of genetic polymorphisms in ABCBA on the incidence of nephrotoxicity and tacrolimus dosage requirements in pediatric patients following liver transplantation.[660] Haplotype analysis showed a significant association between T-T-T haplotypes and an increased incidence of nephrotoxicity at 6 months posttransplantation (haplotype-frequency = 52.9% in nephrotoxic patients vs. 29.4% in controls; P = .029). Furthermore, G2677→T and C3435→T polymorphisms and T-T-T haplotypes were significantly correlated with higher tacrolimus dose-adjusted predose concentrations at various time points examined long after drug initiation. The findings suggest that ABCB1 polymorphisms in the native intestine significantly influence tacrolimus dosage requirement in the stable phase after transplantation. In addition, ABCB1 polymorphisms in pediatric liver transplant recipients may predispose them to nephrotoxicity over the first year posttransplantation. Genotyping future transplant recipients for ABCB1 polymorphisms (G2677→T and C3535→T), therefore, could have the potential to individualize better tacrolimus immunosuppressive therapy and enhance drug safety.

In conclusion, the nephrotoxicity of tacrolimus is functionally and morphologically comparable to the nephrotoxicity of cyclosporine as well in recipients of a renal allograft, as in recipients of a solid nonrenal organ. However, there is evidence that tacrolimus is a more powerful immunosuppressive agent, with a different toxicity profile.

CNI nephrotoxicity was recognized in Cambridge in the late 1970s. The vasoconstrictor impact of CsA, and to a lesser extent tacrolimus, in both acute and chronic settings, results from a decrease in vasodilators and increase in vasconconstrictors whereas direct tubular toxicity results from blockade of mitochondrial permeability transition pores and inhibition of prolyl isomerase. It is thus apparent that we must revisit the data and again question the basis for chronic CNI nephrotoxicity in current clinical practice. This contribution to the debate will focus on the evidence that CNIs are nephrotoxic and that their impact needs to be limited if we are to improve long-term outcomes after transplantation, leaving others to promote the contrary perspective and perhaps also to reflect on the largely unproven impact of the steroid avoidance and other minimization strategies so prevalent today.[661]

MYCOPHENOLATE MOFETIL

MMF, the morpholinoethyl ester of mycophenolic acid (Fig. 31.5), has been developed as an immunosuppressant for prevention of rejection in renal transplantation. In vivo, MMF is deesterified to mycophenolic acid (the active immunosuppressive component), which is a potent and specific inhibitor of the synthesis of guanosine nucleotides and thus a selective suppressor of proliferation of both T and B lymphocytes. MMF, given alone or with corticosteroids or cyclosporine, lowers the frequency of acute rejection after allogeneic organ transplantation in animals.[662,663]

The immunosuppression of MMF appears to be additive with that of cyclosporine and tacrolimus, and MMF does not promote nephrotoxicity.[664] Initial studies indicated that MMF, in combination with cyclosporine and steroids, reduces the incidence of acute rejection in renal transplantation.[665–667]

The Mycophenolate Steroids Sparing (MYSS) study found that in renal transplant recipients who were on immunosuppressive therapy with the cyclosporine microemulsion Neoral, MMF was not better than azathioprine in preventing acute rejection at 21 months after transplantation and was 15 times more expensive. The MYSS Follow-up Study, an extension of MYSS, was aimed at comparing long-term outcome of 248 MYSS patients according to their original randomization to MMF (1 g twice daily) or azathioprine (75 to 100 mg per d). In kidney transplantation, the long-term risk/benefit profile of MMF and azathioprine therapy in combination with cyclosporine Neoral is similar. In view of the cost, standard immunosuppression regimens for kidney transplantation should perhaps include azathioprine rather than MMF.[668]

In the meantime, MMF has been used under several clinical conditions. In cardiac, liver, lung, and pancreas transplantation the use of MMF in association with reduced doses of cyclosporine has resulted in improved renal function and maintained immunosuppression.[669–672] In renal transplantation, Halloran and others[673] summarize the three multicenter trials that confirm, at 1-year posttransplant, MMF is effective in preventing acute renal allograft rejection.[665–667] Two studies address patients with chronic kidney graft dysfunction in whom MMF is introduced and cyclosporine exposure reduced.[674,675] The rationale is that the increased immunosuppressive potency of MMF would allow for a safe reduction of CsA doses. The conclusion from these two studies is that cyclosporine dose reduction in patients on MMF results in an improved graft function with no increased risk of rejection. It must be noted, however, that follow-up was short—less than 1 year—in both studies. In addition, whether MMF does better than azathioprine (AZA) in this setting remains an open question. Indeed, a similar improvement of chronic graft dysfunction is reported when AZA is introduced and cyclosporine doses reduced.[676]

Is there a role for MMF in the attempt to withdraw calcineurin inhibtors in patients with stable renal transplantation? Several randomized,[677–679] as well as nonrandomized,[690] clinical trials in renal transplantation examined the efficacy and safety of calcineurin inhibitors in patients with stable graft function under triple immunosuppressive regimen, consisting of prednisolone, cyclosporine or tacrolimus, and MMF. All these studies reported a significant improvement of the graft function, as well as lower blood pressure, and an improved lipid profile after CNI withdrawal. In contrast, the incidence of acute rejection was higher in the CNI withdrawal group, without any impact on graft survival. Therefore, these studies provide evidence that CNI withdrawal is achievable in renal transplant recipients with stable graft function. CNI withdrawal appears to improve graft function,

hypertension, and hyperlipidemia. However, caution should be paid to the increased incidence of acute rejection after CNI withdrawal, and the short term of follow-up, reported in these studies (6 to 32 months).Whether CNI withdrawal will result in improved graft survival is not yet known.

Because MMF has multiple immunosuppressive and anti-inflammatory modes of actions—including the inhibition of humoral and cellular immunity, antimutagenesis, reduction of mononuclear cell infiltration, and inhibition of vascular smooth muscle and mesangial cell proliferation—it is not surprising that the drug is now used in autoimmune-mediated renal disease.[681] Briggs and colleagues[682,683] report their limited experience in eight patients with different types of nephrotic-type glomerulonephritis and unsatisfactory response to steroids and cyclosporine. Controlled prospective studies are under way to clarify the potential advantages of MMF compared with other immunosuppressive agents in these disease entities.

In conclusion, MMF is a potent, nonnephrotoxic immunosuppressive drug, which significantly reduced the incidence of acute rejection under tripple immunosuppressive regimens with prednisolone and cyclosporine or tacrolimus. In addition, MMF appears to allow dose reduction or even withdrawal of CNI in renal transplant recipients with stable graft function, thereby avoiding the long-term nephrotoxicity, induced by calcineurin inhibtors. Because immunosuppressant-induced nephrotoxicity has been associated with significant financial costs, cyclosporine-sparing and FK506-sparing regimens should result in substantial savings in health care costs (Fig. 31.5).[684] It is important to emphasize here that there are no long-term data for these experimental regimens, but they offer a new direction if these short-term results are confirmed over a more sufficient period of time.

RAPAMYCIN

Rapamycin (sirolimus/SRL) is a macrocyclic fermentation product of Streptomyces hygroscopicus, and was first isolated in 1975.[685] SRL has a similar molecular structure to FK506 and also binds to FKBP12.[686] However, the SRL-FKBP12 complex does not affect the calcineurin phosphatase, but instead binds to a protein, called the mammalian target of rapamycin (mTOR).[687] This binding of the SRL-FKBP12 complex to mTOR inhibits both DNA and protein synthesis, resulting in arrest of the cell cycle in late G1, as it progresses to the S phase.[688]

SRL blocks T-cell proliferation, induced by cytokines, alloantigens, and mitogens in a dose-dependent manner.[689] In addition, SRL acts on B-cells, causing an inhibition of antigen and cytokine driven B-cell proliferation.[690] In vitro studies have demonstrated the synergistic immunosuppressive interaction of CsA and SRL,[691] in contrast to the combination of FK506 and SRL, which produced an antagonistic effect at low doses.[692] Animal studies have confirmed the immunosuppressive potential of SRL,[693] as well as its synergistic interaction with CsA.[694] In contrast to the in vitro studies, FK506 interacted synergistically with SRL in animal studies.[695]

Treatment with CsA or FK alone significantly decreased KLOTHO expression and increased urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) excretion compared with vehicle treatment but sirolimus treatment did not. Treatment SRL+CsA or SRL+FK further decreased KLOTHO expression and increased urinary 8-OHdG excretion compared with treatment of CsA or FK alone. There was a strong correlation between KLOTHO expression and urinary 8-OHdG excretion ($r = -0.893$; $P < .001$). Treatment of CsA or FK alone increased renal ectopic calcification and serum intact parathyroid hormone level and decreased renal FGF23 expression compared with VH treatment ($P < .05$) but SRL treatment did not. Treatment with SRL+CNI aggravated these parameters compared with CNI alone. Sirolimus accelerates the calcineurin inhibitor-induced oxidative process by down-regulating the renal antioxidant KLOTHO expression in the kidney.[696]

Clinical Efficacy of Sirolimus in Renal Transplantation

Based on the results from several multicenter, prospective, randomized trials—including studies based in the United States, globally, a combined European-United States, and two in Europe—SRL was approved in 1999 by the U.S. Food and Drug Administration (FDA) for the prevention of acute rejection in renal transplant recipients. SRL was used in combination with CsA and prednisolone in these studies, and reduced the incidence of acute rejection at 1 year to 10%.[697]

Three prospective, randomized trials in renal transplant recipients compared CsA to SRL in combination with prednisolone and azathioprine or mycophenolate mofetil.[698–700] All these trials showed a comparable incidence of acute rejection in both treatment arms and, more importantly, a superior graft function at 1 year in the SRL-treated patients. In one study, graft function remained significantly better at 3 years in the SRL-treated patients.[701] In addition, the incidence of normal histology in protocol biopsies at 2 years was significantly higher in SRL-treated patients, compared to CsA-treated patients (66.6% vs. 20.8%).[701] These results from three trials provide evidence that avoidance of the long-term nephrotoxicity, induced by calcineurin inhibitors, is achievable in renal transplant recipients.

Several randomized trials in renal transplantation investigated the feasibility and the outcome of early calcineurin inhibitor withdrawal, in triple regimens with SRL and prednisolone.[702–705] Overall, patient and graft survival, as well as the incidence of acute rejection at 1 year, were comparable in both treatment arms (CsA+SRL+ P vs. SRL+P). In contrast, graft function was superior, and the incidence of hypertension was reduced in the patients weaned from CsA. Similarly, the incidence of chronic allograft nephropathy was significantly lower in protocol biopsies at 1 year from patients on SRL+P alone.[705] Again, these data provide strong

evidence that early witdrawal of calcineurin-inhibitors can safely be achieved in renal transplant recipients under SRL, and may avoid long-term nephrotoxicity.

In a recent study of Heilman and colleagues, it was concluded that conversion from tacrolimus-MMF to sirolimus-MMF at 1 month posttransplant in kidney recipients on rapid steroid withdrawal is poorly tolerated and does not improve GFR at 1 year.[706]

The use of SRL in organ transplantation is still a matter of debate as demonstrated by recent pro and contra back-to-back publications.[707] Sirolimus therapy is burdened by a concerning safety profile including high risk of delayed graft function and onset of proteinuria. In addition, several other side effects such as dyslipidemia, diabetes, myelosuppression, delayed wound-healing, infertility, ovarian cysts, and mouth ulcers further limit its use.[708]

A feature of increasing importance is that the mTOR pathway is central for vital aspects of tumor development, including angiogenesis and cell growth; rapamycin, therefore, has anticancer activities, which may prove critical in the fight against high cancer rates in transplant recipients.[707]

Large trials showed that SRL therapy is associated with an increased risk of acute rejections and worse graft function as compared with cyclosporine or tacrolimus.[709] Boosting memory T-cell response by mTOR inhibitor therapy might help induce long-lasting protective immune memory against bacterial or viral pathogens strong enough to prevent their replication,[710] especially in transplant recipients and other immunosuppressed patients with autoimmune disorders. Conversion to mTOR inhibitors may represent a valuable option in those cases with persistent infection resistant to antiviral therapy.[711,712]

A recent intriguing observation[713] adds to the interest of this molecule. Inoki et al. have shown that activity of mTOR complex 1 (mTORC1), a kinase that senses nutrient availability, was enhanced in the podocytes of diabetic animals. Further, podocyte-specific mTORC1 activation induced by ablation of an upstream negative regulator (PcKOTsc1) recapitulated many DN features, including podocyte loss, glomerular basement membrane thickening, mesangial expansion, and proteinuria in nondiabetic young and adult mice. Abnormal mTORC1 activation caused mislocalization of slit diaphragm proteins and induced an epithelial-mesenchymal transition-like phenotypic switch with enhanced ER stress in podocytes. Conversely, reduction of ER stress with a chemical chaperone significantly protected against both the podocyte phenotypic switch and podocyte loss in PcKOTsc1 mice. Finally, genetic reduction of podocyte-specific mTORC1 in diabetic animals suppressed the development of DN. These results indicate that mTORC1 activation in podocytes is a critical event in inducing DN and suggest that reduction of podocyte mTORC1 activity is a potential therapeutic strategy to prevent DN.

One may suggest that no renal transplant patient should receive de novo SRL therapy and that the use of SRL should be restricted to very selected patients, such as those with posttransplant malignancies or, probably, treatment-resistant viral infection. In such patients, the risk/benefit profile of SRL therapy should be carefully considered on a case-by-case basis. On the other hand, in subjects with stable kidney function and no evidence of treatment-related side effects, there is no reason to stop mTOR inhibitor therapy.

Nephrotoxicity of Sirolimus

Studies in pigs and rats have shown that sirolimus has no deleterious effects on GFR or renal blood flow, and caused minimal morphologic signs of toxicity.[714,715] Sirolimus reduced medullary concentrating ability and increased tubular enzymuria in rat kidneys, suggesting that mild tubular injury may occur.[716] In a salt-depleted rat model of CsA toxicity, the combination of CsA with SRL produced a functional and morphologic deterioration.[717]

In clinical studies in renal transplant recipients, sirolimus proved to be an effective immunosuppressive drug, devoid of intrinsic nephrotoxicity (see "Clinical Efficacy of Sirolimus in Renal Transplantation"). However, sirolimus may prolong delayed graft function in renal transplant recipients.[718] In addition, thrombotic microangiopathy has been described under the combination of sirolimus and tacrolimus, after intestinal transplantation.[719] Of concern are the reports on de novo proteinuria, occurring after conversion from a calcineurin inhibitor to sirolimus in renal transplant recipients.[720] Although the exact mechanism for the development of this proteinuria is currently putative, the increased intraglomerular pressure, resulting from the withdrawal of the intrarenal vasoconstriction, induced by CNIs, could be one reasonable explanation.

Recent evidence of a high incidence of proteinuria among de novo sirolimus-based regimens has been reported among renal transplant patients at short-term follow-up. Proteinuria has become a recognized, serious event (5 years incidence of 29%–38%) of primarily sirolimus-treated renal transplants patients, which is most probably of glomerular origin. It has been shown that proteinuria exerts a bad prognostic effect on graft function and subsequent graft survival at 5-year follow-up.[721]

The mechanisms underlying the development of proteinuria in renal transplant recipients converted from calcineurin inhibitors to sirolimus are still unknown. The data suggest that sirolimus-induced proteinuria in humans may be a dose-dependent effect of the drug on key podocyte structures.[722]

In a recent single center experience[723] and confirmed by many other observations, mTORi sirolimus and everolimus associated–pneumonitis is not a rare disease. Pneumonitis is not apparently dependent on the drug dose or the blood levels. Discontinuation of mTORi seems to be the safest treatment option to avoid pulmonary fibrosis or a fatal outcome.

New-onset diabetes after transplantation (NODAT) is a multifactorial, complex metabolic disorder associated with impaired long-term graft function, reduced recipient survival, and increased risks of cardiovascular disease and

infectious complications. Neither calcineurin inhibitor nor SRL or steroids seems to be innocent of contributing to it. Immunosuppressants account for 74% of the occurrence of NODAT. Among modifiable risk factors, obesity is independent and significant, with great prevalence in the population. In additional to lifestyle modifications, the role of bariatric surgery (BS) either before or after transplantation is highlighted herein as a strategy to reduce disease in the view of the results among overweight, nontransplanted patients.[724] Because of the strong association between high glucose values in the early posttransplant period and the development of NODAT, the condition must be recognized early after (or even before) transplantation by intensive screening. Patients at risk for NODAT must modify appropriate risk factors and particularly undergo pretransplant planning and/or posttransplant adjustment individualizing immunosuppressive therapy to mitigate the risk of this serious complication.

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CHAPTER

32 Nephrotoxicity of Nonsteroidal Anti-inflammatory Agents, Analgesics, and Inhibitors of the Renin-Angiotensin System

Biff F. Palmer

NEPHROTOXICITY OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Nonsteroidal anti-inflammatory drugs (NSAIDs) are some of the most widely used therapeutic agents in clinical practice today. Although the gastrointestinal toxicity of these medications is well known, it has become increasingly apparent that the kidney is also an important target for untoward clinical events. The renal toxicity associated with the use of NSAIDs can be divided into one of several distinct clinical syndromes. These include a form of vasomotor acute renal failure, nephrotic syndrome associated with interstitial nephritis, chronic renal injury, and abnormalities in sodium, water, and potassium homeostasis. The common link in these syndromes is a disruption in prostaglandin metabolism, the class of compounds whose synthesis is inhibited by these agents.

PROSTAGLANDIN BIOSYNTHESIS AND COMPARTMENTALIZATION

Prostaglandins are members of a class of compounds termed eicosanoids. Eicosanoids are biologically active fatty acids that are all derived from the oxygenation of arachidonic acid. The particular enzyme involved in the oxygenation process dictates which class of eicosanoid will be synthesized. Oxygenation of arachidonic acid by the enzyme cyclooxygenase is responsible for prostaglandin and thromboxane synthesis (Fig. 32.1). The enzyme lipoxygenase converts arachidonic acid to leukotrienes, lipoxins, and eventually, to hydro fatty acid derivatives such as hydroxyeicosatetraenoic acid (HETE). Finally, oxygenation by the cytochrome P-450 system generates epoxyeicosatrienoic acids (EETs).

The availability of free arachidonic acid is the rate-limiting step in eicosanoid biosynthesis. Normally, arachidonic acid is found esterified to membrane phospholipids, where it undergoes deacylation primarily under the influence of phospholipase A_2. Phospholipase A_2–mediated arachidonic acid release is a calcium-calmodulin–dependent step that is stimulated by vasopressin, bradykinin, angiotensin, and norepinephrine.[1] Once released, free arachidonic acid is either re-esterified back into membrane lipids or is converted into one of the biologically active eicosanoids.

The first step in the synthesis of prostaglandins and thromboxanes is a cyclooxygenase reaction in which arachidonic acid is converted into the cyclic endoperoxide prostaglandin G_2 (PGG_2). PGG_2 then undergoes a peroxidase reaction to form a second endoperoxide called PGH_2, which is accompanied by the formation of a superoxide radical. Both of these reactions are catalyzed by the enzyme cyclooxygenase (COX), also known as prostaglandin endoperoxide H synthase.[2,3] The cyclooxygenase and peroxidase reactions occur on distinct but neighboring sites on the COX enzyme. Once formed, PGH_2 has a short half-life and is rapidly acted on by a series of enzymes that produce biologically active prostaglandins or thromboxane. Prostacyclin synthase acts to form prostacyclin (PGI_2), thromboxane synthase forms thromboxane A_2, and isomerases are responsible for the formation of PGE_2, PGD_2, and $PGF_{2\alpha}$.

Prostaglandins are synthesized on demand and exert physiologic effects in discrete microenvironments along the nephron in close proximity to their points of synthesis (Table 32.1). Due to the virtual absence of distant effects, these compounds are best regarded as autacoids rather than hormones. Variations in the synthetic and degradative machinery along the length of the nephron account for the differing types and amounts of prostaglandins found in any given segment.[4] PGI_2 is the most abundant prostaglandin produced in the cortex and is primarily synthesized in cortical arterioles and glomeruli.[5] This location corresponds to the known effects of PGI_2 in regulating renal vascular tone, the glomerular filtration rate (GFR), and renin release. PGE_2 and thromboxane A_2 are also produced in the glomerulus and therefore may exert effects at this site.

The most abundant prostaglandin found in the tubules is PGE_2.[5] The cortical and especially the medullary portion of the collecting duct are the dominant sites of PGE_2 synthesis. Lesser amounts are found in the thin descending

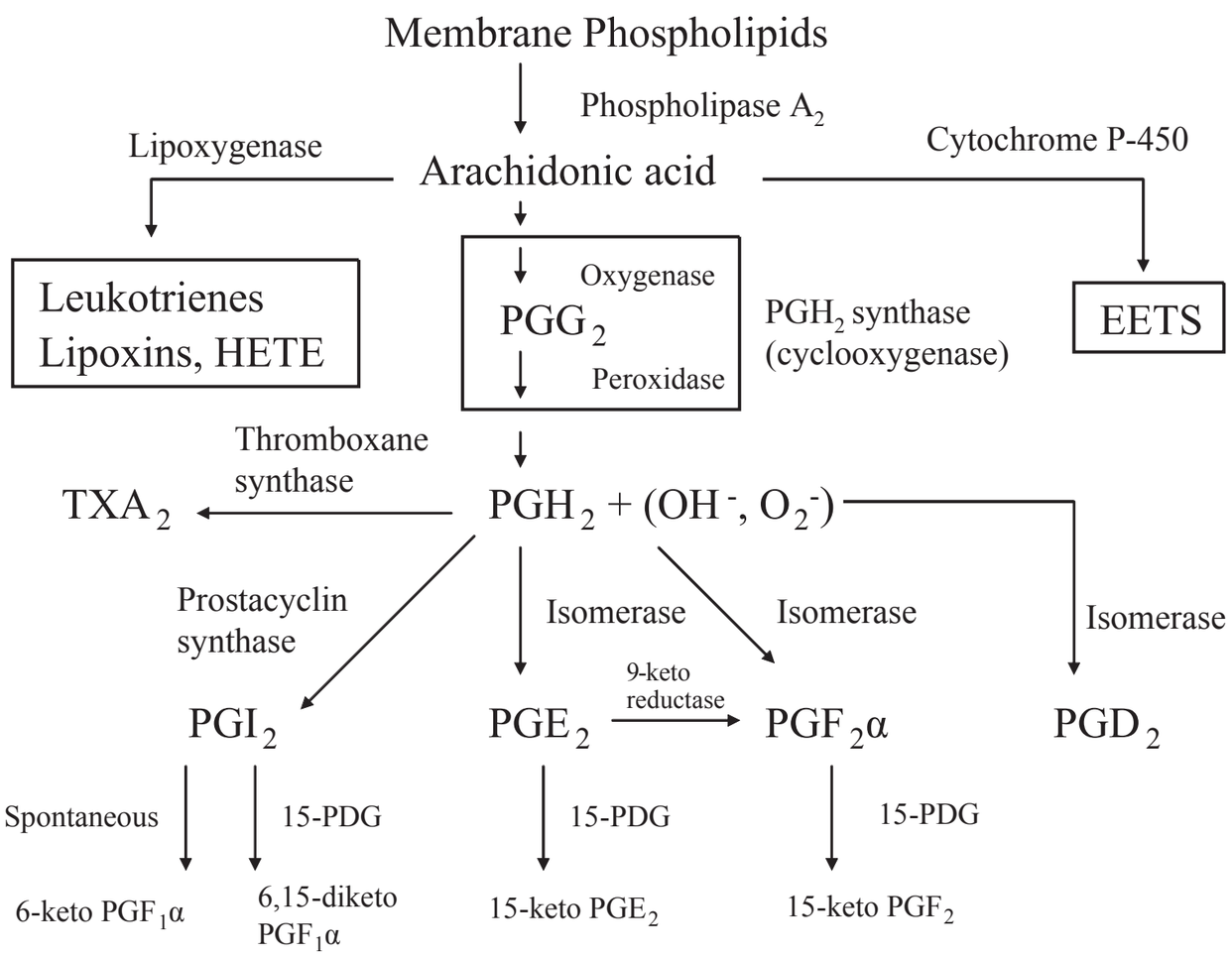

FIGURE 32.1 Synthetic and degradative pathways for the different types of eicosanoids. 15-*PDG*, 15 prostaglandin dehydrogenase; *EETs*, epoxyeicosatrienoic acids; *HETE*, hydroxyeicosatetraenoic acid; *TXA_2*, thromboxane A_2.

and thick ascending limb with the least amount of synthesis found in the proximal tubule. Medullary interstitial cells are also a rich source of PGE_2 production. This distribution provides the anatomic basis for PGE_2 to modulate sodium and chloride transport in the Henle loop, regulate arginine vasopressin-mediated water transport, and control vasa recta blood flow. $PGF_{2\alpha}$ is synthesized primarily by medullary interstitial cells and less by the papillary collecting tubule and glomeruli. Prostaglandin-degradative enzymes are found in both the cortex and medulla but are most abundant in the cortex. Except for PGI_2, which undergoes spontaneous hydrolysis to 6-keto-$PGF_{2\alpha}$, prostaglandins are rapidly metabolized into inactive products by a 15-prostaglandin dehydrogenase. An increased concentration of this enzyme in the proximal nephron may facilitate the degradation of prostaglandins delivered to the proximal tubule by glomerular filtration.[6]

32.1 Compartmentalization and Function of Renal Prostaglandins

Site	Eicosanoid	Action
Arterioles	PGI_2, PGE_2	Vasodilation
Glomeruli	$PGI_2 > PGE_2$ (human)	Maintain GFR
	$PGE_2 > PGI_2$ (rat)	Vasoconstriction
	TXA_2	
Tubules	PGE_2, $PGF_{2\alpha}$	Enhance NaCl and water excretion
Interstitial cells	PGE_2	Enhance NaCl and water excretion, influences regional blood flow
Juxtaglomerular apparatus	PGI_2, PGE_2	Stimulate renin release

PGI, prostaglandin I; PGE, prostaglandin E; PGF, prostaglandin F; TXA, thromboxane A; GFR, glomerular filtration rate.

BIOLOGIC ACTIONS OF PROSTAGLANDINS IN THE KIDNEY

Under baseline euvolemic conditions, prostaglandin synthesis is negligible, and as a result, these compounds play little to no role in the minute-to-minute maintenance of renal function. However, a major role occurs in the setting of a systemic or intrarenal circulatory disturbance. This interaction is best illustrated when examining the renal function under conditions of volume depletion (Fig. 32.2). In this setting, renal blood flow is decreased while sodium reabsorption, renin release, and urinary concentrating ability are increased. To a large extent, these findings are mediated by the effects of increased circulating levels of angiotensin II (AII), arginine vasopressin (AVP), and catecholamines. At the same time, these hormones stimulate the synthesis of renal prostaglandins, which in turn act to dilate the renal vasculature, inhibit salt and water reabsorption, and further stimulate renin release. Prostaglandin release under these conditions serves to dampen and counterbalance the physiologic effects of the hormones that elicit their production. As a result, renal function is maintained near normal levels despite the systemic circulation being clamped down. Predictably, the inhibition of prostaglandin synthesis will lead to unopposed activity

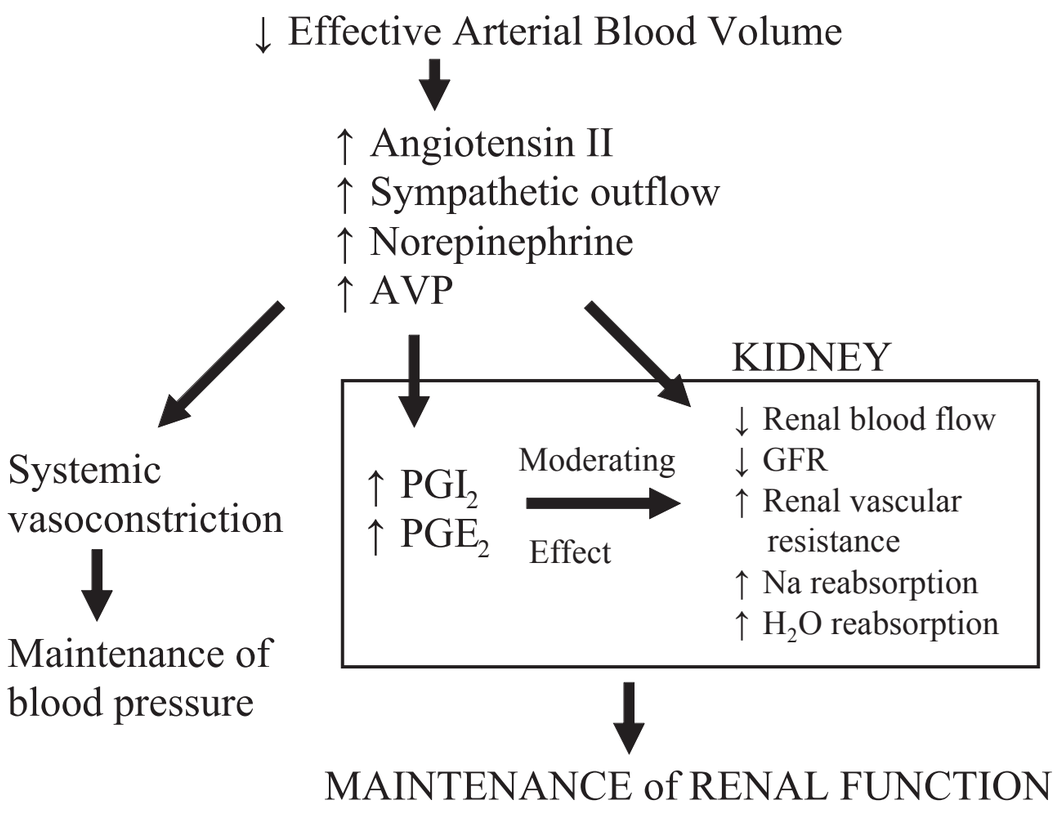

FIGURE 32.2 In the setting of absolute or effective volume depletion, a number of effectors are activated that serve to defend the circulation and, at the same time, stimulate the synthesis of renal prostaglandins. In turn, renal prostaglandins function to moderate the effects of these hormonal systems such that renal function is maintained in the setting of systemic vasoconstriction. *AVP*, arginine vasopressin; *GFR*, glomerular filtration rate.

of these hormonal systems, resulting in exaggerated renal vasoconstriction and magnified antinatriuretic and antidiuretic effects. In fact, many of the renal syndromes that are associated with the use of NSAIDs can be explained by the predictions of this model.

EEXPRESSION AND REGULATION OF CYCLOOXYGENASE-1 AND -2 IN THE KIDNEY

Aspirin and other NSAIDs exert their prostaglandin-inhibitory effects by inhibiting the COX enzyme. The COX enzyme exists as two isoforms termed COX-1 and COX-2. These enzymes are encoded by two different genes and differ significantly in their regulation. The COX-1 enzyme is constitutively expressed in most tissues and is responsible for producing prostaglandins involved in maintaining normal tissue homeostasis. The COX-2 enzyme is principally an inducible enzyme rapidly upregulated in response to a variety of stimuli such as growth factors and cytokines typically found in the setting of inflammation.[3] With the discovery of COX-2, a great deal of effort was put forth to develop compounds to selectively block the activity of this isoform without affecting the activity of COX-1. The availability of a COX-2–specific inhibitor would provide a therapeutic tool to inhibit the synthesis of arachidonic acid metabolites at sites of inflammation and yet leave unperturbed COX-1–derived prostanoids involved in normal homeostasis. In this manner the analgesic, anti-inflammatory, and antipyretic effects of an NSAID could be obtained with minimal to no side effects. Although the initial experience with specific COX-2 inhibitors has been associated with a reduction in gastrointestinal complications, this paradigm is not applicable to the kidney.

COX-1 and COX-2 are both constitutively expressed in the kidney. COX-1 is localized to mesangial cells, arteriolar endothelial cells, parietal epithelial cells of the Bowman capsule, and throughout the cortical and medullary collecting duct.[7] COX-2 is primarily expressed in the macula densa and the adjacent cells in the cortical thick ascending limb with lesser amounts in the podocytes and the arteriolar smooth muscle cells.[8–9] COX-2 is also abundantly expressed in interstitial cells in the inner medulla and the papilla.

The expression of COX-2 in different regions of the kidney varies in response to alterations in intravascular volume. This variation is particularly evident in the macula densa, where studies show COX-2 plays an important stimulatory role in the release of renin via the tubuloglomerular feedback mechanism. Under conditions of low renal perfusion when the chloride concentration at the level of the macula densa is low, renin release is inhibited by a COX-2 selective inhibitor but unaffected by a COX-1 inhibitor.[10] In genetically engineered mice lacking COX-2, there is a failure of renin release in response to a low salt diet, whereas renin release is intact in animals lacking COX-1.[11,12]

Stimulation of renin with the subsequent formation of angiotensin II is part of a feedback loop because angiotensin II exerts an inhibitory effect on COX-2 synthesis in the macula densa via the angiotensin type 1 (AT_1) receptor.[13] In contrast to effects at the macula densa angiotensin II upregulates COX-2 and prostaglandin synthesis in vascular smooth muscle cells and mesangial cells.[14] This latter effect provides a mechanism for COX-2 to both facilitate the tubuloglomerular feedback response to low salt delivery to the macula densa by increasing angiotensin II levels and preserve the glomerular filtration rate through the generation of vasodilatory prostaglandins to antagonize the vasoconstrictive effect of angiotensin II.[15]

The expression of COX-2 is also responsive to changes in volume. COX-2 expression decreases with salt depletion and increases with a high salt diet and dehydration.[9] COX-2–derived prostaglandins may play an important role in facilitating a natriuretic response to salt loading and may help protect against volume overload. The increase in COX-2 in response to dehydration is thought to provide a cytoprotective effect in the setting of hypertonic stress.[16,17] Treatment of water-deprived animals with a selective COX-2 inhibitor is associated with apoptotic patches of renal medullary interstitial cells. By contrast, no such changes are seen in animals treated with the inhibitor alone or in animals undergoing water deprivation without pharmacologic treatment.

In summary, COX-2 is constitutively expressed in the kidney and is highly regulated in response to physiologic perturbations in intravascular volume. The majority of experimental and clinical studies to date suggest that the specific COX-2 inhibitors may not offer any distinct advantage

over traditional NSAIDs with regard to renal toxicity. In fact, most of the renal syndromes that have been linked to nonselective COX inhibitors have now been described with the selective COX-2 inhibitors. The only exception is the development of chronic kidney disease and papillary necrosis. The failure to link COX-2 inhibitors use to these complications is not surprising because these agents have only been available for clinical use for a relatively short time. As with traditional NSAIDs, the COX-2 inhibitors need to be used cautiously and require close monitoring of renal function in patients at high risk for adverse renal outcomes.

EFFECTS OF PROSTAGLANDINS ON THE RENAL CIRCULATION

Prostaglandins primarily exert a vasodilatory effect on the renal vasculature. This vasodilatory effect alters the renal circulation in two major ways. First, these compounds influence the distribution of renal blood flow to different regions of the kidney. Prostaglandin stimulation results in a preferential increase in blood flow to the more juxtamedullary nephrons.[18,19] By contrast, the inhibition of prostaglandin synthesis results in a selective reduction of flow to the inner cortical nephrons while flow remains well preserved in the outer cortex.[20] Second, prostaglandins exert a vasoregulatory effect on the renal microcirculation to include the interlobular, afferent, and efferent arterioles as well as the glomerular mesangium. In isolated renal arterioles, both PGE_2 and PGI_2 attenuate AII-induced and norepinephrine-induced afferent arteriolar vasoconstriction. On the efferent side of the circulation, PGI_2 similarly antagonizes AII-induced and norepinephrine-induced vasoconstriction, but PGE_2 is without effect.[21] In addition to local production, vascular reactivity of the efferent arteriole appears to be influenced by prostaglandins produced in the upstream glomerulus. In this regard, Arima and associates[22] find that the orthograde infusion of AII (afferent arteriole-glomerulus-efferent arteriole) results in less vasoconstriction of the efferent arteriole as compared to when infused in a retrograde fashion (efferent arteriole-glomerulus-afferent arteriole). Pretreatment with indomethacin markedly increases the vasoconstrictive effect during an orthograde infusion but is without effect during the retrograde infusion.

Prostaglandins have also been shown to attenuate mesangial cell contraction induced by AII, endothelin, AVP, and platelet-activating factor.[23,24] Contraction of these cells will normally cause a decrease in the total glomerular capillary surface area and result in a fall in the GFR. Mesangial cell synthesis and the release of PGI_2 in humans and PGE_2 in rats dampen the constrictor effects of these hormones such that the glomerular capillary surface area is maintained, thereby minimizing any fall in GFR. Thus, in the setting of enhanced hormonal constrictor activity, prostaglandins play a major role in maintaining glomerular hemodynamics by exerting a vasodilatory effect at the level of the afferent and efferent arteriole as well as within the glomerular mesangium.

RENAL SYNDROMES ASSOCIATED WITH NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Vasomotor-Induced Acute Renal Failure

Prostaglandins appear to play a negligible role in the maintenance of renal function under normal circumstances. This conclusion is based on studies in both experimental animals as well as humans. In conscious, sodium-replete dogs and rats, the inhibition of renal prostaglandin synthesis with a variety of NSAIDs does not alter baseline renal blood flow or GFR.[25,26] Similarly, renal hemodynamics are unaffected in healthy humans after both the short-term[27,28] and the long-term administration of aspirin.[29] In related studies, the administration of indomethacin to healthy volunteers was also found to produce no change in renal hemodynamics.[30]

A sharply different effect of COX inhibition is observed when systemic hemodynamics are compromised. Under conditions of circulatory distress, renal blood flow represents a balance between vasoconstrictor influences on the one hand and vasodilatory prostaglandins on the other. Predictably, the administration of NSAIDs in this setting will shift this balance toward unopposed vasoconstriction and will potentially result in a precipitous decline in renal function.

This interplay between vasoconstrictive effectors and vasodilatory prostaglandins is particularly well illustrated in a series of studies using a model of hemorrhage in dogs.[31,32] In animals subjected to a hemorrhage, prostaglandin synthesis inhibition was associated with a marked reduction in renal blood flow as compared to prostaglandin-intact dogs. This renal ischemic response was found to be partly reversed after the infusion of an AII antagonist or after renal denervation. When renal denervation was combined with the AII antagonist, renal blood flow was restored to values comparable to that in the nonprostaglandin-inhibited animals. These findings illustrate the pivotal role that prostaglandins play in opposing the renal ischemic effects of AII and renal nerves.

The modulating effect of vasodilatory prostaglandins on renal hemodynamics can be expected to roughly parallel the extent to which vasoconstrictor effectors are activated. In turn, the activity of these effectors will reflect the degree of circulatory distress. With only mild perturbations in the circulation, one can begin to detect a discernible effect of prostaglandins on renal blood flow. For example, unlike subjects ingesting an ad lib sodium diet, normal subjects placed on a salt-restricted diet will demonstrate a modest fall in creatinine clearance and renal blood flow following the administration of aspirin or indomethacin.[33,34]

Diuretic therapy is a common clinical situation where NSAIDs may exert a deleterious effect on renal function in otherwise healthy subjects.[35] Like sodium restriction, diuretics

increase the dependence of renal blood flow and GFR on vasodilatory prostaglandins and potentiate the deleterious effects of prostaglandin inhibition with COX inhibitors. The degree to which renal function is disturbed, however, appears to vary depending on which diuretic–NSAID combination is used. In this regard, Favre and colleagues[35] found that the combination of triamterene and indomethacin given to healthy subjects results in a marked decline in creatinine clearance. By contrast, only a mild decrease in creatinine clearance is found when indomethacin is given in combination with furosemide, hydrochlorothiazide, or spironolactone. Interestingly, triamterene is the only diuretic associated with a marked increase in urinary prostaglandin secretion. Although there is little evidence to suggest that the renal failure patients in this study were volume-depleted, it appears that triamterene, by some unknown mechanism, renders the renal circulation critically dependent on vasodilatory prostaglandins. As a result, triamterene in combination with an NSAID should only be used with extreme caution.

As alterations in the circulation become more pronounced, rendering the renal circulation more dependent on vasodilatory prostaglandins, COX inhibition can be expected to result in more profound changes in renal hemodynamics. In congestive heart failure, a decrease in effective arterial circulatory volume is the proximate cause for activation of neurohumoral vasoconstrictor forces that participate in the maintenance of systemic arterial pressure and result in increased total peripheral vascular resistance. Important to note, the rise in renal vascular resistance is less than that seen in the periphery. Vasodilatory prostaglandins function in a counterregulatory role, attenuating the fall in renal blood flow and GFR that would otherwise occur if vasoconstrictor forces were left unopposed.[36]

Cirrhosis is another clinical condition in which the integrity of the renal circulation can become critically dependent on vasodilatory renal prostaglandins. Cirrhotic patients with a low urinary sodium concentration tend to be the most susceptible to develop acute decrements in renal function following the administration of NSAIDs.[37] These patients have a more marked decrease in effective arterial circulatory volume, primarily due to splanchnic vasodilation, which in turn leads to higher levels of circulating catechols, AII, and AVP.[38,39] As a result, the renal circulation in this subset of patients is more critically dependent on the effect of vasodilatory prostaglandins. As seen in patients with congestive heart failure, these patients have high urinary concentrations of PGE_2, which decline in parallel with the fall in GFR.[37]

Renal prostaglandins may play an important role in the maintenance of renal hemodynamics in nephrotic syndrome. GFR and filtration fraction are moderately decreased in most patients with the nephrotic syndrome.[40,41] Micropuncture studies in an experimental model of the nephrotic syndrome have indicated that the relative preservation of renal plasma flow may serve an important role in attenuating the fall in GFR that would otherwise occur due to a reduction in the ultrafiltration coefficient.[42] In this setting, locally produced vasodilatory prostaglandins may serve to reduce afferent arteriolar resistance, thereby increasing renal plasma flow and increasing filtration pressure.[43,44] The administration of NSAIDs in this setting would lead to increased afferent arteriolar tone. The resulting fall in renal plasma flow and filtration pressure combined with the already decreased ultrafiltration coefficient would result in a dramatic fall in GFR.[43] Indeed, the administration of prostaglandin synthesis inhibitors to nephrotic subjects is commonly associated with a fall in GFR and may precipitate acute renal failure in some patients.[45] Other settings in which there is an increased vasoconstrictive input focused on the kidney rendering it particularly vulnerable to the deleterious effects of NSAIDs include endotoxic shock[46] and anesthesia.[47]

Risk factors for the development of NSAID-induced acute kidney injury are not necessarily confined to conditions characterized by decreases in absolute or effective arterial circulatory volume (Table 32.2). One such example is the presence of underlying chronic kidney disease. In this setting, increased vasodilatory prostaglandins are thought to play an adaptive role in minimizing the decline in global renal function by increasing GFR in surviving nephrons through increased renal blood flow. The signal for increased prostaglandin production is generally not a disturbance in the systemic circulation leading to increased circulating levels of AII and catecholamines but rather intrarenal mechanisms leading to the generation of vasoactive compounds within the glomerular microcirculation.[48]

32.2 Risk Factors for NSAID-Induced Acute Vasomotor Renal Failure

Decreased EABV	Normal or ↑ EABV
Congestive heart failure	Chronic renal failure
Cirrhosis	Glomerulonephritis
Nephrotic syndrome	Elderly
Sepsis	Contrast-induced nephropathy
Hemorrhage	Obstructive uropathy
Diuretic therapy	Cyclosporin A
Postoperative patients with "third space" fluid	
Volume depletion/ hypotension	

EABV, effective arterial blood volume.

32.3 Predisposing Factors for NSAID-Induced Nephrotoxicity in the Elderly

Age-related changes in renal function
↓ in glomerular filtration rate ↓ in renal blood flow ↑ in renal vascular resistance
Age-related changes in pharmacokinetics
↑ free drug concentration - Hypoalbuminemia - Retained metabolites ↓ total body water ↓ hepatic metabolism resulting in longer drug half-life

Increasing age is a risk factor for the development of nephrotoxicity when using NSAIDs.[49,50] This susceptibility, in part, may be related to changes in kidney function that normally accompany the aging process (Table 32.3).[51] Aging is associated with a progressive decline in the GFR and total renal blood flow. In addition, there is an increase in renal vascular resistance. Important to note, the renal vasculature becomes less responsive to vasodilators, whereas the response to vasoconstrictors remains intact. In an analysis of 1,908 patients treated with ibuprofen, renal impairment was found to occur in 343 (18%) patients.[49] The two most important risk factors identified for the development of toxicity is an age greater than 65 years and preexisting renal insufficiency. In a prospective study of 114 older patients (mean age 87 years) started on NSAID therapy, a greater than 50% increase in the serum urea nitrogen concentration was found in 15 (13%) patients.[50] In this study, the concurrent use of a loop diuretic and large doses of NSAIDs were found to be predictive of those who developed significant azotemia.

In addition to age-related changes in kidney function, age-related changes in the pharmacokinetics of NSAIDs may also make this population more susceptible to renal toxicity.[52,53] Older patients, particularly those with chronic illness, often have lower albumin levels, which reduce the protein binding of the drugs and result in higher free-drug concentrations. This binding of the parent compound to circulating albumin is further impaired by retained metabolites, which accumulate as a result of the normal age-related impairment in renal function. Increased drug levels also occur as a result of the age-related decrease in total body water. Finally, decreased hepatic metabolism, which is often present in older adults, contributes to a longer half-life of the parent compound and can result in unexpectedly high drug levels.

Other conditions in which effective arterial circulatory volume is normal or increased and yet renal function is critically dependent on increased synthesis of prostaglandins include immune mediated glomerular injury, urinary obstruction,[54] radiocontrast-induced injury,[55] and the administration of calcineurin inhibitors.[56] In these conditions, the increased production of vasodilatory prostaglandins has been shown to counterbalance the effects of intrarenally generated vasoconstrictors such as thromboxane, leukotrienes, platelet-activating factor, and endothelin. The administration of NSAIDs in each of these settings can be expected to result in an exaggerated fall in renal function.

NSAID-induced acute kidney injury is most commonly an oliguric form of renal failure that begins within several days after the initiation of the drug (Table 32.4). The urinalysis is unremarkable in the majority of cases. Unlike other causes of acute kidney injury, the fractional excretion of sodium is often less than 1%. This low fractional excretion of sodium reflects the underlying hemodynamic nature of the renal failure. Hyperkalemia out of proportion to the decrement in renal function is also a typical feature of this lesion. If recognized early, the renal failure is reversible with the discontinuation of the NSAID. As a result, dialysis is usually not required.

Glomerular and Interstitial Disease

The use of NSAIDs can be associated with the development of a distinct syndrome characterized by the development of interstitial nephritis and nephrotic range proteinuria. The incidence of this lesion is unknown but is thought to be rare. One estimate for fenoprofen-induced interstitial nephritis was 1 case per 5,300 patient-years of treatment.[57] Although virtually all NSAIDs have been reported to cause this syndrome, the vast majority of cases have been reported in association with the use of propionic acid derivatives (fenoprofen, ibuprofen, and naproxen). Of these, fenoprofen has been implicated in greater than 60% of cases.[58] Interstitial nephritis with and without nephrotic syndrome has also been reported with the COX-2 inhibitors, rofecoxib and celecoxib.[59–63]

Unlike hemodynamically mediated ARF, there are no clear-cut risk factors that serve to identify those at risk for

32.4 Clinical Features of NSAID-Induced Vasomotor Renal Failure

• Oliguria
• Usually occurs within a few days of beginning medicine
• Hyperkalemia out of proportion to renal failure
• Low fractional excretion of Na
• Usually does not require dialysis
• Usually reversible

the development of this syndrome. The mean age of patients is 65 years.[58] The presence of an underlying renal disease prior to the exposure of the NSAID has been notably absent. This syndrome has generally been referred to as an example of acute interstitial nephritis. There are, however, a number of features that distinguish this form of interstitial renal disease from that observed with other pharmacologic agents (Table 32.5).[58] First, the average duration of exposure prior to the onset of the disease is typically measured in months and can be as long as a year. By contrast, allergic interstitial nephritis due to other drugs usually presents within several days to weeks after exposure to the drug. Second, nephrotic range proteinuria is found in >90% of cases of NSAID-induced interstitial disease, a degree of proteinuria that is distinctly uncommon in acute allergic interstitial nephritis due to other drugs. Third, symptoms of hypersensitivity that are commonly seen in acute allergic interstitial nephritis such as rash, fever, arthralgias, or peripheral eosinophilia are uncommon in NSAID-associated disease. Fourth, the vast majority of cases associated with NSAIDs have been reported in older patients. On the other hand, allergic interstitial nephritis is seen in all age groups.

Renal biopsy findings typically show a diffuse or focal lymphocytic infiltrate. The number of eosinophils in the infiltrate is variable but generally is not marked. The glomerular changes are most commonly those seen in minimal change disease. In particular, the glomeruli are normal by light microscopy, whereas fusion of the podocytes is seen with electron microscopy. In some cases there is evidence of glomerulosclerosis. Because most patients who develop this syndrome are older, this latter finding may simply represent the normal age-related increase in glomerulosclerosis. Immunofluorescent studies are typically nonspecific. There has been an occasional report of weak and variable staining for immunoglobulin G and C_3 along the tubular basement membrane. Electron microscopy typically shows a diffuse fusion of the podocytes in cases with heavy proteinuria. Mesangial, electron-dense deposits have been observed only rarely, suggesting that this is not an immune-mediated disease.

32.5 Clinical Characteristics of NSAID-Induced Tubulointerstitial Nephritis (TIN) Versus Typical Drug-Induced TIN

Characteristic	NSAID-Induced TIN	Typical Drug-Induced TIN
Duration of exposure	5 days to >1 year	5–26 days
Hypersensitivity symptoms	7%–8%	80%
Eosinophilia	17%–18%	75%–80%
Proteinuria >3.5 gms/24 hours	>90%	<10%
Eosinophiluria	0%–5%	80%–85%
Peak serum creatinine	1.5– >10 mg%	1.5– >10 mg%

Although the combination of interstitial nephritis and nephrotic syndrome is the most common clinical manifestation, a second presentation is the development of nephrotic syndrome without evidence of interstitial renal disease.[64] Once again, the glomerular histology is typical of minimal change disease, although a few patients have been described with changes typical of membranous glomerulopathy.[59,65] It is likely that the pathophysiologic mechanism that underlies the development of glomerular disease in the absence of interstitial disease is similar, if not identical, to the more common finding of combined nephrotic proteinuria and interstitial nephritis.

A third presentation that has uncommonly been reported is the development of interstitial nephritis without nephrotic proteinuria.[66,67] The onset of disease following the initiation of drug therapy tends to be much shorter and, in this respect, resembles the more common form of drug-induced allergic interstitial nephritis. In addition, these patients are more likely to exhibit a systemic hypersensitivity reaction. Given the closer temporal relationship between the administration of the offending NSAID and the development of renal insufficiency, one may confuse this latter presentation with that of NSAID-induced vasomotor ARF. Symptoms of hypersensitivity as well as histopathologic changes typical of interstitial renal disease should allow one to distinguish this lesion from hemodynamically mediated ARF.

Finally, NSAID toxicity may present as an exacerbation of an underlying disease. In a case report of a patient with systemic lupus erythematosus, the development of interstitial nephritis and nephrotic syndrome after the administration of naproxen clinically appeared as a rapidly progressive lupus glomerulonephritis.[68]

The clinical course of patients who present in any one of these manners is to develop a spontaneous remission after the removal of the offending NSAID. The time until resolution is variable but can range from a few days to several weeks. In some patients, the degree of renal insufficiency can be severe enough that dialytic support is required. Steroid therapy has been used in many of the reported cases; however, the efficacy and necessity of this therapy are unknown. It should be noted that relapses have been reported after inadvertent exposure to the same NSAID or after exposure to a different NSAID.[69]

Renal Sodium Retention

Sodium retention is a characteristic feature of virtually all NSAIDs, occurring in as many as 25% of patients who use them. The physiologic basis of this effect is directly related to

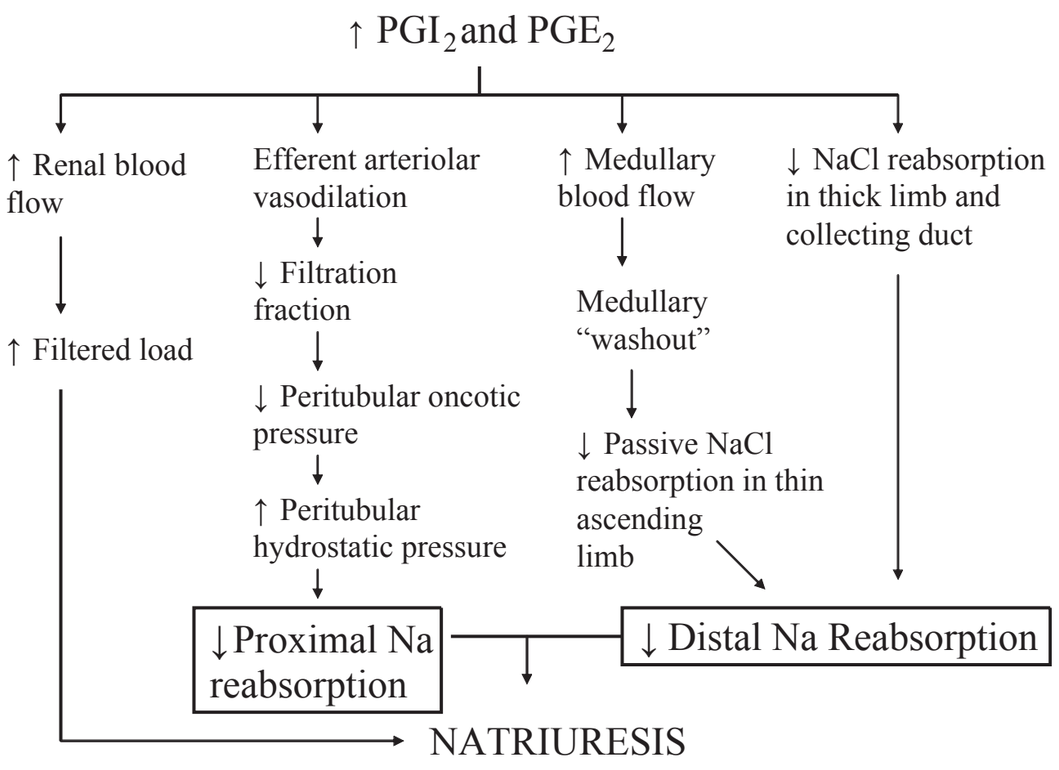

FIGURE 32.3 The direct and indirect mechanisms by which renal prostaglandins exert a natriuretic effect.

the natriuretic properties of prostaglandins. Prostaglandins increase urinary sodium excretion by both indirect and direct mechanisms (Fig. 32.3). Through their activity as renal vasodilators, prostaglandins may cause an increase in the filtered load of sodium. In addition, these compounds preferentially shunt blood flow to the inner cortical and medullary regions of the kidney.[19–21] As a result of increased medullary blood flow, there is a fall in the medullary interstitial solute concentration. Processes that reduce the degree of medullary hypertonicity lead to a concomitant reduction in the osmotic withdrawal of water from the normally sodium-impermeable thin descending limb of Henle. This in turn decreases the sodium concentration of fluid at the hairpin turn. The net effect is less passive reabsorption of sodium across the normally water-impermeable thin ascending limb of Henle. Consistent with this mechanism, the infusion of PGE_1 lowers and the prostaglandin synthesis inhibition raises sodium chloride and total solute concentration in the medulla.[70]

Finally, prostaglandins can affect sodium reabsorption in the proximal tubule by virtue of their ability to influence the tone of the efferent arteriole. Changes in the tone of this vessel play a central role in determining the Starling forces that govern fluid reabsorption in this nephron segment. Increased resistance of this vessel, as that which occurs in the setting of high concentrations of AII, leads to a decrease in the downstream peritubular hydrostatic pressure. In addition, efferent constriction increases the filtration fraction by reducing glomerular plasma flow and increasing the upstream glomerular pressure. The increased filtration fraction leads to an increase in the peritubular oncotic pressure. A decrease in hydrostatic pressure and the increase in oncotic pressure in the peritubular vessel favor fluid reabsorption in the proximal tubule. By modulating the degree to which the efferent arteriole is constricted and thus altering peritubular Starling forces acting on the proximal tubule, prostaglandins can decrease proximal tubular sodium reabsorption. Predictably, in a model of high circulating levels of AII induced by suprarenal aortic constriction, the inhibition of prostaglandin synthesis was found to increase efferent arteriole oncotic pressure and decrease peritubular hydrostatic pressure, resulting in a significant increase in proximal fluid reabsorption.[71]

In addition to these hemodynamically mediated changes in renal sodium handling, prostaglandins have direct effects on tubular sodium transport. In the isolated perfused tubule, PGE_2 has been shown to inhibit sodium transport in the cortical and outer medullary collecting duct.[72,73] Using the same technique, PGE_2 has also been shown to decrease chloride transport in the thick ascending limb of Henle.[74] In vivo studies also support a direct inhibitory effect of prostaglandins on the sodium transport in the loop of Henle, the distal nephron, and the collecting duct.[75,76] The mechanism of this direct inhibitory effect is unclear but may involve decreased activity of the Na^+-K^+-ATPase pump.[77] Prostaglandins have also been shown to mediate the natriuretic response to increased renal interstitial hydrostatic pressure that occurs during renal interstitial volume expansion.[78,79] In addition, these compounds play a permissive role in the sodium excretion that follows volume expansion and an increase in renal perfusion pressure.[80]

It would at first seem paradoxical that under conditions of volume depletion the kidney would elaborate a compound that would have further natriuretic properties. The role of prostaglandins in this setting, however, is to moderate the avid salt retention that would otherwise occur in the setting of unopposed activation of the renin–angiotensin–aldosterone and adrenergic systems. By virtue of their natriuretic properties, prostaglandins play a role in ensuring adequate delivery of filtrate to more distal nephron segments under conditions in which distal delivery is threatened (e.g., renal ischemia, hypovolemia). In addition, diminished NaCl reabsorption in the thick ascending limb of Henle reduces the energy requirements of this segment. This reduction in thick ascending limb workload in conjunction with a prostaglandin-mediated reallocation in renal blood flow helps to maintain an adequate oxygen tension in the medulla under conditions that would otherwise have resulted in substantial hypoxic injury.[81,82]

NSAIDs are thought to cause salt retention primarily by inhibiting prostaglandin synthesis and therefore disrupting the foregoing mechanisms. The extent to which salt retention becomes clinically manifest depends on the degree of baseline prostaglandin production. In normal healthy humans, baseline prostaglandin production is minimal. As a result, NSAID-induced positive sodium balance is transient and usually of no clinical importance. By contrast, NSAID administration in clinical conditions such as congestive heart failure, cirrhosis, or nephrotic syndrome can result in marked sodium retention and potentially adverse clinical consequences.

In addition to causing sodium retention, NSAIDs have been shown to attenuate the natriuretic effect of diuretics.[83] The mechanism of this resistance is multifactorial. The natriuretic effects of loop diuretics have, in part, been linked to

the ability of these drugs to increase renal blood flow, an effect mediated by the stimulation of vasodilatory prostaglandins.[84,85] By inhibiting prostaglandin synthesis, NSAIDs limit sodium excretion by preventing the increase in renal blood flow normally seen after the administration of the diuretic.[85] In addition to this hemodynamic effect, micropuncture and microperfusion studies have shown that prostaglandin inhibition also blunts the effect of furosemide at the level of the thick ascending limb of Henle.[86,87] This latter effect may be related to the inhibition of furosemide-induced stimulation of natriuretic prostaglandins that act within this tubular segment. Finally, NSAIDs may limit the diuretic response to loop diuretics by competing for tubular secretion, thereby limiting the delivery of the drug to the luminal surface of the thick ascending limb.

Indomethacin has also been shown to attenuate the diuretic response to hydrochlorothiazide.[88] The mechanism of this interaction may result from enhanced salt absorption in the loop of Henle, which would then limit the delivery of chloride to the site of the thiazide action in the distal nephron. A similar explanation may underlie the resistance that has been described with NSAIDs and spironolactone.[89]

Sodium Balance and Hypertension

In considering the natriuretic and vasodilatory properties of prostaglandins, it is not surprising that the administration of NSAIDs has been shown to interfere with blood pressure control. In pooled studies, the administration of NSAIDs has been associated with an average increase in blood pressure of between 5 and 10 mm Hg.[90,91] Of the various subgroups examined, this effect is most pronounced in patients who are already hypertensive and much less so in those who are normotensive. Of the hypertensive patients, those treated with β-blockers seem to be the most vulnerable to the hypertensive effect of NSAIDs.[91] In this regard, it is particularly interesting to note that propranolol has been shown to increase prostacyclin formation.[92] There is less of an interaction with diuretics and angiotensin-converting enzyme inhibitors, whereas no effect is seen with calcium channel blockers.

Subgroup analysis shows that patients with low renin hypertension (older adults and blacks) are at higher risk for worsening hypertension in association with NSAID use. Older hypertensive patients have reduced urinary PGE_2 excretion when compared to younger hypertensive patients.[93] The pathogenesis of NSAID-induced hypertension is not known with certainty. In a recent meta-analysis, NSAIDs were found to not alter body weight or urinary sodium excretion significantly, implying that mechanisms other than salt retention were responsible for the increased blood pressure.[91] In this regard, elimination of the vasodilator prostacyclin from the resistance blood vessels is believed to play some role in the development of hypertension in individuals at risk.[93,94]

The use of COX-2 inhibitors is complicated by the development of peripheral edema with a frequency similar to that seen with traditional NSAIDs. In addition, the majority of data suggest no difference between the various COX-2 inhibitors and the tendency to develop increased blood pressure.[95]

Potassium Metabolism

The use of NSAIDs has been associated with the development of hyperkalemia in the setting of chronic kidney disease as well as with normal renal function.[96,97] The physiologic basis for this effect is inhibition of prostaglandin-mediated renin release with the subsequent development of hypoaldosteronism. Both in vivo and in vitro studies have shown a direct stimulatory effect of prostaglandins (primarily PGI_2 and PGE_2) on renin release from the juxtaglomerular cells.[98,99] Clinical studies in salt-restricted subjects have shown the COX-2 selective inhibitors reduced urinary potassium excretion to a similar extent as traditional NSAIDs. Both celecoxib and rofecoxib have been reported to cause significant hyperkalemia in case reports. These findings are consistent with COX-2 in the macula densa playing an important role in stimulating renin release.

In addition to direct effects, these compounds play an essential intermediary role in those pathways that are of primary importance in the regulation of renin release. In particular, renin release stimulated by both decreased perfusion pressure and decreased delivery of filtrate to the macula densa is dependent on an intact cyclooxygenase system.[100] By contrast, β-adrenergic stimulation of renin release can occur independently of prostaglandin synthesis.[101]

NSAID-induced suppression of renin release with the subsequent development of a hyporenin–hypoaldosterone state is thought to be the primary mechanism of hyperkalemia. Decreased renin release leads to decreased circulating levels of angiotensin I, which in turn results in low levels of AII. Because AII normally stimulates aldosterone release from the zona glomerulosa cells in the adrenal gland, serum aldosterone levels fall. In addition to low circulating levels, the effect of any given level of AII on aldosterone release is impaired because prostaglandins have been shown to play an intermediary role in this stimulatory effect.[102] Low circulating levels of AII further contribute to the development of hypoaldosteronism because adequate levels of AII are required for the stimulatory effect of hyperkalemia on aldosterone release at the level of the adrenal gland.[103] In addition to interfering with the renin–angiotensin–aldosterone cascade, NSAIDs favor positive potassium balance in other ways. As discussed earlier, the inhibition of prostaglandin synthesis is associated with increased sodium reabsorption in the loop of Henle and thus decreased distal delivery. A reduction in sodium delivery to the aldosterone-sensitive cortical collecting tubule is a known factor impairing potassium excretion. In addition, tubular flow rates are an important determinant of potassium excretion. Because NSAIDs increase the hydroosmotic effect of AVP, flow rates can fall, further impairing potassium excretion. Finally, decreased synthesis of prostaglandins may have effects of decreasing potassium secretion at the level of the potassium channel.[104]

The development of hyperkalemia in patients receiving an NSAID is most likely to occur in the setting of renal insufficiency or those with baseline abnormalities in the renin–angiotensin–aldosterone system.[98] Diabetic patients are at risk due to the increased incidence of hyporeninemic hypoaldosteronism that occurs in this patient population.[105,106] Similarly, older adults are at higher risk by virtue of the normal age-related decrease in circulating renin and aldosterone levels. Particular caution should be used when NSAIDs are combined with other pharmacologic agents known to interfere with the renin–angiotensin–aldosterone cascade.[107] Examples would include β-blockers, calcineurin inhibitors, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, heparin, ketoconazole, high-dose trimethoprim, and potassium-sparing diuretics.

Water Metabolism

Prostaglandins have important modulatory effects on renal water metabolism. Their primary effect is to impair the ability to maximally concentrate the urine. In doing so, two processes that are central in the elaboration of concentrated urine are interfered with; namely, the generation of a hypertonic interstitium and maximal collecting duct water permeability. The decrease in interstitial hypertonicity is due to a washout effect that results from the ability of prostaglandins to shunt blood flow to the inner cortical and medullary regions of the kidney. In addition, prostaglandins decrease sodium absorption in the thick ascending limb and decrease AVP-induced urea permeability in the medullary collecting duct. The decreased accumulation of sodium and urea in the interstitium further reduces the interstitial osmolality. The impairment in the collecting duct water permeability is the result of prostaglandins opposing the hydro-osmotic effect of AVP.[108] Interesting to note, AVP is known to stimulate PGE_2 synthesis in collecting duct cells; by doing so, AVP induces its own antagonist. This interaction is another example in which prostaglandins exert a moderating effect on an effector mechanism that elicited their synthesis. In this case, prostaglandins play an important role in minimizing the water retention that would otherwise occur if the activity of AVP was unopposed. By opposing the vasoconstrictive action of AVP, prostaglandins also contribute to the maintenance of glomerular perfusion and filtration.

Based on the foregoing discussion, the administration of NSAIDs would predictably impair solute-free water excretion by increasing the hydro-osmotic effect of any given level of circulating AVP and increasing the degree of interstitial hypertonicity (Fig. 32.4). In most circumstances, however, hyponatremia is not associated with the use of NSAIDs. Under normal conditions, any decrease in serum osmolality would be sensed in the hypothalamus and result in the inhibition of AVP release. As a consequence, excess solute-free water would be promptly excreted, restoring the serum osmolality back to normal. On the other hand, the administration of NSAIDs in the setting of nonsuppressible AVP release may result in dramatic falls in the serum sodium concentration. Patients at risk for this complication would include those with high circulating levels of AVP driven by a decreased effective arterial circulatory volume such as congestive heart failure or cirrhosis.[109] Patients with syndrome of inappropriate antidiuretic hormone secretion (SIADH) or those taking medications capable of stimulating AVP secretion or impairing urinary dilution by other mechanisms are also at risk for the development of hyponatremia.[47]

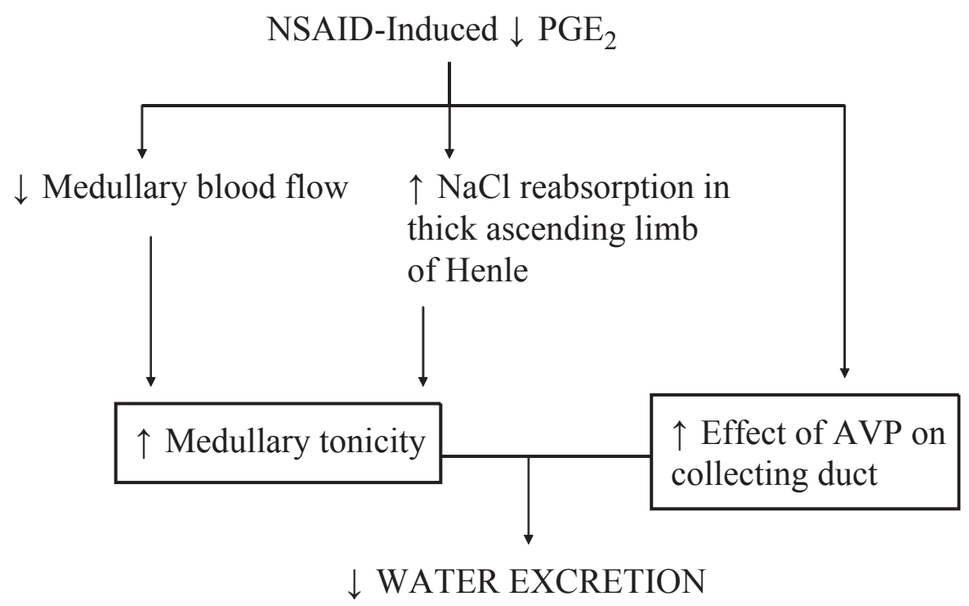

FIGURE 32.4 The mechanisms by which nonsteroidal anti-inflammatory drugs lead to decreased renal water excretion. *AVP*, arginine vasopressin.

In this regard, a recent study examined the effects of ibuprofen and a thiazide diuretic on renal water handling in otherwise healthy young and older volunteers subjected to a water load.[110] Three days of hydrochlorothiazide (100 mg per day) was found to impair both solute-free water clearance and the ability to elaborate a maximally dilute urine. A delay in the recovery of serum osmolality was noted in both the young and older subjects but to a significantly greater extent in the older subjects. When the young subjects were then given a water load after treatment with ibuprofen together with the thiazide, solute-free water clearance and serum osmolality were reduced further and to a degree similar to that seen in the older subjects on the thiazide regimen alone. It was postulated that the susceptibility to thiazide-induced hyponatremia known to occur in some elderly patients may, in part, be related to lower renal prostaglandin production. It can be expected that a greater number of older patients will be taking a combination of NSAIDs and hydrochlorothiazide given the efficacy of thiazide diuretics in the treatment of systolic hypertension.

NONSTEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED CHRONIC KIDNEY DISEASE AND ANALGESIC NEPHROPATHY

The most common form of drug-induced chronic kidney disease is analgesic nephropathy. This lesion has most commonly been linked to the chronic ingestion of compound analgesics containing aspirin, phenacetin, and caffeine.[111]

A still unresolved question is whether long-term use of NSAIDs alone can similarly result in a progressive and irreversible form of chronic kidney disease. In this regard, a number of observations have emerged that would appear to substantiate the belief that long-term use of NSAIDs can lead to a chronic form of renal injury. Furthermore, the clinical characteristics of NSAID-induced chronic kidney disease are sufficiently different from those in analgesic nephropathy to suggest that this is a distinct clinical entity. Before reviewing the data linking chronic NSAID use and renal insufficiency, a brief description of analgesic nephropathy will be provided.

Analgesic nephropathy is a chronic kidney disease characterized by renal papillary necrosis and chronic interstitial nephritis.[111–113] The early reports linking analgesics and renal disease were generally found in patients who consumed combination products containing phenacetin. This fact focused attention on phenacetin as the primary cause of the syndrome and prompted many countries to officially remove the drug from nonprescription analgesics. Significantly, the removal of phenacetin has not been uniformly followed by the expected reduction in the incidence of the syndrome.[114] Given that other agents such as acetaminophen or salicylamide have been substituted for phenacetin in many combination products, the lack of decline in incidence of analgesic nephropathy suggests that the use of combination products is as important as whether the compound contains phenacetin.[114,115] This conclusion is further supported by the experience in Belgium where a strong geographic correlation exists between the prevalence of analgesic nephropathy and sales of analgesic mixtures containing a minimum of two analgesic components.[116,117]

Numerous epidemiologic studies performed in the past demonstrated a wide variation in the geographic incidence of analgesic nephropathy.[114,115,118–121] Much of this variability could be explained by differences in the annual per capita consumption of phenacetin.[113,114] In those countries with the highest consumption such as Australia and Sweden, analgesic nephropathy was found responsible for up to 20% of cases of end-stage renal disease. In Canada, which had the lowest per capita consumption, analgesic nephropathy accounted for only 2% to 5% of end-stage renal disease patients. It has been estimated that between 2% and 4% of all end-stage renal disease cases in the United States can be attributable to habitual analgesic consumption. Within the United States, there are also regional differences in the reported incidence of analgesic nephropathy, which are thought to be reflective of differences in analgesic consumption.[114,115,118,119] For example, the use of combination analgesics is more common in the southeastern United States, and the incidence of analgesic nephropathy is three to five times as common a cause of end-stage renal disease in North Carolina compared to Pennsylvania.[114,115,118,119]

The development of analgesic nephropathy is associated with a number of well-defined clinical characteristics.[122] The disease is more common in women by a factor of 2 to 6 and has a peak incidence at age 53 years. Patients typically consume compound analgesics on a daily basis, often for chronic complaints such as headache, dyspepsia, or to improve work productivity. It has been estimated that nephropathy occurs after the cumulative ingestion of 2 to 3 kg of the index drug. Often, patients will exhibit a typical psychiatric profile characterized by addictive behavior. Gastrointestinal complications, such as peptic ulcer disease, are common. The patients are frequently anemic as a result of gastrointestinal blood loss as well as renal insufficiency. Ischemic heart disease and renal artery stenosis have both been reported to occur with higher frequency in these patients.[113] In fact, regular use of analgesic drugs containing phenacetin is associated with an increased risk of hypertension and mortality and morbidity due to cardiovascular disease.[121,123] Finally, long-term use of analgesics is known to be a risk factor for the subsequent generation of uroepithelial tumors.[124]

Patients with analgesic nephropathy predominantly have tubulomedullary dysfunction characterized by an impaired concentrating ability, acidification defects, and occasionally a salt-losing state. Proteinuria tends to be low to moderate in quantity. Interesting to note, the pattern of proteinuria is typically a mixture of glomerular and tubular origin. Pyuria is common and is often sterile. Occasionally, hematuria is noted, but if persistent should raise the possibility of an uroepithelial tumor.

There are several features of analgesic nephropathy that make it difficult to diagnose. The disease is slowly progressive and the symptoms and signs are nonspecific. Patients are often reluctant to admit to a heavy usage of analgesics and therefore are either misdiagnosed or not diagnosed at all until the renal failure is far advanced. In addition, the lack of a simple and noninvasive test that reliably implicates analgesics as the cause of the renal injury has been an important limiting factor. Noncontrast abdominal computed tomography (CT) may emerge as a useful diagnostic tool in this setting given its usefulness in the diagnosis of papillary necrosis.[125] Characteristic findings by CT suggesting the diagnosis include small kidneys with an irregular contour and intrarenal calcifications, particularly in the medulla.

As mentioned earlier, there are a number of reports that suggest that chronic use of NSAIDs alone may also lead to renal injury. In this regard, several NSAIDs have been associated with the development of papillary necrosis either when administered alone or in combination with aspirin.[126] In addition to inhibiting prostaglandin synthesis, the ability of these agents to redistribute blood flow to the cortex, thus rendering the renal medulla ischemic, may underlie this association. Although the reports linking papillary necrosis and NSAIDs are predominantly anecdotal in nature, more recent observations would suggest that chronic renal failure resulting from long-term use of NSAIDs may be more prevalent than once thought.[127,128] In a multicenter case-control study, Sandler and associates[127] reported a twofold increase in the risk for chronic kidney disease associated with the previous daily use of NSAIDs. Chronic kidney disease in these patients was newly diagnosed and was defined as a serum creatinine concentration of 1.5 mg per deciliter or greater. This increased risk was primarily limited to older men. An

additional report linking the chronic use of NSAIDs with the development of chronic kidney disease described 56 patients from Australia.[113] These patients had taken only NSAIDs over a period of 10 to 20 years for treatment of varying rheumatic diseases. In 19 patients (34%), radiographic evidence of papillary necrosis was found. In 37 patients, renal biopsy material was available that disclosed evidence of chronic interstitial nephritis. The clinical characteristics of these patients were quite different from those with analgesic nephropathy, suggesting that NSAID-induced chronic kidney disease is indeed a distinct entity. In particular, patients with NSAID-associated renal disease were older, had an equal female-to-male ratio, a lower incidence of papillary necrosis, less severe renal insufficiency, and a lower incidence of urinary tract infections.[113] In addition, an increased risk of uroepithelial tumors has not been described in these patients.

Further evidence of chronic toxicity has been reported in a preliminary communication in which patients treated with NSAIDs for rheumatoid arthritis and osteoarthritis were compared to a matched control arthritis population.[129] In this study, the NSAID-treated patients had a rise in the serum creatinine concentration from 1.28 to 2.58 mg per deciliter over a mean period of 47.5 months. The control group not taking NSAIDs had stable renal function. Finally, Segasothy and colleagues[128] report on the risk of chronic renal disease in a prospective study of 259 heavy analgesic abusers. In this study, 69 patients developed radiographic evidence of papillary necrosis. Of these, 29 used NSAIDs either singularly (17 patients) or in combination with another NSAID (12 patients). Another 9 patients used NSAIDs in combination with paracetamol, aspirin, caffeine, or a traditional herbal medicine. Renal insufficiency (serum creatinine concentration 1.4 to 8.8 mg per deciliter) was noted in 26 of the 38 patients who had used an NSAID chronically. Similar to the patients from Australia,[113] this disorder was more common in males (1.9:1.0), distinguishing this disorder from classic analgesic nephropathy, which typically occurs in females. Similarly, these patients did not exhibit the usual psychological profile associated with analgesic abuse.

Thus, although further studies are needed to definitely assess the question of cumulative toxicity, it appears that some chronically treated patients may develop a change in renal function over a long-term period. Given the abuse potential of powerful NSAIDs and the fact that ibuprofen, naproxen, and ketoprofen are now available on an over-the-counter basis, it is possible that chronic NSAID abuse may become a more common cause of chronic kidney disease in the future.

In considering the definite association of compound analgesic abuse and the possible linkage of chronic NSAID use to the development of chronic kidney disease, it has become common clinical practice to recommend acetaminophen whenever possible for analgesia. In this regard, a recent case-control study examining the use of over-the-counter analgesics as a risk factor for end-stage renal disease found that acetaminophen may also cause chronic kidney disease when used on a continual basis.[130] In this study, heavy average use of acetaminophen (>1 pill per day) and medium- to high-cumulative intake (1,000 or more pills in a lifetime) each doubled the odds of end-stage renal disease. These authors conclude that a reduced consumption of acetaminophen could decrease the overall incidence of end-stage renal disease approximately 8% to 10%. The findings in this study confirmed an earlier report that also concluded that long-term daily use of acetaminophen is associated with an increased risk of chronic kidney disease.[118] Although these studies do not establish a cause-and-effect relationship between acetaminophen ingestion and chronic kidney disease, the data do suggest that the ingestion of acetaminophen on a continual and chronic basis should be discouraged.

An organized review was conducted by a consensus panel of the National Kidney Foundation (NKF) in which over 600 articles were surveyed. This review studied the implications of several different kinds of analgesic ingestion and renal failure risks.[131,132] The highlights of the recommendations from the NKF consensus panel based on this review are that:

1. The ingestion of aspirin and nonsteroidal combinations are not encouraged because of an increased risk of renal failure when those combinations are ingested together.
2. The habitual consumption of analgesics is discouraged, and monitoring is recommended when such use is mandatory.
3. Combination analgesics are recommended to be available by prescription only with an explicit warning to physicians that the habitual consumption of these combination products could lead to the insidious development of chronic kidney disease.
4. There should be an explicit warning to consumers regarding NSAID ingestion.

The panel concluded that there is negligible clinical evidence that suggests the habitual use of acetaminophen alone causes the clinical entity of analgesic nephropathy and that there is no evidence that the occasional use of acetaminophen causes renal injury. Finally, the panel points out that there is no risk from the regular use of aspirin in the relatively small doses recommended for the prevention of cardiovascular events.

NEPHROTOXICITY OF INHIBITORS OF THE RENIN-ANGIOTENSIN SYSTEM: ANGIOTENSIN CONVERTING ENZYME INHIBITORS, ANGIOTENSIN RECEPTOR BLOCKERS, AND DIRECT RENIN INHIBITORS

The most common form of nephrotoxicity associated with inhibitors of the renin-angiotensin system is an increase in the serum creatinine concentration occurring in the setting

of antihypertensive therapy.[133] This complication is becoming more common in clinical practice because guidelines governing adequate blood pressure control have been made more stringent. This decline in renal function is hemodynamic in origin and not secondary to structural injury to the kidney and can be traced to changes in renal autoregulation that accompany chronic kidney disease.

Normal renal autoregulation enables the kidney to maintain fairly constant renal blood flow and glomerular filtration rates in the setting of varying systemic blood pressure. One component of this process is an intrinsic property of the afferent arteriole called the myogenic reflex. The myogenic reflex causes this vessel to either constrict or dilate in response to changes in intraluminal pressure. An increase in arterial pressure elicits a vasoconstrictive response, whereas decreased arterial pressure results in vasodilation. These changes in afferent tone provide an immediate response to maintain intraglomerular pressure and glomerular filtration rate relatively constant in the face of everyday fluctuations in systemic blood pressure.[134]

In the setting of chronic hypertension, the small arteries of the kidney, including the afferent arteriole, undergo a number of pathologic changes that give rise to alterations in the way the kidney autoregulates.[135] As with vessels elsewhere, the afferent arteriole initially demonstrates evidence of endothelial dysfunction leading to impaired vasodilation. Over time, this impairment is exaggerated by histologic changes of hyaline arteriosclerosis and myointimal hyperplasia. These changes lead to a blunted ability of the preglomerular circulation to either constrict or dilate in response to changes in renal perfusion pressure. In essence, these vessels take on the characteristics of a pressure-passive vasculature where changes in mean arterial pressure are matched by proportional change in GFR (Fig. 32.5).[135,136]

A blunted ability of the preglomerular circulation to dilate in response to a drop in the mean arterial pressure will cause an exaggerated decrease in the intraglomerular pressure and GFR. This impairment in autoregulation explains why patients with hypertension and chronic kidney disease are more likely to have an increase in the serum creatinine concentration when blood pressure is lowered. Any drug that lowers blood pressure can cause an increase in the serum creatinine concentration through this mechanism but inhibitors of the renin-angiotensin system are more commonly associated with this complication. Angiotensin-converting-enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and the direct renin inhibitor will exaggerate the decline in intraglomerular pressure due to blood pressure reduction by concomitant vasodilation of the efferent side of the glomerular circulation.

As long as the increase in serum creatinine concentration is not excessive (>30% above the baseline value) or progressive, discontinuation of these drugs is not necessarily warranted particularly considering the potential benefit these agents have in slowing the progression of chronic kidney disease. Long-term trials in both diabetic and nondiabetic patients have shown that the initial decline in renal function reaches a plateau within several weeks and is reversible with discontinuation of the blocker even after several years of therapy.[138,139] Thus a small, stable increase in the serum creatinine concentration after the start of an ACE inhibitor, ARB, or the direct renin inhibitor is hemodynamic in nature and reflects a fall in intraglomerular pressure.

If the rise in serum creatinine is >30% or the repeat value shows a progressive rise, then the appropriate response is to discontinue the drug and initiate a search for other causes of renal dysfunction. There are several conditions in which use of renin-angiotensin blockers may cause exaggerated or progressive declines in renal function. The first setting involves significant (usually >70%) bilateral renal artery obstruction or unilateral renal artery obstruction to a solitary functioning kidney. Under these conditions, increased tone of the efferent arteriole acts to attenuate the decline in intraglomerular pressure that results from the arterial obstruction. The trade-off is that renal function and glomerular filtration rate become dependent upon sustained constriction of the efferent vessel by AII. A similar physiology can develop in patients with polycystic kidney disease where the renal arteries become extrinsically compressed by large cysts.[140] Unless the underlying obstruction can be treated, other classes of antihypertensive agents will have to be used.

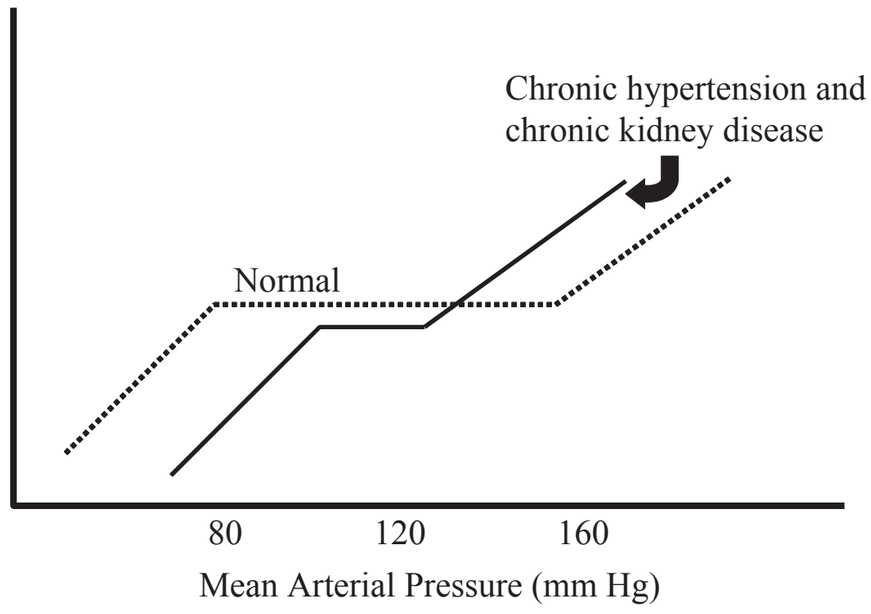

FIGURE 32.5 Renal autoregulation normally maintains relatively constant intraglomerular pressure despite variations in renal perfusion pressure. In patients with chronic hypertension or mild chronic kidney disease renal autoregulation changes in such a way that intraglomerular pressure begins to vary more directly with changes in mean arterial blood pressure. One can conceptualize this change as if the normal sigmoidal relationship between systemic blood pressure and intraglomerular pressure becomes progressively more linear. As a result, increases in mean pressure cause exaggerated rises in intraglomerular pressure, whereas declines in mean pressure will cause exaggerated falls in intraglomerular pressure. The decline in intraglomerular pressure accompanying more stringent levels of blood pressure control will manifest itself by an increase in the serum creatinine concentration. Renal dysfunction that occurs in this setting is hemodynamic in origin and is reflective of a lower intraglomerular pressure.

Blockade of the renin-angiotensin system can also cause an azotemic response under conditions of an absolute (gastroenteritis, aggressive diuresis, poor oral intake) or effective reduction in arterial circulatory volume (moderate to severe congestive heart failure). In these settings, AII–mediated constriction of the efferent arteriole serves to minimize the decline in glomerular filtration rate that would otherwise occur as a result of the fall in renal perfusion pressure. In the volume-contracted patient, the appropriate response is to hold the drug and then restart the medication once the extracellular fluid volume has been replenished. In a patient with congestive heart failure, renin-angiotensin blockade will increase the serum creatinine when the decrease in intraglomerular pressure resulting from efferent vasodilation is not offset by an increase in renal perfusion. This can occur in patients with severely depressed cardiac function in which afterload reduction can no longer increase cardiac output or in the setting of aggressive diuresis.

A similar mechanism is responsible for renal dysfunction that occurs in patients given renin-angiotensin system blockers in the setting of NSAIDs, cyclosporin A, or early sepsis.[141–143] In these settings, there is increased vasoconstriction of the renal vasculature. Inhibiting AII-mediated efferent vasoconstriction in the face of decreased renal perfusion pressure accounts for the fall in GFR.

A few cases of membranous nephropathy have been attributed to the use of ACE inhibitors,[144,145] but the overall incidence of this complication and that of tubulointerstitial nephritis are believed to be low. To date, there are no such reports linking ARBs or the direct renin inhibitor to the development of glomerular or interstitial renal disease.

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CHAPTER

33 Contrast-Induced Acute Kidney Injury

Steven D. Weisbord • Paul M. Palevsky

Acute kidney injury is a well-recognized complication of intravascular iodinated contrast administration.[1–4] Epidemiologic trends and clinical factors suggest that contrast-induced acute kidney injury (CIAKI), which is associated with serious adverse short- and long-term outcomes, will continue to be an important clinical entity for the foreseeable future. First, patients are living longer with a greater burden of chronic illness, suggesting that there will be an increasing demand for radiographic procedures that use intravascular (IV) radiocontrast. Second, chronic kidney disease (CKD), which is the principal risk factor for CIAKI, and diabetes mellitus, which amplifies the risk for CIAKI in patients with baseline renal impairment, are increasing in prevalence.[5] Third, recent studies linking gadolinium-containing contrast agents used to enhance the diagnostic accuracy of magnetic resonance imaging (MRI) studies with nephrogenic systemic fibrosis, a potentially severe fibrosing disorder in patients with advanced CKD, has led to the performance of fewer contrast-enhanced MRI procedures in patients with kidney disease and is likely to result in a greater reliance on imaging modalities that use iodinated radiocontrast in patients at risk for CIAKI.[6–8] Lastly, advancements in modern imaging technology have led to an increasing array of diagnostic and therapeutic procedures that employ iodinated contrast. As a result of these factors, CIAKI is likely to remain a common iatrogenic complication.

This chapter reviews the pathophysiology, risk factors, clinical presentation, and incidence of CIAKI; discusses the adverse outcomes associated with the development of CIAKI; and summarizes the data regarding interventions to prevent this iatrogenic condition.

THE PATHOPHYSIOLOGY OF CONTRAST-INDUCED ACUTE KIDNEY INJURY

Our current understanding of the pathophysiology of CIAKI principally derives from animal studies examining the effect of contrast media on renal hemodynamics, oxygen delivery, and kidney function. These studies suggest that multiple overlapping pathophysiologic processes triggered by contrast administration collectively contribute to renal injury. These include mismatched oxygen supply and demand resulting in medullary hypoxia, direct toxic effects of contrast on tubular epithelial cells, and the generation of cytotoxic oxygen free radicals within the kidney that intensify renal injury (Fig. 33.1). Animal models used to investigate the pathophysiology of CIAKI have relied upon additional nephrotoxic insults such as volume depletion or concomitant nonsteroidal anti-inflammatory administration to enhance the susceptibility of the kidneys to contrast-induced injury. For example, Heyman et al.[9] compared the effect of contrast media on renal vasoconstriction in intact rats and uninephrectomized, salt-depleted rats that were injected with intravenous indomethacin. Renal blood flow dropped to a substantially greater degree following contrast administration in the preconditioned rats. Similarly, Agmon and colleagues[10] pretreated rats with indomethacin and nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis to examine the effect of iodinated contrast on renal medullary blood flow. Whether such additional nephrotoxic insults in animals accurately reflect the mechanisms underlying the increased susceptibility of the human kidney to iodinated contrast from renal impairment is not clear. Nonetheless, such studies form much of the basis for our current understanding of the pathophysiology of CIAKI.

Vasoconstriction and Medullary Hypoxia

Multiple studies have demonstrated that the administration of iodinated contrast media induces vasoconstriction of the renal vasculature.[11–13] However, this effect is not uniform throughout the kidney but rather regional because blood flow to the cortex appears to be maintained but decreases significantly in the renal medulla. Oxygen delivery to the renal cortex is high, yet delivery to the medulla is considerably lower, resulting in low medullary tissue oxygen tension, with values as low as 30 mm Hg detected under normal physiologic conditions in rats, dogs, and humans. At baseline, oxygen extraction by the renal medulla is near maximal, reaching 79% of regional oxygen delivery. Thus, medullary oxygen

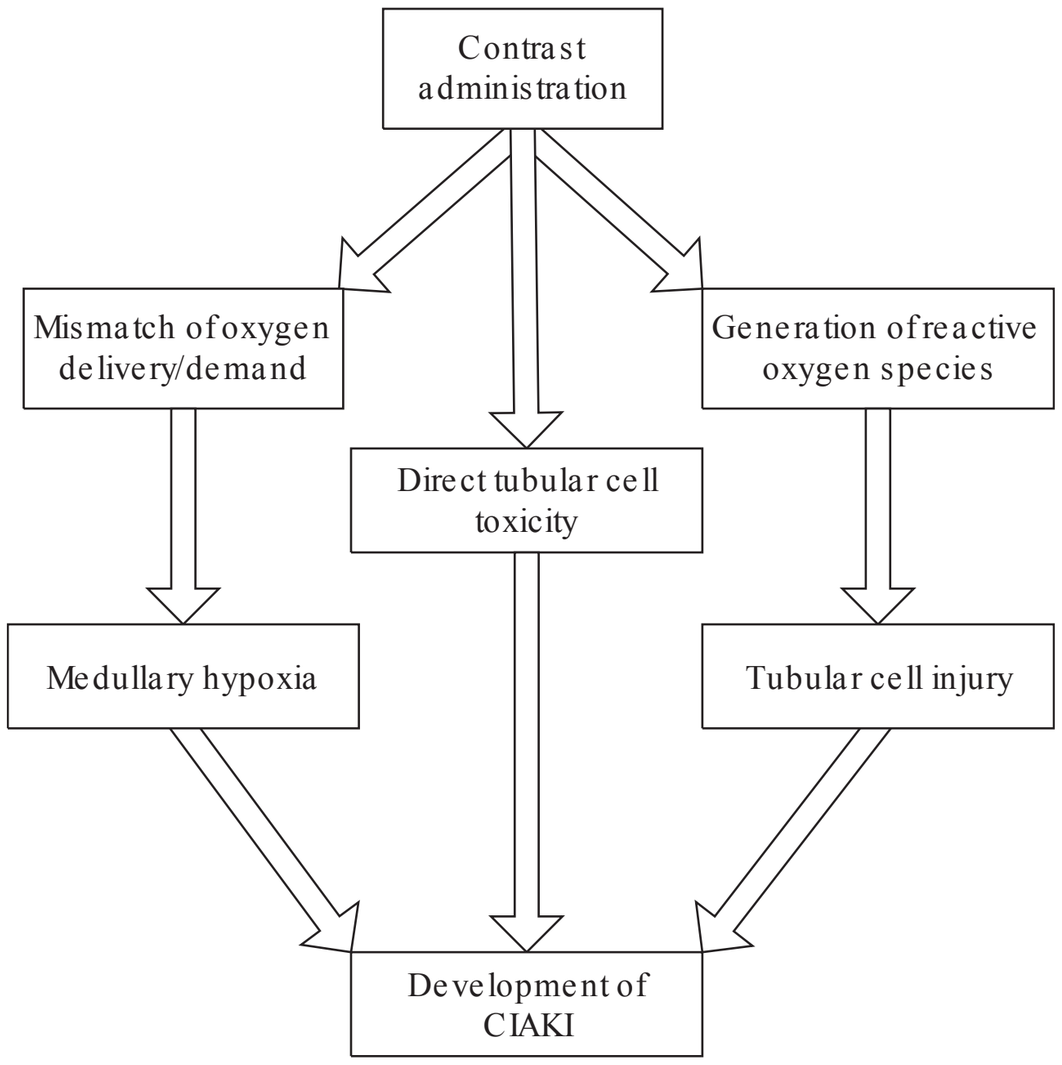


FIGURE 33.1 Pathophysiology of contrast-induced acute kidney injury.

reserve is marginal under normal conditions.[14,15] In the intact kidney, medullary blood flow is usually maintained, even in the presence of systemic and local vasoconstrictive stimuli, by the combined effects of vasodilators including nitric oxide and prostaglandin E_2, as well as the unique regional vasodilatory effects of renal vasoconstrictors.[16] However, in certain settings, including altered renal reserve, vasoconstriction can overwhelm the kidney's capacity to preserve oxygen delivery, leading to medullary hypoxia. In particular, the vasoconstrictors endothelin and adenosine have been implicated in the reduction in outer medullary blood flow following the administration of iodinated contrast.[17–20] However, the precise role of and degree to which renal injury from contrast is directly attributable to these vasoconstrictors is unknown. Nonetheless, a series of studies have examined the role of medullary hypoxia in the pathogenesis of CIAKI.[21,22] Liss and colleagues[22] demonstrated a fall in medullary oxygen tension from approximately 30 mm Hg to 15 mm Hg after the administration of low-osmolal and iso-osmolal contrast agents. The contrast-induced exacerbation of medullary hypoxemia has also been suggested by noninvasive blood-oxygen level dependent magnetic resonance imaging (BOLD MRI), which detects an increased unsaturated hemoglobin concentration within the renal medulla and by the detection of increased levels of hypoxia-inducible factors (HIF) shortly after contrast administration.[23,24] Systemic effects of iodinated contrast media may also contribute to the decline in renal medullary oxygen tension. Contrast administration is associated with the induction of pulmonary ventilation–perfusion mismatch, reduced cardiac output with a secondary decrease in renal perfusion pressure, rheologic alterations of blood, and a leftward shift of the oxygen-hemoglobin dissociation curve.[25–29] However, these systemic effects appear to play a less prominent role in the reduction in medullary oxygen tension than the intrarenal mechanisms.

Simultaneous with its effect on medullary oxygen tension, iodinated contrast increases oxygen demand within the kidney. The administration of contrast media induces an abrupt and transient natriuresis and increases glomerular filtration and urinary output, effects that are mediated, at least in part, by an increase in plasma volume and the release of natriuretic peptides.[17,30,31] These effects, along with the increased osmotic load following contrast media administration, lead to enhanced solute delivery to the distal nephron. The increased active transport in the distal nephron increases oxygen demand. Thus, the contrast-induced decrease in medullary oxygen tension is accompanied by a concomitant increase in oxygen demand, particularly at the corticomedullary junction, resulting in a mismatch between oxygen supply and demand, tissue hypoxia, and cellular injury.

Direct Tubular Toxicity and Generation of Reactive Oxygen Species

Radiocontrast agents result in tubular cell injury directly through direct cytotoxicity and indirectly through the generation of reactive oxygen species (ROS).[32–37] Following filtration at the glomerulus, iodinated contrast enters the urinary space and increases the viscosity of the tubular fluid. The increased urine viscosity combined with intratubular cast formation by cellular debris and the precipitation of contrast medium with urinary proteins may increase exposure of the tubules to the contrast medium, thus increasing the risk for direct cytotoxicity. Evidence supporting the role of direct tubular cell toxicity in the pathogenesis of CIAKI derives from renal biopsy specimens demonstrating vacuolization and necrosis of tubular cells in patients who recently underwent contrast-enhanced procedures.[38] Additionally, data from in vitro studies of renal epithelial cells demonstrate contrast-associated DNA fragmentation, altered cell polarity, inhibition of mitochondrial function, increased brush border marker enzyme activity, and apoptosis—effects that support the direct nephrotoxic effects of contrast.[34–36]

In vitro studies demonstrate that iodinated contrast agents also induce oxygen free radical mediated injury through the oxidation of cell membranes, cellular proteins, and nucleic acids.[39–43] Secondary activation of reparative processes, such as the DNA mending poly-(ADP-ribose) polymerase (PARP) may, in turn, precipitate additional depletion of intracellular energy stores and tubular damage.[44,45] Endothelial cells may also be injured by the evolving hypoxic stress. Following exposure to contrast media, HIF-2α accumulates in medullary endothelial capillaries.[46] Resultant endothelial damage, induced by reactive oxygen species and energy consuming reparative mechanisms, such as PARP, leads to endothelial dysfunction, further exacerbating regional tissue hypoxia.[40,47–49] Thus, the hemodynamic and toxic effects of

iodinated contrast media are synergistic, leading to the amplification of kidney injury.

RISK FACTORS FOR CONTRAST-INDUCED ACUTE KIDNEY INJURY

Patient-Related Risk Factors

Risk factors for CIAKI can be categorized as patient related or procedure related (Table 33.1). CIAKI rarely develops in the absence of patient-related risk factors, which are collectively characterized by functional and structural changes impairing the capacity of the kidneys to adequately compensate for the hemodynamic and microcirculatory stresses caused by iodinated contrast media. Preexisting disease of the renal parenchyma (i.e., CKD) is characterized by an abnormal medullary microcirculation and a diminished capacity to compensate for hemodynamic perturbation. Clinical studies confirm that underlying renal impairment is the major patient-related risk factor for CIAKI, with increasing levels of dysfunction associated with escalating levels of risk.[50] In a study of 378 hospitalized patients undergoing nonrenal angiography, D'Elia et al.[51] found that preexisting renal insufficiency was the only risk factor predisposing to CIAKI. In an analysis of nearly 3,700 patients, McCullough and colleagues[52] found a strong inverse relationship between the baseline kidney function and a risk of both CIAKI and the need for acute dialysis. Schemata outlining the risk associated with varying levels of CKD have been proposed. For patients with mild to moderate underlying renal insufficiency, the incidence of CIAKI is approximately 5% to 10%. Superimposition of diabetes mellitus on mild to moderate renal insufficiency heightens this risk, whereas the incidence of CIAKI increases to as much as 50% or more in patients with very advanced CKD.

Although diabetes mellitus substantially amplifies the risk of CIAKI in patients with underlying renal impairment, the presence of diabetes in the setting of intact kidney function does not appear to be associated with an increased risk of CKAKI.[51–55] For example, in a study by Rudnick et al.[53] that compared the effects of high- and low-osmolal contrast agents in 1,196 patients undergoing coronary angiography, CIAKI—defined by a postprocedure increase in serum creatinine (SCr) of ≥1.0 mg per deciliter—occurred in none of 359 patients without diabetes or underlying CKD and in just 2 of 315 (0.6%) patients with diabetes but no underlying CKD. However, in study participants with baseline renal impairment, 17 of 296 nondiabetics (6%) and 42 of 213 diabetics (20%) developed CIAKI. Thus, diabetes substantially amplifies the risk of CIAKI in patients with impaired kidney function, but does not appear to represent a notable risk factor in the setting of intact kidney function.

Patients with absolute or effective intravascular volume depletion have increased susceptibility to renal injury from iodinated radiocontrast media.[56] Both clinical states are associated with reduced renal blood flow, which can exacerbate the impact of renal vasoconstriction following intravascular radiocontrast administration. Absolute extracellular volume depletion due to gastrointestinal (GI) losses or diuresis and effective intravascular volume depletion due to advanced heart failure or end-stage liver disease, which are associated with an increased reliance on the vasodilatory effects of prostaglandins to maintain renal microperfusion, also augment the risk for CIAKI.[56,57] Similarly, nephrotoxic medications, specifically nonselective and cyclo-oxygenase-2 selective nonsteroidal anti-inflammatory medications, which inhibit vasodilatory prostaglandins in the kidney, are associated with an increased risk of CIAKI.[58] Other medications that may also increase the likelihood of contrast-associated renal injury include aminoglycosides and calcineurin inhibitors.

Additional factors that have been reported to increase the risk of CIAKI include older age, hypertension, and anemia.[59,60] However, the independent impact of these factors on the risk for CIAKI is uncertain because each is strongly correlated with the presence of underlying CKD. Recent studies suggest that an elevated serum glucose concentration at the time of contrast administration may confer an added risk of CIAKI, particularly among nondiabetics.[61] Likewise, elevated urinary protein excretion has been shown to be associated with increased risk of AKI in several clinical settings, although its role in the context of contrast administration is currently unknown.[62] In animal studies, intratubular light chains, particularly if accompanied by intravascular volume depletion, augment the nephrotoxic potential of radiocontrast media.[34] However, more recent studies in humans do not support an association of multiple myeloma with an increased risk for CIAKI.[63] An analysis of 476 patients with multiple myeloma who received iodinated contrast revealed

33.1 Risk Factors for Contrast-Induced Acute Kidney Injury

Patient Related	Procedure Related
Renal impairment	High-osmolal contrast media
Diabetes mellitus[a]	Large contrast volume
Absolute intravascular volume depletion	Multiple sequential procedures
Effective intravascular volume depletion	Intra-arterial administration
Concomitant nephrotoxic medication use	

[a]Amplifies risk in the setting of renal impairment; not a strong independent risk factor.

an incidence of CIAKI of just 0.6% to 1.25%.[64] Early reports of CIAKI following contrast exposure in patients with multiple myeloma may not have fully accounted for other comorbid factors such as sepsis and volume depletion.

In summary, the administration of iodinated contrast media leads to hemodynamic and toxic effects that, in healthy subjects, are balanced by protective regulatory systems that maintain renal parenchymal oxygenation, function, and integrity. These protective regulatory systems are impaired in the setting of patient-related risk factors, particularly preexisting CKD, leading to an increased susceptibility to renal injury and the development of CIAKI.

Procedure-Related Risk Factors

A series of procedure-related factors have been identified that increase the risk for CIAKI. The dose of contrast has been the subject of considerable attention, with some studies demonstrating an association of higher volumes of contrast with greater risk and other studies showing no such association.[65–67] Miller and colleagues[67] prospectively evaluated 200 patients undergoing procedures with intravenous or intra-arterial contrast and reported no consistent change in renal function with increasing doses of contrast media. Conversely, Cigarroa et al.[65] demonstrated that decreasing the volume of contrast administered during coronary angiography was associated with a reduction in the incidence of CIAKI. Although a specific threshold volume of contrast above which the risk for CIAKI increases substantially has not been definitively determined, multiple sequential procedures or procedures employing larger volumes of contrast appear to pose a greater risk. Similarly, higher doses of iodine have also been associated with a greater risk for CIAKI, which has led to the development of formulas that incorporate the dose of iodine to estimate risk.[68,69] However, the role and importance of an iodine dose relative to the overall volume of contrast requires further study.

The type of contrast agent has also been strongly associated with risk for CIAKI. The first iodinated contrast media widely used in clinical practice were high-osmolal ionic derivatives of triiodobenzene, such as diatrizoate, meglumine, or metrizoate.[70] These high-osmolal contrast media (HOCM) were characterized by osmolalities that were five to eight times greater than blood (approximately 1,500 to 2,000 mOsm per kilogram of water). A series of studies in the early 1990s demonstrated that contrast agents with osmolalities of approximately 600 to 850 mOsm per kilogram (low-osmolal contrast media [LOCM]) were less nephrotoxic than conventional HOCM.[53,71] A third-generation iso-osmolal agent (approximately 300 mOsm per kilogram), iodixanol, was introduced in the late 1990s. Several clinical trials and meta-analyses have compared the nephrotoxicity of this agent with various LOCM, with conflicting results (see section on prevention).[72–75]

Iodinated contrast media are administered intra-arterially in the setting of an angiography and intravenously with computed tomography (CT). Direct comparisons of the incidence of CIAKI across different procedure types have been scarce. In a study of 660 patients with CKD, Weisbord et al.[76] demonstrated that the incidence of CIAKI, defined by an increase in SCr ≥25%, was higher following noncoronary angiography (13.2%) than both coronary angiography (8.5%) and CT imaging (6.5%). However, these analyses did not account for variation in baseline clinical risk factors such as diabetes mellitus, heart failure, and the severity of CKD or in differential application of preventive interventions such as IV fluids. Differences in such factors may account for the higher observed rate of CIAKI following angiography in this study and may also underlie the perception that procedures involving intra-arterial contrast administration are associated with a higher risk of CIAKI than procedures that use IV injection.

THE CLINICAL PRESENTATION OF CONTRAST-INDUCED ACUTE KIDNEY INJURY

CIAKI presents as an acute decline in renal function that characteristically develops within 72 hours following contrast administration. Serum creatinine typically peaks within 3 to 5 days and returns toward baseline levels within 7 to 10 days. Most patients with CIAKI remain nonoliguric, although oliguric acute kidney injury (AKI) may occur. CIAKI is a form of acute tubular necrosis (ATN), for which the principal differential diagnoses include ischemic ATN and renal atheroembolic disease.[77] Differentiation from ischemic ATN is generally based on the clinical setting, although distinguishing between these two forms of AKI may be difficult in hemodynamically unstable patients who sustain episodes of hypotension contemporaneous with contrast administration. The urine sediment in CIAKI commonly demonstrates coarsely granular "muddy brown" casts, as is seen in other etiologies of ATN. The fractional excretion of sodium in CIAKI is often less than 1%, although this does not have sufficient diagnostic reliability to definitively differentiate CIAKI from ischemic or other forms of ATN.

Renal atheroembolic disease, which is considerably less common than CIAKI and ischemic ATN, results from the release of cholesterol crystals and other atheromatous debris into the systemic circulation from ulcerated atherosclerotic plaques.[78] Although renal atheroembolic disease can occur spontaneously, it is more common following angiographic procedures.[78] The time course of angiography-associated atheroembolic disease and CIAKI differ; although acute atheroembolism can occur immediately following vascular catheterization, more commonly it is delayed, typically developing days to weeks following vascular instrumentation.[78] Unlike CIAKI, atheroembolic disease is generally associated with specific systemic manifestations including mesenteric ischemia, digital ischemia ("blue toe" syndrome) and livedo reticularis, and laboratory abnormalities that include eosinophilia, eosinophiluria, and hypocomplementemia.[79]

In clinical practice, the identification of patients with CIAKI is based on observing a rise in SCr that occurs in the characteristic time frame following contrast administration. Because clinically detectable elevations in SCr are not evident for many hours to days after renal injury, the diagnosis and implementation of supportive care are typically delayed. Earlier identification of renal injury in the incipient stage of AKI could lead to more immediate implementation of supportive care in the postprocedure period. A series of candidate urinary and serum biomarkers have been identified for the early diagnosis of AKI, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and kidney injury molecule-1 (KIM-1).[80,81] In addition, these biomarkers have the potential to differentiate volume responsive AKI (prerenal azotemia) from intrinsic AKI. Given their early expression in the urine following tubular injury, these or other biomarkers may ultimately assist in the diagnosis of CIAKI prior to a change in SCr.

THE INCIDENCE OF CONTRAST-INDUCED ACUTE KIDNEY INJURY

The reported incidence of CIAKI is highly dependent on the patient population studied and on the criteria employed to define renal injury. Serologic criteria that have been used in past studies to define CIAKI include an increase in SCr of at least 0.3 mg per deciliter, 0.5 mg per deciliter, 1.0 mg per deciliter, or 2.0 mg per deciliter, or a relative increase of at least 10%, 25%, or 50% within 5 days following contrast administration.[51,65,72,82–91] D'Elia and coworkers[51] reported that 0.68% of nonazotemic patients and 17.4% of azotemic patients experienced a 1.0 mg per deciliter rise in SCr following nonrenal angiography. A 12% incidence of CIAKI was reported in seriously ill, hospitalized patients, using an elevation in SCr of ≥1 mg per deciliter within 48 hours as the criterion for nephrotoxicity.[92] A study of 537 patients undergoing angiography that defined CIAKI as an increase in SCr of at least 1.0 mg per deciliter within 24 hours demonstrated no episodes of CIAKI.[93] More recently, an observational cohort study by Weisbord and colleagues[76] enrolled patients with baseline estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73m^2 who were undergoing nonurgent coronary or noncoronary angiography or CT, and reported on the rates of CIAKI with these procedures. The incidence of CIAKI, defined by an increase in SCr of ≥25%, was 13.2% following noncoronary angiography, 8.5% following coronary angiography, and 6.5% following CT scans.[76] Using more robust increments in SCr to define CIAKI resulted in considerably lower rates of renal injury with less than 1% of patients overall experiencing a rise in SCr of ≥1.0 mg per deciliter. In a recent observational study of 1,111 hospitalized patients who underwent procedures with intravascular contrast, the incidence of CIAKI, defined as an increase in SCr of ≥0.5 mg per deciliter within 1 to 5 days, was as high as 44% among patients with baseline renal insufficiency and concomitant diabetes.[94]

The substantial variability in the reported rates of CIAKI across these and other studies highlights the important impact that patient population, procedure type, and criteria used to define renal injury have on the reported disease incidence. Determining accurate estimates of the incidence of CIAKI, particularly if defined by diminutive increments in SCr, is further confounded by the underlying fluctuation in SCr that occurs independently of iodinated contrast administration. Bruce et al.[95] studied the incidence of AKI, defined by an increase in SCr ≥0.5 mg per deciliter or a decrease in eGFR ≥25%, among 11,588 patients who underwent a total of 13,274 CT scans either with iodinated contrast (n = 5,790) or without iodinated contrast (n = 7,484). Among patients with baseline CKD, the overall incidence of renal injury in patients who did not receive iodinated contrast (8.8%) was comparable to that of patients who received iodinated contrast (9.7% with iso-osmolal iodixanol and 9.9% with low-osmolal iohexol). Thus, baseline variability in SCr and factors other than iodinated contrast should be considered when estimating the incidence of acute kidney injury from contrast administration.

OUTCOMES ASSOCIATED WITH CONTRAST-INDUCED ACUTE KIDNEY INJURY

Short-Term Outcomes Associated with Contrast-Induced Acute Kidney Injury

Several studies have demonstrated that CIAKI is associated with increased short-term mortality (Table 33.2).[52,96–100] In a retrospective study of 183 hospitalized patients, Levy et al.[96] demonstrated that CIAKI was associated with an increased risk of in-hospital mortality after adjustment for underlying level of comorbid illness (odds ratio [OR] = 5.5, P< .001). McCullough et al.[52] evaluated 1,826 patients who had undergone percutaneous coronary intervention and documented an in-hospital mortality rate of 7.1% among patients with CIAKI (defined by an increase in SCr of >25%) compared to 1.1% in those without CIAKI (P< .0001). Patients who developed CIAKI that required renal replacement therapy experienced an in-hospital mortality rate greater than 35%. In a retrospective study, Rihal et al.[100] examined outcomes in 7,586 patients undergoing percutaneous coronary intervention and reported that patients who developed CIAKI had a marked increased risk for in-hospital mortality (OR = 10.8, P< .0001). Among more than 20,000 patients who underwent percutaneous coronary intervention, Bartholomew and colleagues[97] demonstrated that CIAKI, defined by increases in SCr ≥1.0 mg per deciliter, was associated with a striking increase in in-hospital mortality (OR = 22, 95% confidence interval [CI]: 16 to 31). In a recent analysis, Shema et al.[94] demonstrated that among over 1,100 hospitalized patients who underwent contrast-enhanced radiographic procedures, CIAKI was independently associated with a nearly 10-fold increase in in-hospital mortality (OR = 9.8, 95% CI: 4.4 to 22.0).

33.2 Association of Contrast-Induced Acute Kidney Injury with Short-Term Risk of Mortality

Study Authors	# Study Patients	Definition of CIAKI	Adjusted OR for Death	95% CI
Levy et al.	357	↑ SCr ≥25% to ≥2.0 mg/dL	5.5	2.9–13.2
Gruberg et al.	439	↑ SCr >25%	3.9	2.0–7.6
Shema et al.	1,111	↑ SCr ≥50% or ↓ eGFR ≥25%	3.9	1.2–12.0
McCullough et al.	1,826	↑ SCr >25%	6.6	3.3–12.9
From et al.	3,236	↑ SCr ≥25% or ≥0.5 mg/dL	3.4	2.6–4.4
Rihal et al.	7,586	↑ SCr >0.5 mg/dL	10.8	6.9–17.0
Bartholomew et al.	20,479	↑ SCr ≥1.0 mg/dL	22	16–31
Weisbord et al.	27,608	↑ SCr 0.25–0.5 mg/dL	1.8	1.4–2.5

CIAKI, contrast-induced acute kidney injury; OR, odds ratio; CI, confidence interval; SCr, serum creatinine; eGFR, estimated glomerular filtration rate.

Finally, in a retrospective analysis of over 27,000 patients who underwent coronary angiography, Weisbord et al.[99] found that postangiography increases in SCr greater than 0.25 mg per deciliter but no higher than 0.5 mg per deciliter were independently associated with increased in-hospital mortality (OR = 1.83, 95% CI: 1.35 to 2.49).

Prospective observational studies and clinical trials reveal comparable findings. In a study of 439 patients with CKD undergoing percutaneous coronary intervention, Gruberg et al.[101] found that in-hospital mortality occurred more frequently among patients who developed CIAKI than in comparable patients without CIAKI (14.9% versus 4.9%, P= .001). In a clinical trial, Marenzi et al.[102] also found that patients who developed CIAKI had a significantly increased incidence of in-hospital mortality compared to patients who did not sustain a postangiography decline in renal function (26% versus 1.4%, P< .001). Finally, Maioli et al.[103] demonstrated that in-hospital mortality among patients who developed CIAKI was markedly higher than among patients who did not develop this postprocedure complication (11.1% versus 0.2%, P= .001).

CIAKI is also associated with prolonged hospitalization.[97,99,104–107] Bartholomew et al.[97] found that patients who developed CIAKI after percutaneous coronary intervention (PCI) were 15 times more likely to have their hospitalization prolonged more than 4 days. In the aforementioned study by Shema et al.,[94] CIAKI was associated with a marked increase in hospital length of stay (24 days with CIAKI versus 13 days without CIAKI, P< .001). Adolph et al.[104] demonstrated that the development of CIAKI resulted in an increased mean duration of hospitalization of 2 days. The extended length of a hospital stay associated with CIAKI translates into increased health care expenditures. Using decision analytic techniques, Subramanian et al.[105] reported that a single episode of CIAKI results in an average increase in hospital-related costs of more than $10,000.

Notwithstanding the findings of these studies that demonstrate that CIAKI is associated with increased short-term mortality and a prolonged duration of hospitalization, it is important to note that the causal nature of such associations has not been established. Risk factors that increase the risk for CIAKI (i.e., CKD, heart failure) are also independently associated with these adverse short-term outcomes.

Long-Term Outcomes Associated with Contrast-Induced Acute Kidney Injury

In several recent analyses, CIAKI has also been linked with increased longer term mortality (Table 33.3).[100,108–112] Solomon et al.[111] demonstrated that the development of CIAKI following angiography was associated with a greater than threefold increased risk of death, stroke, myocardial infarction, and end-stage renal disease at 1 year of follow-up. In a study of 985 patients, Harjai et al.[109] demonstrated that CIAKI following coronary angiography was independently associated with an increased likelihood of death at 24 months (hazard ratio [HR] = 2.6; 95% CI: 1.5 to 4.4). A study by Brown et al.[112] that examined long-term outcomes among 7,856 patients found that patients with either transient or persistent postangiography decrements in kidney function had a two- to threefold increase in long-term mortality. In a smaller study of 78 patients, Goldenberg and colleagues[108] documented that CIAKI that fully recovered within 7 days of angiography was associated with a greater than twofold increase in the 5-year mortality (HR = 2.66, 95% CI: 1.72 to 4.46). The previously described study by Rihal et al.[100] also demonstrated that the

33.3 Association of Contrast-Induced Acute Kidney Injury with Long-Term Risk of Mortality

Study Authors	# Study Patients	Definition of CIAKI	Follow-Up (Months)	Adjusted HR	95% CI
Goldenberg et al.	78	↑SCr ≥0.5 mg/dL or ≥25%	60	2.7	1.7–4.5
Solomon et al.	294	↑SCr ≥0.3 mg/dL	12	3.2[a]	1.1–8.7
Harjai et al.	985	↑SCr ≥0.5 mg/dL	24	2.6	1.5–4.4
Roghi et al.	2,860	↑SCr ≥0.5 mg/dL	24	1.8	1.0–3.4
Rihal et al.	7,075	↑SCr >0.5 mg/dL	6	[b]	[b]
Brown et al.	7,856	↑SCr ≥0.5 mg/dL	90	3.1	2.4–4.0

[a]Reflects incident rate ratio of death, cerebrovascular accident, acute myocardial infarction, and end-stage renal disease.
[b]Six-month mortality of 9.8% with CIAKI versus 2.3% without CIAKI ($p < 0.0001$).
CIAKI, contrast-induced acute kidney injury; HR, hazard ratio; CI, confidence interval; SCr, serum creatinine.

5-year mortality rate among patients who underwent coronary angiography and survived to hospital discharge was significantly higher in those who had experienced CIAKI (44.6% versus 14.5%). Finally, James et al.[113] recently demonstrated that patients sustaining a 50% to 100% increase in SCr following coronary angiography doubled their risk of death (HR = 2.0; 95% CI: 1.69 to 2.36) over the ensuing 3 years, whereas patients sustaining an acute increase in SCr >100% experienced a nearly fourfold increased risk of death (HR = 3.72; 95% CI: 2.92 to 4.76).

Past studies have also demonstrated a relationship between CIAKI and accelerated progression of CKD.[103,108,113,114] In a study of 78 patients, Goldenberg et al.[108] found that patients with a transient postprocedure rise in SCr ≥25% or ≥0.5 mg per deciliter following coronary angiography experienced a larger decrement in eGFR 2 years following the procedure than patients without these transient elevations in SCr (Δ eGFR of −20 ± 11 mL/min/1.73 m^2 versus −6 ± 16 mL/min/1.73 m^2, P= .02). Maioli et al.[103] reported that patients with CIAKI had a 0.2 mg/dL higher mean SCr 1 month postangiography than did patients without CIAKI (P= .001). Finally, James et al.[114] recently documented that patients who developed an increase in SCr ≥0.3 mg/dL or between 50% and 99% following coronary angiography experienced a more rapid loss of kidney function compared to patients without such changes in kidney function (loss of eGFR 0.8 mL/min/1.73m^2 per year versus 0.2 mL/min/1.73m^2 per year). For patients sustaining increases in SCr ≥100%, the long-term rate of loss of eGFR was even more marked (2.8 mL/min/1.73m^2 per year).[114] These investigations also demonstrated an increased risk of mortality and end-stage renal disease during 3 years of follow-up in patients who developed CIAKI with graded risks based on the severity of AKI (P< .001 for the trend in mortality and ESRD) (Fig. 33.2).[113] As was noted with the associations of CIAKI with shorter term outcomes, although there is a strong association of CIAKI with longer term mortality and more rapid progression of CKD and ESRD, the causality of this association has not been determined.

THE PREVENTION OF CONTRAST-INDUCED ACUTE KIDNEY INJURY

Overview of Prevention

With a large body of evidence demonstrating associations of CIAKI with adverse short and longer term adverse outcomes, there has been a substantial emphasis placed on identifying interventions to prevent this form of renal injury. CIAKI is one of the few forms of AKI that is potentially preventable because patients at high risk are easily identifiable and most procedures that involve the intravascular administration of iodinated contrast are scheduled sufficiently in advance to allow implementation of prophylactic measures.

The initial step in the prevention of CIAKI is the identification of patients at an increased risk. Alternative imaging procedures that do not require the use of iodinated contrast media, including ultrasonography, nonenhanced CT scans, and MRIs and nuclear imaging, should be considered in patients at increased risk for CIAKI. This involves a careful assessment of both the risk of CIAKI and any potential loss of diagnostic yield with alternative imaging procedures. The use of gadolinium enhancement of MRI procedures is particularly problematic in patients with advanced CKD or ongoing AKI because gadolinium-containing agents are associated with the development of nephrogenic systemic fibrosis in patients with markedly decreased GFR—the subset of patients at highest risk of developing CIAKI.[6,7]

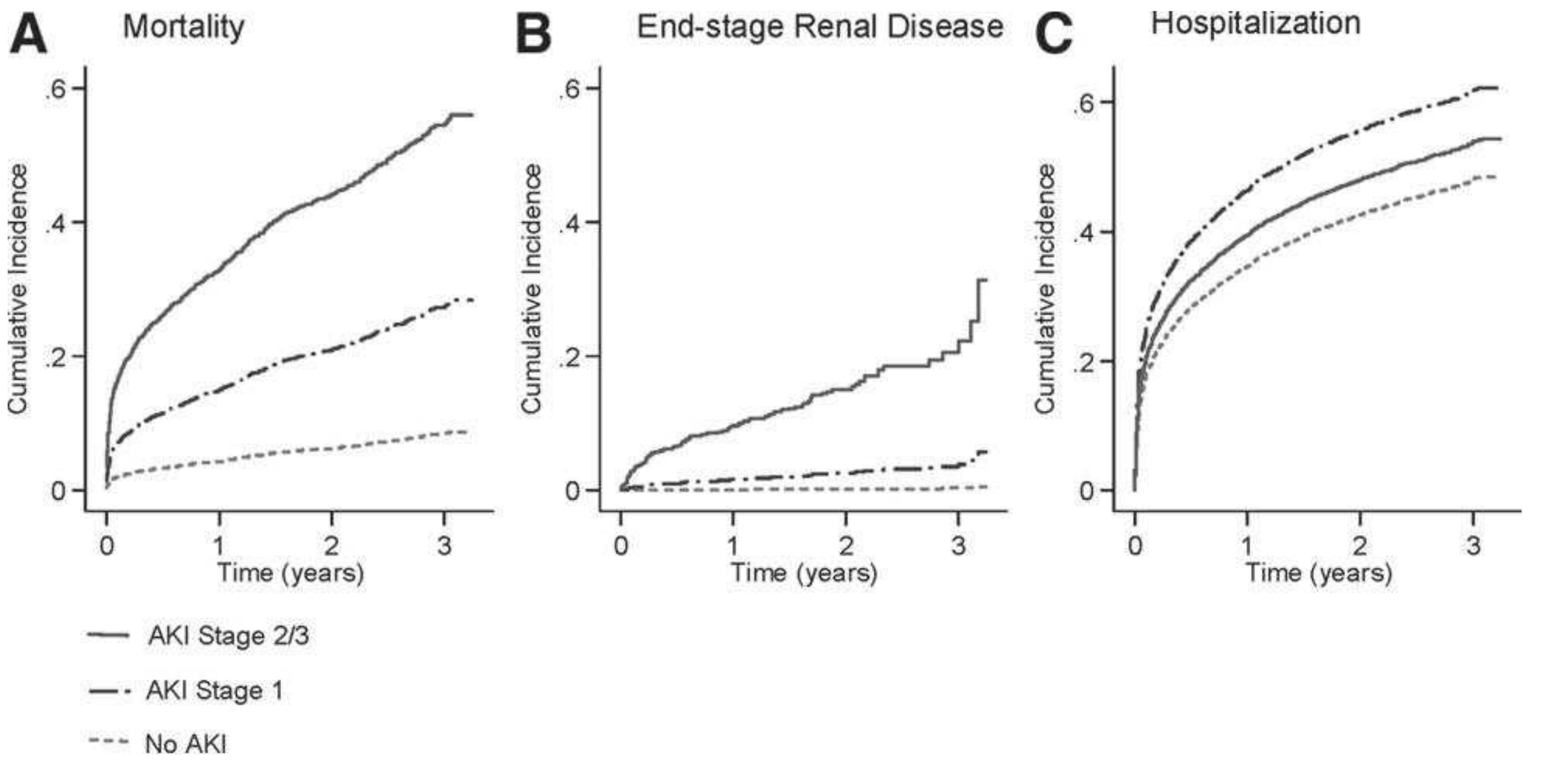


FIGURE 33.2 Cumulative incidence of **(A)** mortality, **(B)** end-stage renal disease (ESRD), and **(C)** hospitalization for all causes according to stage of acute kidney injury (AKI) defined according to the Acute Kidney Injury Network criteria (AKI stage 1: ≥0.3 mg/dL absolute or 1.5- to twofold relative increase in serum creatinine; AKI stage 2: >two- to threefold increase in serum creatinine; AKI stage 3: >threefold increase in serum creatinine or serum creatinine ≥4.0 mg/dL with an acute rise of >0.5 mg/dL). Reprinted with permission from James MT, et al. Associations between acute kidney injury and cardiovascular and renal outcomes after coronary angiography. *Circulation*. 123(4):409–416.

Preventive measures for CIAKI can be categorized into four principal strategies: (1) the use of less nephrotoxic contrast agents; (2) the provision of preemptive renal replacement therapy to remove contrast from the circulation; (3) the use of pharmacologic agents to counteract the nephrotoxic effects of contrast media; and (4) the administration of IV fluids to expand the intravascular space and enhance diuresis.

The Choice of Contrast Agent

Various physicochemical properties of iodinated contrast media, including ionicity, osmolality, and viscosity, have been implicated in the development of CIAKI. All iodinated contrast agents contain either a single triiodobenzene ring or are dimeric structures with two triiodobenzene rings (Fig. 33.3).[70] The initial contrast media were monomeric sodium or meglumine salts of triiodobenzoic acid derivatives. These agents are characterized as ionic because they dissociate in aqueous solution, and as high osmolal, with osmolalities ranging from approximately 1,500 mOsm per kilogram to greater than 2,000 mOsm per kilogram. The next generation of contrast media included nonionic derivatives of triiodobenzene. Because these agents do not dissociate in an aqueous solution, an equivalent dose of iodine is provided with approximately half the osmolal load (approximately 600 mOsm per kilogram to 1,000 mOsm per kilogram), resulting in these agents being characterized as low osmolal, although they still have a much higher osmolality than plasma. Low-osmolal agents can also be ionic, consisting of a dimeric structure derived from triiodobenzene and triiodobenzoic acid. Most recently, nonionic, iso-osmolal (osmolality approximately 300 mOsm per kilogram) agents have been synthesized as dimeric derivatives of triiodobenzene. Although these newer

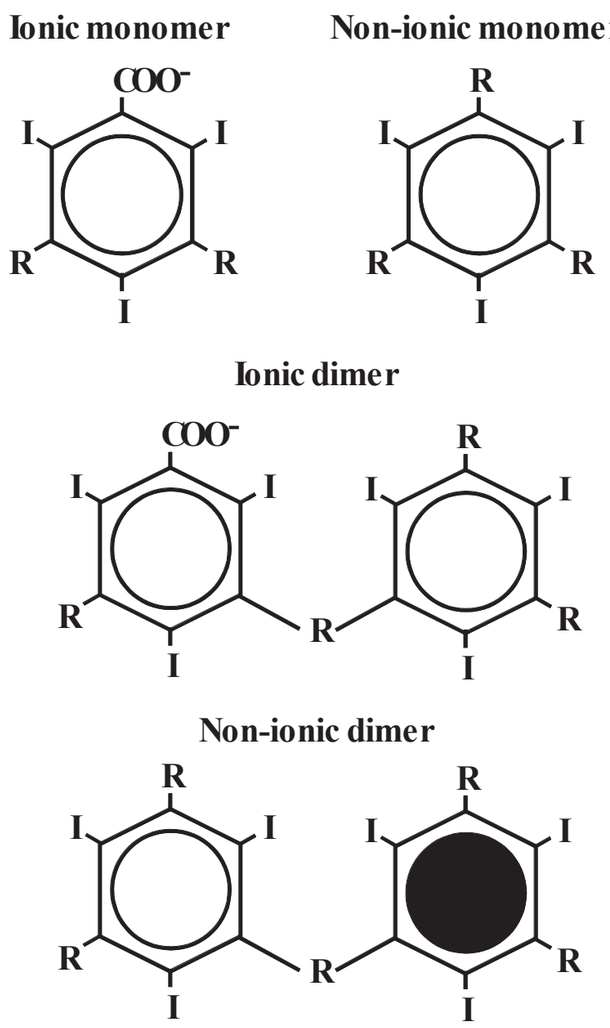


FIGURE 33.3 The molecular structure of iodinated contrast agents.

agents have a lower osmolality than "low-osmolal contrast media," which is thought to decrease their toxicity, their larger molecular size results in increased viscosity. Studies in animal models demonstrate that higher viscosity increases the transit time of contrast in the renal tubular fluid, raises hydrostatic pressure, and reduces glomerular filtration.[115] It has also been suggested that higher viscosity leads to a more pronounced aggregation of red blood cells and decreased red blood cell velocity in the medullary vessels.[116] Although HOCM and LOCM have lower viscosities than iso-osmolal contrast media (IOCM) particularly at room temperature, preheating may represent a means to reduce viscosity prior to administration.[117]

A substantial body of research has been devoted to discerning the relationship between the osmolality of various contrast media and the risk for CIAKI. Early studies focused on the comparative nephrotoxicity of HOCM and LOCM.[53,71] Following the performance of a series of small studies, Rudnick et al.[53] reported the results of the Iohexol Cooperative Study, a multicenter clinical trial comparing high-osmolal diatrizoate with low-osmolal iohexol in 1,196 patients undergoing nonemergent coronary angiography. Overall, CIAKI was less common with iohexol than with diatrizoate (3.2% versus 7.1%, $p=0.002$). The lower risk of CIAKI with iohexol was particularly notable among the subset of patients with both diabetes mellitus and baseline renal impairment. Using data from this and several other trials, Barrett and Carlisle[71] conducted a meta-analysis demonstrating a lower risk of CIAKI, defined by a rise in SCr >0.5 mg per deciliter, with LOCM compared to HOCM (OR = 0.61; 95% CI: 0.48 to 0.77). Among patients with underlying renal impairment, the odds of CIAKI with LOCM decreased further to 0.5 (95% CI: 0.36 to 0.68). These studies helped establish a sound evidence basis for the preferential use of LOCM rather than HOCM in patients with impaired kidney function.

More recently, clinical trials have examined the relative nephrotoxicity of LOCM and the iso-osmolar agent iodixanol. Although some of these studies demonstrated a lower incidence of CIAKI associated with iodixanol than with certain LOCM (i.e., iohexol, ioxaglate), the results have not been consistent across studies, with other studies demonstrating comparable rates of CIAKI with iodixanol and LOCM.[72,75,90,118–122] Meta-analyses of these data suggest that although iodixanol may be associated with a lower risk of CIAKI than specific low-osmolal agents (e.g., iohexol, ioxaglate), this benefit is not seen across the entire spectrum of LOCM.[73,74,123–125] The composite findings of the clinical trials and meta-analyses to date are reflected in clinical practice guidelines issued by the American College of Cardiology/American Heart Association in 2009, which recommend the use of iso-osmolal iodixanol or LOCM exclusive of iohexol or ioxaglate in patients with chronic kidney disease undergoing angiography.[126] The European Society of Urogenital Radiology has recommended the use of either LOCM or IOCM in patients at an increased risk of CIAKI.[127]

Use of Renal Replacement Therapies to Prevent Contrast-Induced Acute Kidney Injury

Because of their water solubility, limited protein binding, and restriction of distribution to the extracellular space, iodinated contrast media are efficiently cleared by hemodialysis, an observation that has led to studies investigating the role of prophylactic renal replacement therapy for the prevention of CIAKI. These studies were based on the hypothesis that accelerated removal of iodinated contrast from the circulation will reduce renal exposure and minimize the development of CIAKI. Lee et al.[128] found a smaller decrement in creatinine clearance and a less frequent requirement for chronic hemodialysis among 82 patients with advanced CKD undergoing a coronary angiography who were randomized to receive prophylactic hemodialysis compared to patients who did not receive prophylactic hemodialysis. However, the small sample size, inaccuracy of the primary endpoint that was based on 24-hour urine collection for the calculation of creatinine clearance, and paucity of patients who progressed to ESRD preclude meaningful conclusions from this trial. However, the benefit seen in this study has not been supported by the majority of other trials conducted over the past 2 decades, which found either no benefit or increased rates of CIAKI in patients who received prophylactic hemodialysis.[129–134] Collectively, available data to date indicate that prophylactic hemodialysis does not reduce the incidence of CIAKI and may be associated with potential harm and increased cost.[135,136]

Continuous renal replacement therapy (CRRT) also effectively removes iodinated contrast from the circulation, albeit at a slower rate than conventional intermittent hemodialysis. Two trials conducted by Marenzi and colleagues[137,138] studied the role of continuous veno-venous hemofiltration (CVVH) for the prevention of CIAKI, and both studies reported that this therapy reduces the risk for CIAKI and death. However, the primary endpoint in both trials was defined by small increases in SCr, an endpoint of questionable validity given that CVVH directly lowers the serum creatinine concentration. Therefore, given the level of existing evidence as well as issues related to cost and risks of CRRT, the preemptive use of this therapy to prevent the development of CIAKI or its attendant complications cannot be recommended.

Pharmacologic Agents to Prevent Contrast-Induced Acute Kidney Injury

A large number of pharmacologic agents have been investigated for the prevention of CIAKI. Although none have been definitively shown to be effective, these agents can be categorized into those that have been shown to be ineffective (and in some cases harmful) and those for which the evidence basis upon which to determine effectiveness is inadequate (Table 33.4).

33.4 Pharmacologic Agents for the Prevention of Contrast-Induced Acute Kidney Injury

Ineffective	Indeterminate Effectiveness
Loop diuretics[a]	Atrial natriuretic peptide
Dopamine[a]	Theophylline/aminophylline
Fenoldopam[a]	Statins
Calcium channel blockers	Prostaglandin analogs N-acetylcysteine Bosentan Allopurinol Acetazolamide

[a]Potentially deleterious.

Diuretics

By virtue of its inhibition of active sodium transport in the ascending limb of the Henle loop, furosemide was proposed as a potential intervention that would reduce oxygen use and, hence, decrease the risk of nephrotoxicity of iodinated contrast. This theoretical benefit of furosemide has not been borne out in clinical trials. In a study by Solomon et al.,[17] the addition of furosemide to hypotonic sodium chloride was associated with a higher incidence of CIAKI than periprocedural hypotonic saline alone (40% versus 11%, P≤ .02). The ineffectiveness of furosemide was consistent with an earlier report that also demonstrated furosemide to be associated with a higher risk of CIAKI.[139] Several studies have also investigated the role of mannitol for the prevention of CIAKI.[84,140,141] Mannitol results in an osmotic dieresis, increases medullary blood flow, and serves as a scavenger of reactive oxygen species. However, clinical studies have failed to demonstrate a beneficial effect of mannitol for this purpose. In the aforementioned trial by Solomon et al.,[17] patients who received mannitol in combination with hypotonic saline had a greater risk of developing CIAKI than did patients who received hypotonic saline alone (relative risk [RR] 2.61, 95% CI: 0.76 to 9.03). In a subsequent study, Majumdar et al.[142] evaluated the effect of forced isovolemic diuresis with a combination of furosemide and mannitol to volume administration with hypotonic saline. Similar to the results in the prior study, the use of mannitol and saline was associated with a significantly increased risk of CIAKI despite the use of a protocol to ensure that intravascular volume was maintained. The carbonic anhydrase inhibitor acetazolamide was shown to be renoprotective in a rat model of CIAKI, perhaps by means of attenuating oxygen free radical formation through alkalinization of the urine.[143] Two studies demonstrated lower rates of CIAKI among patients who were administered acetazolamide, but the studies were insufficient in size to permit meaningful conclusions on the effectiveness of this agent.[144,145]

Calcium Channel Blockers

Calcium channel blockers have vasodilatory effects, which have led to their evaluation for the prevention of CIAKI. Cacoub and colleagues[146] retrospectively studied 26 patients, 11 of whom received nifedipine before and after iodinated contrast administration, and reported comparable rates of CIAKI among patients who had and had not received this agent. Khoury et al.[147] randomized 85 patients to receive IV fluids with or without nifedipine and failed to demonstrate a reduced incidence of CIAKI in patients who received this calcium channel blocker.

Dopamine and Fenoldopam

At low doses, dopamine increases renal blood flow and inhibits sodium reabsorption in the proximal tubule.[148] Several studies have investigated the use of prophylactic dopamine to reduce the propensity for iodinated contrast media to induce renal injury.[148–150] Although small studies suggested a beneficial effect, larger clinical trials have failed to confirm these findings.[148–151] In a study of patients with CKD undergoing coronary angiography, Weisberg and colleagues[148] compared renal blood flow and the incidence of CIAKI between subjects treated with low dose dopamine coupled with 0.45% saline and patients who received 0.45% saline alone. Despite observing a sustained increase in renal blood flow with dopamine, overall rates of CIAKI between the two groups were similar (6 out of 15 versus 5 out of 15, p=1.0). Of note, diabetics treated with dopamine experienced more CIAKI than diabetics treated with saline alone. More recently, Abizaid et al.[152] conducted a randomized trial comparing the effects of dopamine added to IV saline on rates of CIAKI following coronary angiography. No benefit was observed for subjects who received dopamine. Thus, although low dose dopamine increases renal blood flow, this hemodynamic effect has no impact on diminishing the nephrotoxic effect of iodinated contrast and may be detrimental to patients with underlying diabetes mellitus.

Fenoldopam is a selective dopamine receptor agonist and vasodilator that is used for the treatment of hypertensive emergency. Studies in humans have shown that fenoldopam dilates renal vessels and augments renal perfusion, properties that informed studies investigating its potential to prevent CIAKI.[153–156] Several studies reported that periprocedural fenoldopam administration reduced the incidence of CIAKI.[155–160] However, most were uncontrolled, nonrandomized or of limited sample size. Two randomized controlled trials found no benefit to fenoldopam for the prevention of CIAKI. In a randomized trial of 123 patients, Allaqaband et al.[161] compared half-isotonic saline alone to either half-isotonic saline plus fenoldopam or half-isotonic

saline plus N-acetylcysteine. There was no difference in the incidence of CIAKI across the three treatment arms (P= .92). In a subsequent study, Stone et al.[162] randomized 315 patients with CKD to receive IV fluids with or without IV fenoldopam before and after coronary angiography. The incidence of CIAKI, defined as an increase in SCr of at least 25% within 96 hours, was 33.6% in patients assigned to receive fenoldopam as compared to 30.1% in patients assigned to placebo (P= .61). There were also no significant differences in the rates of rehospitalization, the need for dialysis, or 30-day mortality. The data therefore do not support the use of fenoldopam for the prevention of CIAKI.

Atrial Natriuretic Peptide

The renal vasodilatory properties of atrial natriuretic peptide (ANP) have led to interest in this agent for the prevention and treatment of many etiologies of AKI, including CIAKI. Although early studies using animal models suggested a benefit with ANP, clinical trials of ANP in CIAKI have yielded conflicting results.[31,55,163–165] In a multicenter, randomized controlled trial, Kurnik et al.[163] assigned 247 patients with baseline CKD to placebo or one of three doses of ANP (0.01 mcg per kilogram per minute, 0.05 mcg per kilogram per minute, or 0.1 mcg per kilogram per minute) beginning 30 minutes prior to and continuing until 30 minutes after radiocontrast administration. There was no beneficial effect of ANP on the development of CIAKI, with an incidence of CIAKI of 19% in patients receiving placebo as compared to 25% in patients receiving the highest dose of ANP. In a more recent study, Morikawa and colleagues[164] randomized 254 patients with CKD undergoing coronary angiography with or without PCI in an unblinded fashion to ANP at a dose of 0.042 mcg per kilogram per minute and IV fluids or to IV fluids alone. CIAKI occurred less frequently among patients who received ANP than among patients in the control group (3.2% versus 11.7%, P= .015), and the incidence of persistent decline in kidney function at 1 month was lower among patients randomized to ANP. The difference in the results in these two trials may relate to differences in the study protocols; in the study by Morikawa et al., the ANP infusion rate was lower than the maximal rate in the Kurnik study, and the duration of infusion was more prolonged. Although these data suggest a potential benefit to ANP for the prevention of CIAKI, further study is required to establish the potential role for this agent.

Theophylline/Aminophylline

Research demonstrating that adenosine may be an important mediator in the pathogenesis of CIAKI prompted an investigation of theophylline, an adenosine antagonist, as a potential protective agent in patients receiving radiocontrast.[166,167] Initial human studies suggested that kidney function was preserved by IV theophylline in patients receiving contrast.[168] These observations led Erley et al.[166] to conduct a prospective randomized clinical trial of theophylline in combination with IV saline in 80 patients. In this study, theophylline offered no benefit in preventing CIAKI, although the incidence was low in both the theophylline and the placebo groups. Similarly, a randomized trial of patients undergoing angiography compared aminophylline, dopamine, and saline, and demonstrated no reduction in the incidence of renal injury with aminophylline.[69] In contrast, Huber et al.[134] observed a reduction in the incidence of CIAKI from 20% to 4% following pretreatment with oral theophylline in a study of 100 patients with CKD. In an effort to reconcile these conflicting studies, Ix et al.[169] conducted a meta-analysis that included seven randomized trials of theophylline or aminophylline encompassing 480 patients. In this pooled analysis, patients treated with theophylline or aminophylline experienced a smaller mean increase in SCr than did patients in the study control arms (0.13 mg per deciliter; 95% CI: 0.06 to 0.22 mg per deciliter; P= .004). Adverse effects of therapy and the incidence of longer term outcomes of CIAKI were not reported in the majority of studies. In a second meta-analysis of nine randomized controlled trials involving 585 patients, Bagshaw and Ghali[170] reported an odds ratio for the development of CIAKI of 0.40 (95% CI: 0.14 to 1.16; P= .09) associated with theophylline administration using a random-effects model. They cautioned, however, that there was significant heterogeneity across trials and that the pooled results required cautious interpretation. Kelly and colleagues[171] reached a similar conclusion in a meta-analysis of six studies that included 531 patients, with an odds ratio for the development of CIAKI associated with theophylline of 0.49 (95% CI: 0.23 to 1.06). Given the potential side effects of theophylline and the limited quality of existing data, it is premature to conclude that this agent is protective in the setting of contrast administration.

Statins

By virtue of their antioxidant and anti-inflammatory properties and their effects on endothelial function, statins have been hypothesized to be potentially protective against the development of CIAKI.[172–175] Preliminary observational analyses suggested that statins are effective at reducing the incidence of renal injury from contrast. However, a trial by Jo et al.[175] that randomized patients to four 40 mg doses of simvastatin or placebo at the time of coronary angiography failed to demonstrate a benefit to simvastatin therapy. A recent single-center trial by Toso et al.[176] randomized 304 patients with CKD to receive 80 mg of atorvastatin or placebo daily for 2 days prior to and 2 days following coronary angiography. CIAKI, defined as an increase in SCr $\geq$0.5 mg per deciliter within 5 days, developed in 10% of atorvastatin-treated patients and 11% of placebo-treated patients (P= .86). No differences were seen between the groups in the incidence of persistent renal injury at 30 days following angiography. Thus, data to date are insufficient to support the administration of statins for the prevention of CIAKI.

Prostaglandins

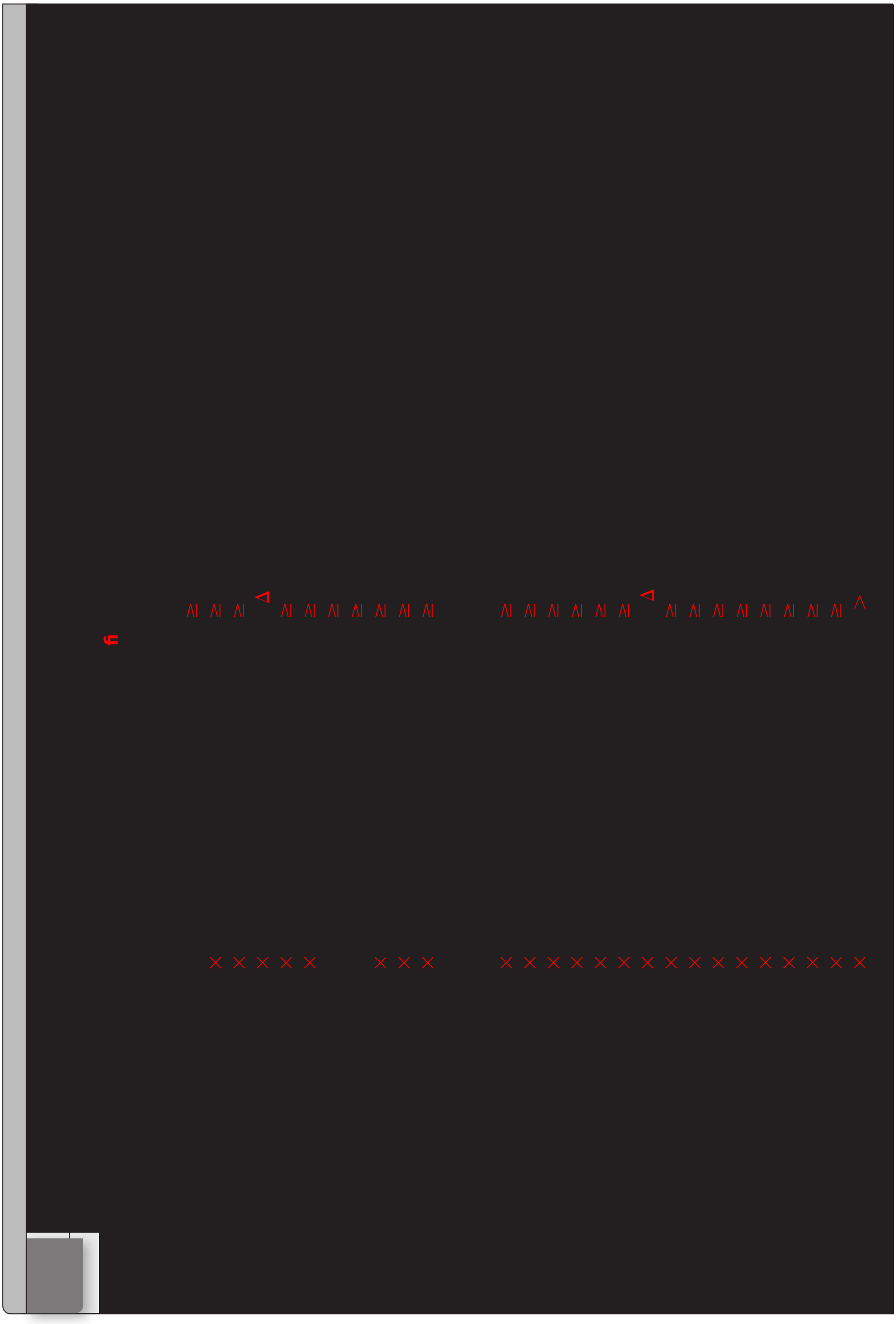
Prostaglandins (PGI_2 and PGE_2) have vasodilatory actions in the kidney and may attenuate the vasoconstrictive effects of iodinated contrast. Studies in animal models suggest a potential beneficial role of prostaglandins for the prevention of nephrotoxicity caused by iodinated contrast.[10,23] Spargias and colleagues[177] conducted a randomized clinical trial in 208 patients with CKD who were undergoing coronary angiography. Patients were randomized to receive placebo or iloprost, a PGI_2 analog, at a dose of 1 ng per kilogram per minute prior to and following the angiography. The investigators found a lower incidence of CIAKI, defined by an increase in SCr ≥0.5 mg per deciliter or ≥25% within 2 to 5 days, with iloprost compared to placebo (8% versus 22%, P= .005) with few adverse events. Notwithstanding the reasonably substantial reduction in the incidence of CIAKI, several caveats must be considered in interpreting the results of this trial. First, the study's power was based on the assumption of a 70% reduction in the incidence of the primary outcome with iloprost, a biologically and clinically implausible effect size. Second, the effects of iloprost on longer term adverse outcomes were not tracked. Until adequately powered studies that comprehensively investigate the short- and long-term risk/benefit ratio of prostaglandins, the routine use of these agents for the prevention of CIAKI cannot be recommended.

N-Acetylcysteine (NAC)

The rationale for the use of NAC for the prevention of CIAKI relates to its capacity to serve as a ROS scavenger, reduce the depletion of glutathione, and stimulate the production of vasodilatory agents such as nitric oxide.[178,179] Studies in animal models of AKI demonstrate that the administration of NAC reduces oxidative damage, attenuates renal medullary vasoconstriction, and decreases renal injury.[178,180] These animal models provided the scientific basis for human studies of NAC for the prevention of CIAKI. An initial trial by Tepel et al.,[181] published in 2000, described the efficacy of NAC for the prevention of CIAKI. In this study, 83 patients undergoing contrast-enhanced CT scans were randomized to receive 600 mg of NAC or placebo twice daily on the day prior to and the day of the procedure. A lower proportion of patients randomized to NAC developed CIAKI than did patients randomized to placebo (2% versus 21%, P= .01). Similar results were reported by Diaz-Sandoval et al.,[182] who randomized 54 patients undergoing coronary angiography to NAC or placebo and also demonstrated a markedly lower incidence of CIAKI among patients who had received NAC (8% versus 45%, P= .005). Similarly, Shyu and colleagues[183] randomized 121 patients with significant baseline CKD (SCr ≥2.8 mg per deciliter) who were undergoing coronary intervention to NAC or placebo and also demonstrated a lower rate of CIAKI among patients who had received NAC (3% versus 25%; P< .001). These initial positive findings were followed by multiple additional trials that yielded highly conflicting results (Table 33.5).[86,102,161,181–203] In an effort to reconcile the incongruent clinical trial findings, multiple meta-analyses have analyzed the pooled results of these studies.[204–221] In one of the larger meta-analyses, Kelly and colleagues[171] included 26 trials encompassing 3,393 patients and found a 38% reduction in the risk of CIAKI associated with NAC (RR=0.62; 95% CI: 0.44 to 0.88). Although the investigators reported heterogeneity among the pooled studies, they found no evidence of publication bias. In another meta-analysis of 22 trials that included 2,746 patients published nearly simultaneously with the Kelly study, Gonzalez et al.[211] found significant heterogeneity among the trials. Using cluster analyses, the investigators divided the trials into two groups that had minimal heterogeneity. One cluster included trials in which NAC was found to be protective, and was characterized by an observed decline in SCr following contrast administration among patients treated with NAC, whereas the other cluster consisted of studies in which there was no fall in SCr in the NAC-treated patients and no overall benefit for the prevention of CIAKI. The investigators concluded that NAC was not effective for the prevention of CIAKI. Trevidi and colleagues[219] performed a meta-analysis of 16 studies, encompassing 1,677 patients, in which the dose of NAC was greater than 1,200 mg. In this analysis of "high-dose" therapy, NAC was associated with an odds ratio for the development of CIAKI of 0.46 (95% CI: 0.33 to 0.63). Thus, the conclusions of multiple meta-analyses of NAC are as contrasting and inconclusive as the findings of the primary clinical trials. Since the publication of these meta-analyses, Berwanger and colleagues[221a] reported the results of a randomized clinical trial comparing NAC to placebo in 2,308 patients undergoing angiography. The study did not demonstrate a benefit to NAC; however, only a small minority of trial participants had abnormal baseline kidney function, rendering the study population at low overall risk for CIAKI. However, given the low cost and minimal risk associated with oral NAC, the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury suggests its use in conjunction with isotonic crystalloid administration in patients at high risk for CIAKI.[222]

Additional Pharmacologic Agents

A series of additional pharmacologic agents have been investigated for their capacity to prevent the development of CIAKI. Wang et al.[223] randomized 158 patients undergoing coronary angiography to receive the nonselective endothelin (ET_A/ET_B) receptor antagonist bosentan or placebo. The mean increase in SCr 48 hours following angiography was significantly higher in the treatment group than in the placebo group (0.7 ± 0.7 versus 0.4 ± 0.6 mg per deciliter; P= .002), and the incidence of CIAKI was also higher among patients who received bosentan (56% versus 29%; P= .002). Katholi et al.[224] studied the effect of allopurinol in 39 patients with mild kidney disease. Allopurinol-treated patients with low plasma Mg^{2+} had somewhat milder decrements in creatinine clearance following contrast administration than control, hypomagnesemic patients (33% versus 79%,



respectively). However, in normomagnesemic patients, allopurinol did not affect the decline in kidney function. At present, the data are insufficient to establish any role for either of these agents for the prevention of CIAKI.

Intravenous Fluids for the Prevention of Contrast-Induced Acute Kidney Injury

The principal intervention that has been shown to be effective in reducing the risk for CIAKI is the administration of IV fluid.[84,87,91,225] Intravascular volume expansion is believed to have two physiologic effects that protect against the development of CIAKI. First, expansion of the intravascular space may attenuate the vasoconstrictive effect of contrast by means of suppressing vasopressin, inhibiting the renin–angiotensin–aldosterone system, and inducing the synthesis of vasodilatory renal prostaglandins.[226] Second, intravascular volume expansion may counteract the direct toxicity of iodinated contrast on tubular epithelial cells by decreasing the concentration and viscosity of contrast media in the tubular lumen.[226]

Early animal studies provided preliminary, albeit indirect, support for the benefit of intravascular volume expansion. Larson and colleagues[227] evaluated the degree and duration of contrast-induced vasoconstriction in sodium-depleted and sodium-replete dogs. Renal blood flow was reduced to a considerably greater degree (42.2% versus 12.2%) and for a longer period of time (343 seconds versus 147 seconds, $P < .01$) in sodium-depleted compared to sodium-replete dogs. Yoshioka et al.[43] assessed the impact of contrast administration on glomerular filtration in water-deprived and non-water-deprived rats and in water-deprived rats that received sodium chloride. An early (24 hours) and a sustained reduction in glomerular filtration was observed in water-deprived rats, whereas non-water-deprived rats and water-deprived rats that were administered sodium chloride experienced no reduction in GFR. Finally, Erley et al.[20] studied the impact of contrast on rats, including a group that was administered L-NAME, which contributes to a nitric oxide–depleted state. Among volume-depleted rats administered L-NAME, iodinated contrast reduced GFR (1.25 mL per minute to 0.89 mL per minute, $P < .05$). This effect was diminished in volume-depleted rats in the absence of L-NAME and was absent in volume-expanded L-NAME rats. Collectively, these studies highlight the association of volume-depleted states with an increased risk for CIAKI.

Human data on the benefit of intravascular volume expansion derived initially from observational studies. In 1981, Eisenberg et al.[88] reported the results of a single arm observational study of 537 patients who received intravascular volume expansion with an average volume of 550 mL of isotonic NaCl during each hour of angiography. CIAKI did not develop in any study patients compared to 12% of patients in a historic cohort who had received 80 mL per hour of 5% dextrose in water. Subsequently, Kerstein and Puyau[228] studied the effect of 0.5% NaCl in 5% dextrose administered prior to and following noncoronary angiography in 150 patients. Study participants with baseline renal insufficiency received an additional 300 to 500 mL of IV fluid and 20 to 40 mg of furosemide prior to the procedure. None of the patients were observed to have developed CIAKI. However, this study had no control group and enrolled a relatively low-risk patient population. Nonetheless, these observational studies provided early insights into the potential benefit of IV volume expansion for the prevention of CIAKI.

Several randomized clinical trials over the past 2 decades have examined the effect of IV volume expansion in the prevention of CIAKI, and provide preliminary data on the comparative effects of IV fluid composition. In 1994, Solomon et al.[84] published the results of a three-armed clinical trial of patients undergoing coronary angiography comparing prophylactic IV 0.45% saline to prophylactic IV 0.45% saline with 25 g of IV mannitol or 80 mg of IV furosemide. Overall, 11% of patients who received IV fluid alone developed CIAKI compared to 28% of patients who also received mannitol and 40% in those who also received furosemide. The absence of a control group who received no IV fluid precluded a conclusion that IV fluids were definitively beneficial. However, Trivedi et al.[225] subsequently confirmed the benefit of IV volume expansion in a clinical trial that enrolled patients undergoing nonemergent coronary angiography. Patients were randomized to receive either IV isotonic sodium chloride for 12 hours prior to and 12 hours following angiography or unrestricted oral fluids. The study was stopped by the safety monitor after just 53 patients had been enrolled because the rate of CIAKI was markedly lower in the IV saline group than in the oral fluid group (3.7% versus 34.6%; $P = .005$).

Mueller et al.[87] compared the effects of hypotonic and isotonic saline in over 1,600 low-risk patients undergoing coronary angiography who were randomized to receive periprocedural IV 0.45% saline or 0.9% saline. The overall rate of CIAKI was greater in patients who received half-isotonic saline compared to those who were administered isotonic saline (2% versus 0.7%, $P = .04$). Notwithstanding the decidedly low-risk profile of this study's patient population, this trial has served as the justification for the widespread belief that isotonic saline is superior to hypotonic saline for the prevention of CIAKI.

Recent research on IV fluid composition has focused on the comparison of isotonic sodium bicarbonate with isotonic sodium chloride. The belief that the generation of ROS in the kidney, which is augmented by acidic urine and contributes to renal tubular cell injury, and that the alkalization of the urine with the administration of IV bicarbonate attenuates this process, forms the physiologic framework for clinical studies comparing isotonic IV fluids that differ in respect to the anion component (HCO_3^- v. Cl^-). Merten and colleagues[91] conducted the initial clinical trial comparing the benefits of isotonic bicarbonate to isotonic saline. One hundred and nineteen patients were randomized to one of two fluid regimens; 8 of 59 patients (13.6%) randomized to receive isotonic saline developed CIAKI as compared to

33.6 Clinical Trials of Intravenous Isotonic Sodium Bicarbonate and Saline for the Prevention of Contrast-Induced Acute Kidney Injury

Authors	# Study Patients	Diabetes (%)	Baseline SCr (mg/dL)	Definition of CIAKI	% CIAKI Bicarbonate	% CIAKI Saline
Positive studies						
Briguori et al.	219	52%	2.0	↑ SCr ≥25%	1.9%	9.9%
Masuda et al.	59	31%	1.3	↑ SCr ≥0.5 mg/dL or ≥25%	6.6%	34.5%
Merten et al.	119	48%	1.7–1.9	↑ SCr ≥25%	1.7%	13.6%
Ozcan et al.	176	45%	1.4	↑ SCr ≥0.5 mg/dL or ≥25%	4.2%	16.6%
Pakfetrat et al.	192	30%	1.1	[a]	4.2%	12.5%
Recio-Mayoral et al.	111	30%	1.0	↑ SCr ≥0.5 mg/dL	1.8%	21.8%
Negative studies						
Adolph et al.	145	34%	1.5–1.6	↑ SCr ≥0.5 mg/dL or ≥25%	4.2%	2.7%
Brar et al.	353	44%	1.5	↓ eGFR ≥25%	13.3%	14.6%
Maioli et al.	502	24%	1.2	↑ SCr ≥0.5 mg/dL	10%	11.5%
Vasheghani et al.[b]	265	22%	1.6–1.6	↑ SCr ≥0.5 mg/dL or ≥25%	7.4%	5.9%

[a]Three definitions of CIAKI assessed; differences between bicarbonate and saline based on ↑ SCr ≥0.3 mg/dL.
[b]Bicarbonate administered as 75 mL of 8.4% sodium bicarbonate added to 1 L isotonic saline.
CIAKI, contrast-induced acute kidney injury; SCr, serum creatinine; eGFR, estimated glomerular filtration rate.

1 of 60 patients randomized to receive isotonic bicarbonate (P = .02). Subsequently, at least nine other clinical trials comparing IV isotonic bicarbonate with IV isotonic saline have been published, slightly more than half of which have demonstrated a lower incidence of CIAKI with bicarbonate (Table 33.6).[103,104,144, 229–234]

In response to the disparate results of these clinical trials, nearly a dozen systematic reviews and meta-analyses comparing the effectiveness of bicarbonate and saline have been performed.[207,229,230,235–242] Zoungas et al.[235] pooled the findings of 23 published and unpublished trials that included more than 3,500 patients and found a reduction in the risk of CIAKI with IV bicarbonate compared to saline (RR = 0.62; 95% CI: 0.45 to 0.86). However, they also noted significant study heterogeneity and evidence of publication bias. Sensitivity analyses restricted to published studies demonstrated a significantly lower risk of CIAKI with bicarbonate (RR = 0.43, 95% CI: 0.25 to 0.75), whereas analyses focused on unpublished trials failed to demonstrate such a benefit (RR = 0.78, 95% CI: 0.52 to 1.17) (Fig. 33.4). Another recent meta-analysis by Kunadian et al.[242] included data from seven published trials that also demonstrated a lower risk of CIAKI with IV isotonic bicarbonate (OR = 0.33, 95% CI: 0.16 to 0.69). However, the investigators also reported study heterogeneity and publication bias, which impact the interpretation of benefit. Thus, based on the current level of evidence, there is considerable uncertainty regarding the true benefit of isotonic bicarbonate as compared to saline. Given this uncertainty, and the potential for medical errors in compounding isotonic bicarbonate solutions in the absence of commercially available preparations, the KDIGO Clinical Practice Guideline for Acute Kidney Injury did not make a recommendation favoring either isotonic bicarbonate or saline.[222]

Current Recommendations for the Prevention of Contrast-Induced Acute Kidney Injury

Once a determination has been made that a procedure using intravascular iodinated contrast is required in a patient at increased risk for CIAKI, steps to attenuate that risk should be implemented based on our current understanding of the preventive modalities discussed previously. Modifiable patient-related risk factors should be addressed. Overt intravascular volume depletion should be corrected in all patients prior to the administration of iodinated radiocontrast. Based on our understanding of the pathophysiology of CIAKI and data from animal studies, nonselective nonsteroidal anti-inflammatory medications and selective cyclooxygenase-2

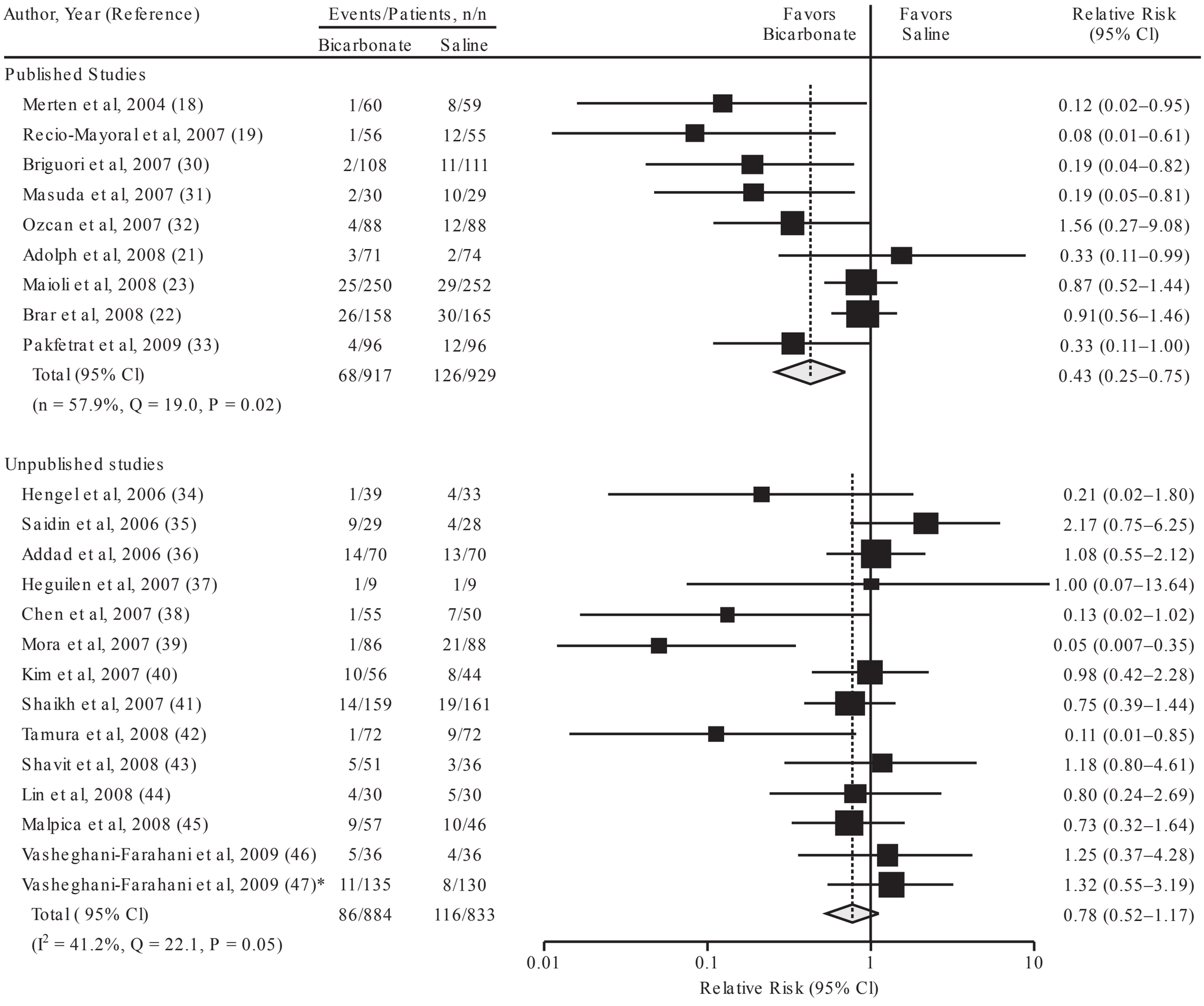


FIGURE 33.4 The forest plot of relative risks for contrast-induced nephropathy from 23 studies. Reprinted with permission from Zoungas S, et al. Systematic review: sodium bicarbonate treatment regimens for the prevention of contrast-induced nephropathy. *Ann Intern Med*. 2009;151(9):631–638.

inhibitors, which inhibit the production of vasodilatory prostaglandins, should be discontinued at least 24 hours prior to contrast administration and held until confirmation has been made that CIAKI has not developed. Additionally, modifiable procedure-related risk factors should be considered. The volume of contrast administered should be limited to the minimum required dose that does not compromise diagnostic accuracy. Iso-osmolal or nonionic, low-osmolality contrast other than iohexol or ioxaglate should be employed. IV fluids consisting of isotonic sodium chloride or sodium bicarbonate should be administered prior to and following the administration of contrast. Among inpatients undergoing nonemergent procedures, an IV fluid regimen of 1 mL per kilogram per hour for 6 to 12 hours preceding and 6 to 12 hours following the administration of iodinated contrast has been most rigorously validated and should be considered. For high-risk patients undergoing elective outpatient procedures or urgent inpatient studies, comparable volumes of isotonic saline or sodium bicarbonate, administered over 1 to 3 hours prior to and 4 to 6 hours following contrast administration, may provide a more practical alternative, although this approach has not been formally compared to more prolonged periods of IV fluid administration. Although IV volume expansion would be inappropriate in the setting of acute decompensated heart failure, fluids should not be withheld merely because of a history of heart failure or low cardiac ejection fraction; rather, cautious IV fluid administration should be accompanied by close monitoring for adverse pulmonary effects.

Although the role of N-acetylcysteine for the prevention of CIAKI remains unclear, this antioxidant agent has minimal toxicity and is inexpensive when administered orally. Certain studies that examined the effect of the dose demonstrated a potential benefit to a higher dose NAC. Therefore, oral NAC administered at a dose of 1,200 mg twice daily prior to and following contrast administration should be considered. However, NAC should not be used in lieu of

more effective proven preventative interventions such as isotonic IV fluids. There is currently no role for prophylactic renal replacement therapy for the prevention of CIAKI. Diuretics, dopamine, and fenoldopam have been shown to be ineffective and, in certain instances, may be deleterious and therefore should not be used. Until further evidence from large, methodologically rigorous clinical trials is available, atrial natriuretic peptides, theophylline, statins, and prostaglandin analogs are not recommended. There is currently no role for allopurinol or endothelin receptor antagonists.

There is little evidence that lactic acidosis develops in patients taking metformin who have normal renal function before receiving iodinated contrast. Concern arises for the development of metformin-induced lactic acidosis when diabetics with other risk factors for CIAKI develop renal injury and delayed excretion of the drug occurs. Therefore, current recommendations call for the discontinuation of metformin in patients at an increased risk of CIAKI with the reinstitution of the medication once postprocedure renal function has been confirmed to be stable. Additional steps to maintain serum glucose concentrations while metformin is being held may be necessary. There are no data to support the discontinuation of diuretics at the time of contrast administration. Small studies investigating the impact of discontinuing angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor blockers prior to contrast administration have been conflicting.[243,244] Rosenstock and colleagues[244] randomized 220 patients who were on chronic ACE inhibitor or angiotensin receptor blocker therapy and who were scheduled to undergo coronary angiography to either continuation or discontinuation of renin-angiotensin-aldosterone system (RAAS) blockade. The incidence of CIAKI was 6.2% in the 113 patients who continued RAAS blockade and was 3.7% in the 107 patients who discontinued RAAS blockade (P= .66) as compared to 3.3% in 63 matched patients who were naïve to RAAS blockade. Therefore, no evidence-based recommendations to discontinue such agents can be made.

In patients at risk for CIAKI, the monitoring of renal function following the administration of iodinated radiocontrast is essential. Renal function should be measured between 48 and 72 hours following the procedure to assess for the development of renal injury and to permit timely implementation of supportive care.

CONCLUSION

Acute kidney injury resulting from the administration of intravascular iodinated contrast media is a leading cause of nephrotoxic ATN. It develops almost exclusively in patients with underlying risk factors. With multiple studies demonstrating an association of CIAKI with serious adverse short- and long-term outcomes, a careful evaluation of each patient who is to receive intravascular radiocontrast for the presence of underlying risk factors for CIAKI and implementation of evidence-based preventive measures will facilitate the prevention of this iatrogenic condition.

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CHAPTER

34 Nephrotoxicity Secondary to Environmental Agents, Heavy Metals, Drug Abuse, and Lithium

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Environmental kidney diseases are a consequence of occupational exposure, a particular form of environmental disease. Recognized chronic occupational renal diseases include those caused by exposure to heavy metals, organic solvents (aliphatic, aromatic, and halogenated hydrocarbons), and silica. Minamata disease from mercury and Itai-Itai disease from cadmium are two environmental diseases caused by industrial elements. Another disease is Balkan nephritis presumed to be of environmental origin and the nephropathy caused by the ingestion of germanium compounds.

Several heavy metals are generally recognized as nephrotoxic following environmental or occupational exposure, including: lead, cadmium, mercury, uranium, chromium, copper, and arsenic. However, chronic renal failure has been described only for lead, mercury, cadmium, uranium, and arsenic. Therapeutic forms of platinum, gold, lithium, and bismuth may also induce kidney damage and these aspects are explored in other chapters of this book. Other heavy metals with potentially nephrotoxic effects are barium, cobalt, manganese, nickel, silver, thallium, thorium, tin, and vanadium but there is no definitive evidence they can actually lead to renal disease.

URINARY BIOMARKERS

Urinary proteins and biochemical markers were associated with toxic renal injury. Urinary biomarkers may reflect specific sites of renal injury: (1) low molecular weight proteins and intracellular enzymes—proximal tubule damage; (2) Tamm-Horsfall glycoprotein and kallikrein—distal tubule injury; (3) high molecular weight proteins—increased glomerular permeability (if >200 mg per g creatinine); and (4) biochemical markers—eicosanoids suggesting vascular injury.

A group of urinary markers (human intestinal alkaline phosphatase [HIAP], total nonspecific alkaline phosphatase [TNAP], N-acetyl-β-D-glucosaminidase [NAG], retinol binding protein [RBP], Tamm-Horsfall glycoprotein [THG], β_2-microglobulin, microalbumin, thromboxane B_2 [TBX_2], and three prostaglandins [prostaglandin E_2, PGE_2; prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$; 6-keto-prostaglandin $F_{1\alpha}$, 6-keto-$PGF_{1\alpha}$]) are detailed to demonstrate the differentiation of the toxic nephropathies (Table 34.1).[1–5]

The excretion patterns represent occupational exposure levels indicated by the specified mean blood or urine concentrations. Mercury and cadmium exposure[3–5] is associated with the isoenzyme HIAP, a sensitive and specific indicator of injury to the S3 segment of the proximal tubule. Total nonspecific alkaline phosphatase is increased after perchloroethylene exposure[6,7] and NAG and RBP are elevated after cadmium exposure.[3,8] The urinary eicosanoids PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ seem to be associated to the development of hypertension and injury to the glomeruli or renal medulla. Also, low levels of urinary albumin can also express proximal tubular dysfunction and the failure to reabsorb or metabolize albumin that passes through the glomerular filter.

The ability of these urinary markers to discriminate among the diverse nephrotoxins enhances with increasing exposure levels. Urinary markers should be collected in fasting fresh voided specimens (spot urines) at 8 AM, and expressed relative to the creatinine concentration. The specificity of tubular injury decreases in the presence of renal damage.

LEAD NEPHROPATHY

The first description of lead nephrotoxicity was made by Lancereaux in 1862, who reported a patient with saturnine (lead-induced) gout with kidneys showing interstitial nephritis at postmortem examination. Nevertheless, the demonstration of lead nephrotoxicity has presented some issues. There are difficulties proving the connection between late sequelae of chronic absorption to relatively low levels of lead, and distinguishing between glomerular and extraglomerular renal disease.[9] Another difficult aspect is how to separate the transient Fanconi syndrome of acute childhood lead poisoning from the chronic interstitial nephritis characteristic of lead nephropathy in adults. Finally, one confounding aspect is the differentiation of late complications of excessive lead

34.1 Urinary Markers in Toxic Nephropathies—European Cooperative Study

	HIAP	TNAP	NAG	RBP	THG	β_2-M	mAlb	TXB_2	PG
Pb	−	−	+	−	−	−	−	++	6F−
Cd	+++	−	+++	+	−	+	++	−	6F++
Hg	+++	+	+	−	−	−	−	−	F−
					−			−	E−
PCE	−	+++	−	−	±	−	+		F−
									E–

HIAP, human intestinal alkaline phosphatase; TNAP, total nonspecific alkaline phosphatase; NAG, N-acetyl-β-D-glucosaminidase; RBP, retinol binding protein; THG, Tamm-Horsfall glycoprotein; β_2-M, β_2-microglobulin; mAlb, microalbumin; TXB_2, thromboxane B_2; PG, prostaglandin; Pb, lead; Cd, cadmium; Hg, mercury; PCE, perchloroethylene; 6F, 6-keto-$PGF_{1\alpha}$; F, $PGF2_{1\alpha}$; E, PGE_2.

absorption and gout and hypertension renal lesions, which are other important causes of renal disease.

Diagnosis

The mainstay of laboratory diagnosis is the blood lead concentration, which is usually over 60 μg per dL, although recent evidence of lead-induced organ damage occurred with blood levels over 10 μg per dL.[10] Blood lead concentrations usually decrease significantly within 4 weeks of removal from exposure making the blood lead concentration relatively insensitive to cumulative body stores acquired over years. Around 95% of the body stores of lead are accumulated in the bone with a mean residence time of approximating 20 years[11] and cumulative past lead absorption is best assessed by the calcium disodium edetate ($CaNa_2EDTA$) lead-mobilization test.[12]

The ethylenediamine tetraacetic acid (EDTA) test is performed in adults by parenteral administration of 1 to 3 g (intravenous or intramuscular) of $CaNa_2EDTA$ over 4 to 12 hours with subsequent collection of 24-hour urine samples over 1 to 4 days. The intramuscular administration of 2 g of $CaNa_2EDTA$ (1 g of EDTA mixed with local anesthetic in each of two injections, 12 hours apart) seems to be a better option of performing the chelation test because it has been well standardized in both normal subjects and patients with renal failure.[13–18] In the presence of renal damage (serum creatinine > 1.5 mg per dL), urinary excretion of lead chelate should be extended to at least 3 consecutive days and the adequacy of collection checked by simultaneous measurement of urinary creatinine excretion (1.3 g of creatinine per day is an acceptable lower limit in normal adult males). Adults without undue prior lead absorption excrete up to 650 μg of lead-chelate in the urine.

Chelatable lead correlates well with bone lead[19,20] which reflects cumulative body lead stores. Bone lead concentrations can be accurately diagnosed by a new noninvasive technique, the in vivo tibial K X-ray fluorescence.[21,22]

Acute Lead Nephropathy

In acute lead nephropathy, a proximal tubule reabsorptive defect characterized by aminoaciduria, phosphaturia, and glycosuria (Fanconi syndrome) is observed,[23] usually in the presence of blood lead levels in excess of 150 μg per dL. An increase in urinary NAG is observed, which correlates positively with the blood lead concentration.[24] Tubular dysfunction is often reversed after chelation therapy is initiated to treat the more dangerous encephalopathy. Acute lead nephropathy is associated with acid-fast intranuclear inclusions in proximal tubule epithelial cells[25] which are a lead–protein complex, also observed in the urinary sediment.[25] These inclusions can occur in other organs such as in liver, neural tissue, and osteoclasts. Morphologic and functional defects in mitochondria are also observed in acute poisoning.

Chronic Lead Nephropathy

Chronic lead nephropathy is the slowly progressive interstitial nephritis observed in adults after prolonged lead exposure. The disease is more frequently recognized in lead workers after long periods (decades) of exposure but other groups have been described such as young adults who sustained acute childhood lead poisoning,[26] illicit whiskey ("moonshine") consumers, U.S. armed service veterans suffering from renal failure attributed to gout or essential hypertension,[13,14] and sporadic case reports such as geophagia,[27] Asian folk remedies, and cosmetics.

Chronic lead nephropathy causes chronic renal damage less responsive to chelating agents. There is evidence of associated functional impairment such as inhibition of both the renin-angiotensin system and Na^+-K^+-ATPase,[17,28] and these effects may be changed by chelation therapy. This explains why some exposed individuals can restore previous reductions in glomerular filtration rate (GFR) after long-term low-dose chelation therapy (1 g of $CaNa_2EDTA$ with local anesthetic three times weekly until the chelation test returns to normal). Proteinuria and glycosuria are initially absent and an increase in TXB_2, and a decrease in PGE_2 and 6-keto-$PGF_1\alpha$, in the urine is observed.[2,24]

Renal biopsies in chronic lead nephropathy show nonspecific tubular atrophy and interstitial fibrosis with minimal inflammatory response as well as mitochondrial swelling, loss of cristae, and increased lysosomal dense bodies within proximal tubule cells (Fig. 34.1).[15,19] Arteriolar changes indistinguishable from nephrosclerosis are found, often in the absence of clinical hypertension. Intranuclear inclusion bodies are often absent when the renal disease is long-standing or following the administration of chelating agents. Clumped chromatin and nuclear invaginations of cytoplasmic contents may be found even in the absence of intranuclear inclusions. Morphologic alterations are minimal in glomeruli until the reduction in GFR is advanced. This helps explain the association between lead toxicity and hypertension.

The association between lead and gout nephropathy has long been described.[29,30] Hyperuricemia and gout are common among individuals with excessive exposure to lead, apparently the result of decreased excretion and increased production of uric acid, and half of uremic patients with lead nephropathy have clinical gout.[26] There is substantial evidence that renal failure in gout is sometimes secondary to overt or unsuspected lead poisoning. In Queensland, Australia, as many as 80% of gout patients with renal failure have elevated EDTA lead-mobilization tests.[26] In New Jersey chelatable lead was found to be significantly greater among gout patients with renal failure than among gout patients with normal renal function.[13] Therefore, unrecognized lead poisoning can be an explanation for renal failure in some gout patients with no evidence of urinary calculi or intratubular uric acid deposition disease.

The initial renal injury from lead seems to be in the microvascular endothelium.[31] Although the mechanism of injury is not completely clear, it is well known that metabolism of lead is similar to that of calcium and other cations. Also, lead interacts with vasoactive substances that may modulate blood pressure and induce endothelial injury.[32,33] These aspects help to explain the association between lead toxicity and hypertension as suggested in several reports.[34,35] Some patients presenting as "essential hypertension with nephrosclerosis" may have evidence of lead nephropathy by the EDTA lead-mobilization test.[14] Mortality from hypertensive cardiovascular disease is more frequent among lead workers than the general population.[36] In these patients, lead seems to contribute to hypertension particularly in the presence of renal dysfunction.[17]

Treatment

Lead nephropathy is one of the few renal diseases that is preventable and potentially reversible by judicious use of chelation therapy.[16,37,38] However, there is no evidence that such therapy reverses established interstitial nephritis especially if serum creatinine concentration exceeds 3 mg per dL.[39] Reports of partial remissions may be the reversal of acute poisoning superimposed on chronic lead nephropathy.

Before chelation therapy is undertaken, it may be necessary to perform the EDTA lead-mobilization test and other possible causes of renal disease should be excluded. Long-term, low-dose EDTA therapy should be undertaken until an endpoint is achieved, such as reversion of the EDTA test to normal and restoration of renal function. The cumulative nephrotoxicity of prolonged EDTA therapy in patients with advanced renal failure is unknown. Reports of deterioration of renal function after $CaNa_2EDTA$ therapy have been described and treated patients warrant careful follow-up.[40,41]

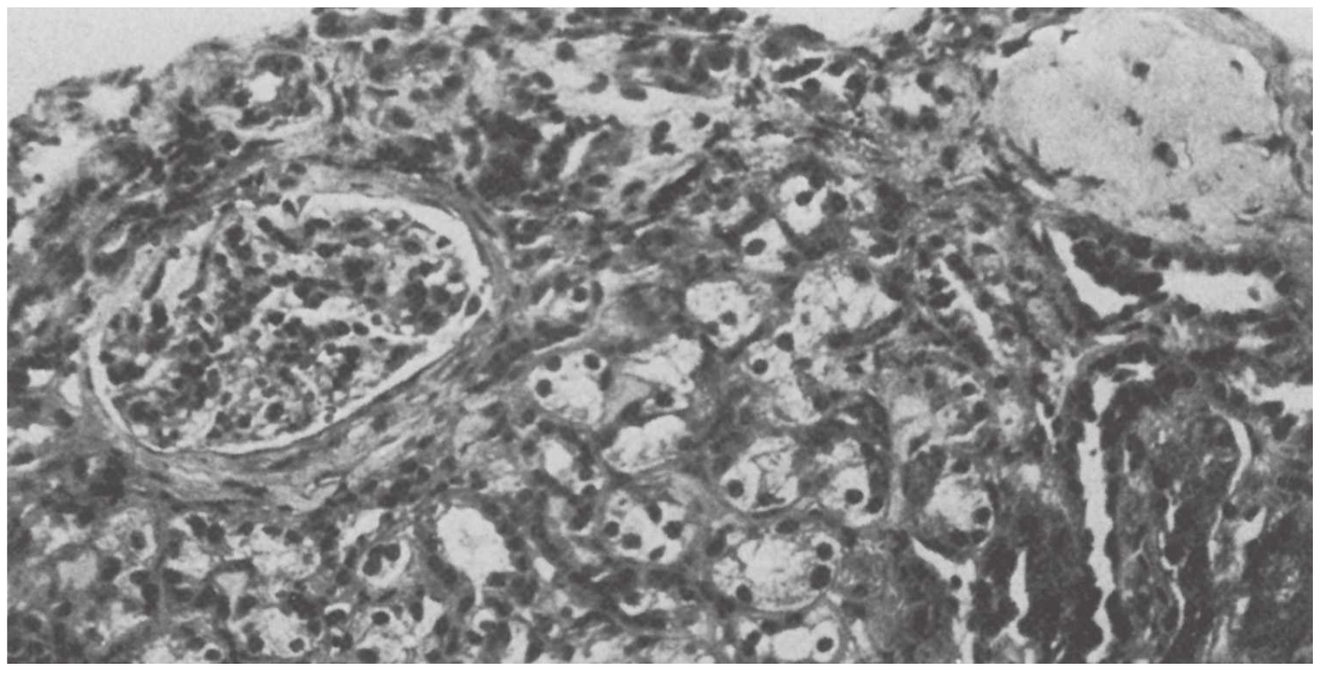

FIGURE 34.1 Renal biopsy obtained from a 28-year-old man who had prepared lead solder for 5 years. His ^{125}I-iothalamate clearance was 52 mL/min/1.73 m^2; hemoglobin, 9.6 g per dL; uric acid, 13.2 mg per dL; and blood lead, 48 μg per dL when he was initially seen. Lead-chelate excretion following 2 g of $CaNa_2EDTA$ intramuscularly was 5.2 mg for 24 hours. Light microscopy shows periglomerular fibrosis, a sclerotic glomerulus, and tubular atrophy. (Trichrome stain; magnification $\times$304.) (From Wedeen RP, Maesaka JK, Weiner B, et al. Occupational lead nephropathy. *Am J Med.* 1975;59:630, with permission.)

CADMIUM NEPHROPATHY

Several compounds containing cadmium are widely used in the manufacturing of pigments, plastics, glass, metal alloys, and electrical equipment. Acute absorption of small quantities as 10 mg of dust or fumes may cause severe gastrointestinal symptoms and fatal pulmonary edema, after a delay of 8 to 24 hours.[42] Chronic low dose exposure leads to slowly progressive emphysema, anosmia, and proximal tubular reabsorption defects characterized by low molecular weight proteinuria, enzymuria, aminoaciduria, and renal glycosuria.[43–46] Hypercalciuria (with normocalcemia), phosphaturia, and distal renal tubular acidosis result in clinically important osteomalacia, pseudofractures, and urinary tract stones.[47,48] Proximal tubular dysfunction can progress to chronic renal failure over years.[49,50]

Metabolism

Nonoccupationally exposed individuals can accumulate cadmium through food and cigarettes. The biologic half-life of cadmium in humans exceeds 15 years, and one third of the total body stores (10 to 20 mg) are accumulated in the kidneys.

Absorbed cadmium is initially sequestered in liver and kidney, where it is bound to metallothionein, a cysteine-rich apoprotein.[42,43] The cadmium–thionein complex is filtered at the glomerulus, taken up in the proximal tubule by endocytosis, and transferred to lysosomes, where it is rapidly degraded. Most of the cadmium accumulated in the proximal tubules is bound to protein and after a "critical concentration" of 200 μg per g of renal cortex is reached, renal effects become evident. Normal urinary cadmium excretion is usually under 2 μg per day and values over 10 μg per day are associated with cadmium accumulation. Urinary cadmium excretion in excess of 30 μg per day correlates with significant abnormalities of proximal tubular function.[51] Although blood cadmium concentration is less reliable as an indicator of health effects and cumulative absorption, blood levels greater than 1 μg per dL are considered evidence of excessive exposure.

β-2 microglobulin has been the most extensively examined urinary protein in cadmium nephropathy. Its excretion is an early renal effect of cadmium[42] but, considering its instability in acid urine, measurement of urinary RBP or NAG is probably more reliable.[3,8,24,51] Low level of albumin and transferrin are observed in the urine of cadmium workers with low molecular weight proteinuria and enzymuria,[3,52] but it is not clear whether this means glomerular injury or impaired tubular reabsorption. Proteinuria in cadmium workers rarely exceeds a few hundred milligrams per day and does not approach nephrotic levels.

Calcium Wasting

The leading feature of cadmium tubular dysfunction is increased calcium excretion.[53] Although osteomalacia is uncommon in cadmium workers, it can be observed, associated with diminished renal tubular reabsorption of calcium and phosphate, elevated circulating parathormone levels, and reduced hydroxylation of vitamin D metabolites.[54,55] Urinary calculi have been reported in up to 40% of those subjected to industrial exposure and ureteral colic is more likely to be the cadmium worker's chief complaint.[43,47,56]

Itai-Itai Disease

Itai-Itai disease is a painful bone condition associated with pseudofractures caused by cadmium-induced renal calcium wasting, first described in Japan. The origin is attributed to local contamination of food staples by river water polluted with industrial effluents, particularly cadmium. The syndrome afflicts postmenopausal, multiparous women presenting with reduced GFR, anemia, lymphopenia, and hypotension as well as osteomalacia. They also exhibit a waddling gait, short stature, anemia, glucosuria, and elevated serum alkaline phosphatase levels. β_2-microglobulin urinary excretion exceeds the normal maximum (1 mg per g of creatinine) by almost 100-fold, predicting the later development of renal failure. The renal damage progresses even after exposure has ceased.

Chronic Interstitial Nephritis

Although the role of cadmium in the induction of chronic interstitial nephritis has been controversial, analysis of postmortem tissue or renal biopsy specimens of exposed individuals was able to find tubulointerstitial nephritis.[57] These findings associated with recent epidemiologic studies in the United States[56] and Belgium,[58,59] and the long-term follow-up of Itai-Itai disease in Japan,[54] have consolidated cadmium as a cause of chronic interstitial nephritis. These studies presented some important evidence: there was an association between cumulative cadmium exposure and the later increase in serum creatinine after a latent period of several decades; and among exposed workers, there was an increase in serum creatinine concentrations over 5 years accompanied by an important increase in mean urinary β_2-microglobulin and loss of glomerular filtration (around 30 mL per minute, 30 times the predicted loss of kidney function for the group).

Diagnosis

Cadmium nephropathy is usually diagnosed based in a history of exposure associated with laboratory tests indicative of proximal tubule injury (e.g., increased excretion of urinary biomarkers, hypercalciuria, or renal glycosuria). Cadmium concentration over 10 μg per g of creatinine confirms the diagnosis. Assessment of renal and hepatic accumulation of cadmium by neutron-activation analysis has been explored and organ cadmium content correlates well with tissue and urinary cadmium levels and β_2-microglobulin excretion. When the hepatic cadmium level exceeds about 60 ppm, and renal cortical content exceeds 200 ppm (20 mg per kidney), tubular proteinuria is likely to occur. The diagnostic value of neutron-activation analysis of kidney is decreased in uremic patients because renal cadmium concentration tends to fall with the development of renal failure.[59]

Treatment

The chelating agent $CaNa_2EDTA$ has little effect after cadmium has been complexed with metallothionein[60] and it is not usually recommended. Progression of renal disease may occur despite removal from exposure.[54] Osteomalacia may be controlled by calcium and vitamin D replacement[51] and urinary tract stones are not a contraindication to such therapy.

MERCURY

The toxicity of mercury depends on both its chemical form and the route of absorption. Elemental mercury produces neurologic disease and even death but it does not cause nephrologic damage. However, the mercuric salt corrosive sublimate ($HgCl_2$) is the most nephrotoxic form which is accumulated in the cells of proximal tubules inducing acute tubular necrosis (ATN).[61] This mercurial compound binds avidly to sulfhydryl groups in circulating proteins and amino acids as well as intracellular glutathione, cysteine, and metallothionein. Mercury accumulates in the pars recta of proximal tubules which is accomplished by transport primarily from the luminal side of mercury bound to amino acids or proteins. Mercury-ligand complexes reach lysosomes by endocytosis[62] with subsequent release into the cytosol by intralysosomal enzymatic degradation.

Diagnosis

The diagnosis of mercury-induced renal disease is usually dependent on known exposure in the presence of renal dysfunction. Although blood mercury levels over 3 μg per dL or urine levels above 50 μg per g of creatinine are considered abnormal, the correlation of blood and urine concentrations with renal disease is poor.[63] Mercury exposure is associated with increased HIAP urinary excretion but little increase in TNAP, NAG, RBP, THG, β_2 microglobulin, or microalbuminuria (Table 34.1).[1,5] There is no evidence that enzymuria from mercury exposure predicts the development of renal failure.

Ingestion of as little as 0.5 g of $HgCl_2$ produces ATN in humans. Initially, the clinical picture is dominated by gastrointestinal symptoms including erosive gastritis with hematemesis and melena. Diuresis should be induced by hydration, mannitol, and furosemide to prevent the development of oliguric acute renal failure. Persisting oliguria in the face of adequate therapy indicates renal parenchymal damage. An elevated urinary sodium concentration (>40 mEq per L) and diminished concentrating capacity (UOsm <450 mOsm per L) in an acutely oliguric patient with adequate therapy confirms the diagnosis of ATN. Acute oliguric renal failure may rapidly lead to death unless dialysis is provided.

Histologic examination of the kidneys reveals necrosis of the proximal tubules, particularly the pars recta. Tubular necrosis extends to more proximal segments after larger doses although the extent of damage to individual nephrons is highly variable.[64]

Oliguria is replaced by polyuria, during the recovery phase, whereas the GFR is still low. Urine flow rates may double daily, achieving a maximum of 5 L per day whereas the serum creatinine continues to rise. Spontaneous regeneration of tubular epithelium occurs with subsequent recovery although dystrophic calcification of necrotic tubules may limit restoration of function, and the kidneys may show residual interstitial nephritis.[62] The process from acute oliguria through polyuria and recovery may last from a few days to many months.

Nephrotic Syndrome

Although there are some sporadic case reports of nephrotic syndrome following exposure to elemental or organic mercury since the 20th century,[65] the causal relationship of mercury exposure to proteinuria and the nephrotic syndrome has been controversial.[66] The dose-response is unpredictable and also the etiology of nephrotic syndrome unrelated to mercury is rarely known.

Renal biopsies have most often shown deposits within glomerular capillaries consistent with membranous nephropathy (Fig. 34.2),[67] but normal glomeruli and antiglomerular basement membrane (anti-GBM) antibody deposition also have been described. In most patients, proteinuria is self-limited and disappears spontaneously when the source of exposure is removed.

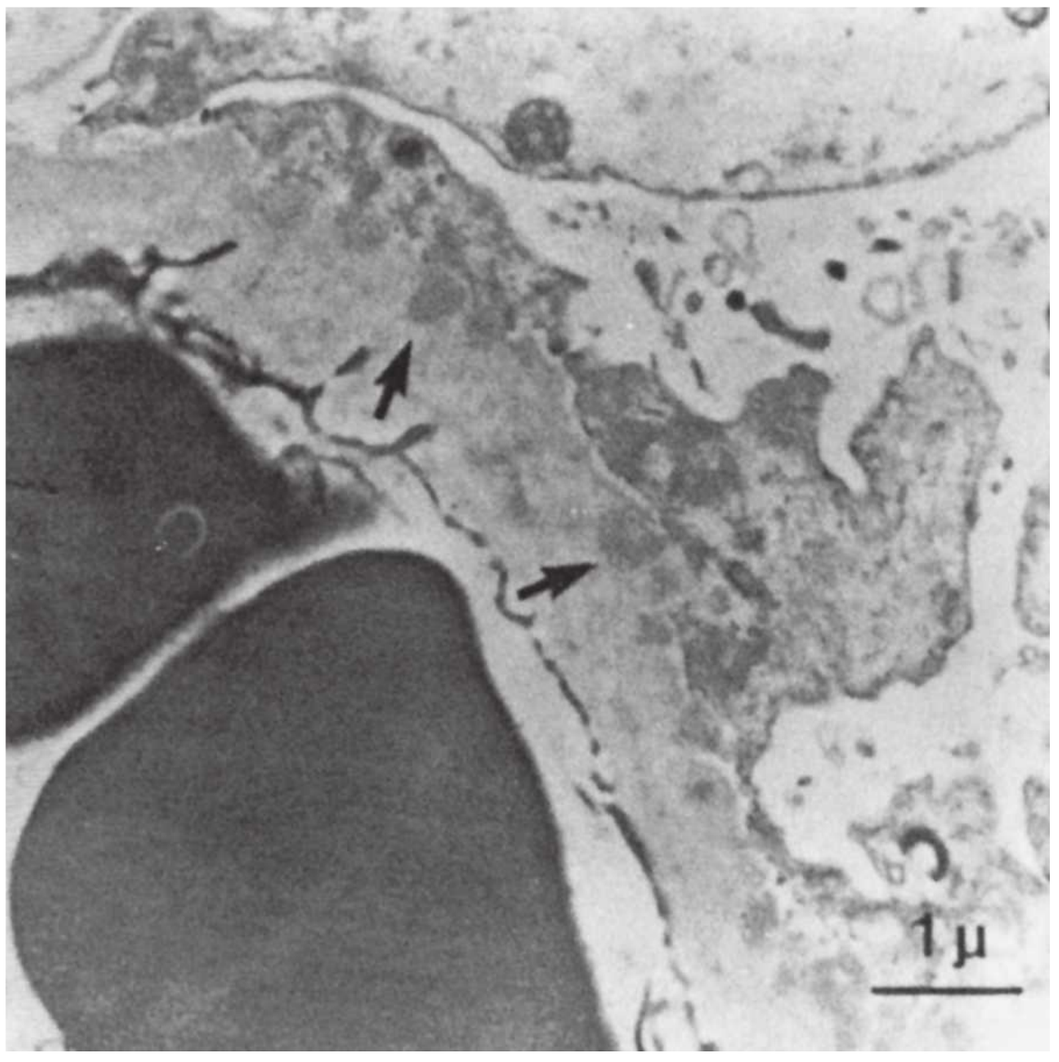

FIGURE 34.2 Electron micrograph of kidney from a 24-year-old man exposed to mercury vapor in an industrial electrolysis unit. The urinary mercury was 174 μg per 24 hours; urinary protein, 3.11 μg per 24 hours. Creatinine clearance was 116 mL/min/1.73 m^2. Subepithelial electron-dense deposits (*arrows*), presumably immune complexes, overlie the glomerular basement membrane. (Lead citrate and uranyl acetate; magnification ×11,000.) (From Tubbs RR, Gephardt GN, McMahon JT, et al. Membranous glomerulonephritis associated with industrial mercury exposure. *Am J Clin Pathol.* 1982;77:409, with permission.)

MINAMATA DISEASE

Endemic methyl mercury poisoning was recognized in Japan in the area of Minamata Bay in 1956, arising from the contamination of food by industrial effluents.[68] The mercury pollution had been going on for a decade and fish from Minamata Bay contained up to 36 mg of mercury per kg. Affected people presented neurologic defects including visual, speech, and gait disturbances. Cerebral palsy was common among the children of affected mothers. Similar clusters of cases were subsequently identified in Niigata, Japan, and in Iraq, where the disease was the result of bread prepared from grain that had been treated with methyl mercury fungicide.

Although the kidney manifestations of Minamata disease are minor, tubular proteinuria occurs[69] but clinically important albuminuria and azotemia are not common.

Treatment

Acute inorganic mercury poisoning is treated with the effective chelator British antilewisite (BAL). Recommended regimen is 5 mg per kg given by the intramuscular route, followed by 2.5 mg per kg twice daily for 10 days. In the presence of acute renal failure the mercury chelate can be removed by hemodialysis. BAL is not used in chronic poisoning, in which treatment is removal from the source of exposure. Succimer (DMSA), an oral chelating agent used for the treatment of lead poisoning in children, is not an effective chelator of mercury and its use is not recommended.[70]

OTHER HEAVY METALS

Uranium is selectively accumulated in the proximal tubule with a biologic half-life approximating 1 week for 95% of the renal stores.[71] After inhalation, the uranyl ion binds to circulating transferrin and to proteins and phospholipids in the second and third segments of the proximal tubule. ATN and increased β_2-microglobulin urinary excretion has been reported after uranium exposure, especially when urinary uranium levels were over the upper acceptable limit of 30 μg per L.[72] There is no evidence of association between uranium exposure and the development of chronic kidney disease (CKD).

Copper sulfate has been associated with ATN in young female science students attempting suicide in Delhi, India.[73]

Like other heavy metals, chromium is selectively accumulated in the proximal tubule and the hexavalent form is able to induce ATN, but there is no convincing evidence of tubular injury from usual occupational exposure.[74–78] Minimal tubular proteinuria has also been reported in chrome platers, but CKD was not documented.

Bismuth compounds prepared as therapeutic agents have produced unequivocal ATN. Lower dosages induce Fanconi syndrome with reduced glomerular filtration and bismuth-containing intranuclear inclusions in proximal tubule cells that are similar to, but distinguishable from, lead inclusions.[79–81]

Arsenic poisoning is associated with renal failure.[82,83] Usually associated with an industrial accident, arsine inhalation produces hemolysis, hematuria, and abdominal pain within a few hours, followed by acute oliguric renal failure and jaundice within 2 days.[84] Reticulocytosis, basophilic stippling, bilirubinemia, and free hemoglobin in the plasma may assist diagnosis, which is established by detecting arsenic in the urine. Patchy cortical necrosis with persistent residual renal failure has been reported.[82,85] BAL is ineffective once renal failure is present. Besides hemodialysis, exchange transfusions may be useful to eliminate hemoglobin-bound arsenic from the body.

SILICON

Silicon is a semimetal found as dioxide silica (SiO_2) in 28% of the earth's crust. Silicon is not a heavy metal because it has a specific gravity of only 2.3. It is present in the serum at concentrations of 20 to 50 μg per dL in an unbound form as silicic acid and is cleared in the urine at the rate of glomerular filtration. Silica is believed to induce renal disease by direct deposition of crystalline material in the renal parenchyma and by immunologic mechanisms acting as an adjuvant to stimulate the immune response.[86,87] Tubular proteinuria is found in workers exposed to silica dust,[88,89] and an increased risk to develop end-stage renal disease (ESRD) has been described.[90] In the accelerated form of silicosis known as silicoproteinosis, rapidly progressive, immune complex-mediated focal glomerulosclerosis, which simulates lupus erythematosus (Caplan syndrome), may appear.[89] Antineutrophil cytoplasmic antibody (c-ANCA)-positive Wegener granulomatosis has been associated with exposure to silica dust as well as to silicon containing compounds such as grain dust.[91]

GERMANIUM

Germanium has been used in the treatment of cancer, a variety of medical ailments, and in unproved remedies for conditions such as arthritis, acquired immunodeficiency syndrome (AIDS), and the chronic fatigue syndrome. Germanium-containing elixirs and health foods have been described to cause chronic tubulointerstitial nephritis first in Japan in the 1980s and more recently in Europe and the United States.[92–97] The kidney disease is different from that induced by other heavy metals in that widening of the interstitium and distal tubular atrophy has been evident after prolonged (6 to 36 months), high-dose (16 g to hundreds of grams) consumption. Electron-dense, periodic acid-Schiff (PAS) reagent-positive granules (containing germanium in the experimental rat) are found in distal tubular mitochondria.[95] The tubulointerstitial disease is slowly progressive even after exposure elimination. Fatal outcomes have been reported. The pathophysiologic mechanism of tubular damage is not defined because selective accumulation in the kidney was not found and immunologic mechanisms have not been implicated. There is no evidence of primary proximal tubular injury and proteinuria is absent.[92,94]

BALKAN NEPHROPATHY

Balkan endemic nephropathy is a slowly progressive tubulointerstitial nephritis of unknown etiology described about 40 years ago among middle-aged men and women living in farming villages along the Danube River in Croatia, Serbia (the former Yugoslavia), Romania, and Bulgaria.[98] Early clinical manifestations included clinically silent tubular dysfunction such as glucosuria and/or aminoaciduria. More advanced disease leads to decreased concentrating ability and progression to ESRD.[99] A variety of environmental factors have been suspected including lead, cadmium, and mycotoxins, but no single etiologic agent has been found. The prevalence of generally recognized kidney diseases has not been established in the Danube region, making differentiation of Balkan nephropathy from known renal diseases identified in other regions of the world problematic.

DRUG ABUSE

Substance abuse is common, involving lifetime exposure of 46% of the general population.[100] Substances with the potential to be abused include alcohol, opiates, sedatives and hypnotics, cocaine, cannabis, hallucinogens and psychedelic drugs, psychotropic, stimulant and anxiolytic medications, analgesics, and amphetamines. Such drugs have been associated with several kidney syndromes by varied mechanisms. Although some substances are directly nephrotoxic, other mechanisms are also involved, including glomerular, interstitial, and vascular diseases (Table 34.2).[101]

34.2 Renal Disease Associated with Drug Abuse

1.	Focal glomerulosclerosis in intravenous heroin users
2.	Amyloidosis in subcutaneous heroin abusers
3.	Endocarditis-associated glomerulonephritis in intravenous drug users
4.	Acute renal failure due to nontraumatic rhabdomyolysis
5	Cocaine-associated nephropathy
6.	Systemic necrotizing vasculitis
7.	Nephropathy in glue and solvent "sniffers"
8.	Hepatitis-related glomerulonephritis in drug abusers
9.	Focal glomerulosclerosis in drug abusers infected with HIV

Since the first reports in the late 1960s and early 1970s there have been numerous studies describing the clinical and pathologic features of renal diseases associated with chronic parenteral abuse of heroin, cocaine, morphine, amphetamine, and other narcotic and hallucinogenic drugs, including several adulterants. Renal disease in cocaine and heroin users is associated with the nephrotic syndrome, acute glomerulonephritis, amyloidosis, interstitial nephritis, and rhabdomyolysis.

In the past few decades an explosive growth in illicit drug use has occurred in many parts of the world. Reports indicate that in some areas of the United States the percentage of cocaine addicts among young people is as high as 20%. In a university population in the United States, 6% were found to be cocaine users as documented by hair analysis.[102] Other than the more common acute renal effects of the abuse of multiple drugs, chronic abuse may also be associated with CKD and progression to ESRD.

HEROIN

Heroin is processed from a naturally occurring substance, morphine, extracted from various poppy plants. It can be sniffed ("snorting"), eaten, smoked ("chasing the dragon"), injected subcutaneously ("skin popping"), or injected intravenously ("mainlining").[103] The purity of heroin depends on the presence of adulterants. Commonly used adulterants include sucrose, dextrose, mannitol, lactose, starches, powdered milk, quinine, caffeine, inositol, lidocaine, procaine, acetylprocaine, methapyrilene, and strychnine.[104]

Heroin is the most commonly abused opiate in the United States and has become a growing health-related problem in large metropolitan areas.[100] The term "heroin-associated nephropathy" includes different morphologic findings following chronic drug abuse.[105] Although the prevalence of heroin use in the United States has increased, the incidence of "heroin nephropathy" has declined. Socioeconomic conditions, cultural and behavioral practices, or differences in genetic susceptibilities may be more associated with the development of nephropathy in heroin users than the drug's pharmacologic properties.[103]

There are several renal complications from heroin abuse varying from hypotension to coma complicated by pressure-induced muscle damage and rhabdomyolysis. This latter event in the absence of coma, or evidence of muscle compression, could also occur as a direct toxic effect or an allergic response to heroin or the components in adulterated heroin.[106] The high rate of viral, bacterial, and fungal contamination associated with intravenous drug use increases the risk for glomerulonephritis (GN) associated with chronic infections. Local abscesses, due to Staphylococcus aureus, have been associated with GN. Bacterial and fungal endocarditis are also associated with immune-complex mediated GN. Other causes of GN in these patients are associated with hepatitis B and hepatitis C.

Secondary (AA) amyloidosis is also a cause of kidney disease in chronic drug users, particularly among those who inject drugs subcutaneously ("skin poppers").[107] In the majority of these patients, continued abuse leads to progression to end-stage renal failure and resolution of the lesions following abstinence from subcutaneous drug abuse has been reported.[108]

In the 1970s and 1980s, heroin-associated nephropathy (HAN) was described, presenting as nephrotic syndrome and progressing rapidly to end-stage renal failure. Kidney biopsy usually showed a focal segmental glomerulosclerosis.[109] Earlier studies suggested that heroin, or one of its adulterants, acted as an antigen leading to renal deposition of immune complexes in the kidney, but animal studies have shown that morphine has a direct effect on the glomerulus, causing proliferation of fibroblasts and a decrease in degradation of type IV collagen.[105,110] As heroin sold in streets became more pure a decrease in the incidence of HAN among intravenous heroin addicts was described.[111] At the same time, HIV-associated nephropathy (HIVAN) started to be diagnosed more frequently among heroin addicts with HIV infection.[112]

Recent studies suggest that morphine has direct effects on mesangial and glomerular epithelial cells (GEC), kidney fibroblasts, and the interaction of mesangial cells with circulating and resident macrophages. The classic lesion of focal segmental glomerulosclerosis (FSGS) starts with mesangial cell hyperplasia and GEC hypertrophy.[113] In the course of the FSGS, there is eventual loss of mesangial epithelial cells and GEC. The loss of GEC, or apoptosis, has been suggested as an underlying mechanism in the development of FSGS.[114] The glomerulosclerosis results from mesangial cell expansion, increased matrix deposition, and secretory factors that are modulated by macrophages. Morphine induces both inhibitory and proliferative effects on fibroblasts, mesangial cells, and macrophage activity.[115] At lower concentrations morphine predominantly stimulates mesangial cells and fibroblast proliferation, mesangial matrix deposition, and macrophage activity. At higher concentrations, morphine inhibits mesangial cells and fibroblast proliferation, mesangial matrix deposition, and macrophage activity.[115]

COCAINE-ASSOCIATED NEPHROPATHY

Cocaine (benzoyl methylecgonine) is extracted from the leaves of the South American plant Erythroxylum coca.[116] It exists in two major forms: Cocaine hydrochloride and alkaloidal freebase (crack) cocaine.[116] Cocaine abuse is epidemic in the United States, with an estimate that 34.3 million Americans have used cocaine at some time.[100]

Although the most frequent renal manifestation of cocaine abuse is acute kidney injury due to nontraumatic rhabdomyolysis, cocaine may induce nephropathy by other mechanisms.[101] The pathophysiologic basis of cocaine-related renal injury involves renal hemodynamic changes, glomerular matrix synthesis and degradation, oxidative stress, and induction of kidney atherogenesis.

Cocaine has potent vasoconstrictive effects on vascular smooth muscle.[117] It directly affects smooth muscle vascular cell calcium influx.[118–120] Endothelins (ET) have also been implicated in the vascular dysfunction that is induced by cocaine intoxication.[116] There is a high density of ET-1 receptors in the vascular smooth muscle of all renal resistance vessels.[121]

Cocaine inhibits catecholamine reuptake at the presynaptic nerve terminal, blocks norepinephrine reuptake in sympathetically innervated tissues, and releases norepinephrine and epinephrine from the adrenal medulla resulting in development of hypertension and tachycardia.[122,123]

The involvement of the renin-angiotensin-aldosterone system (RAAS) has been suggested because cocaine-induced ET release is inhibited by captopril and lisinopril in cultured human and bovine endothelial cells.[124] Activation of the RAAS and angiotensin II by cocaine may lead to renal fibrosis from stimulation of TGF-β.[116]

The resultant effect of cocaine on the kidney, through ET-1, the RAAS, and the l-arginine–NO pathway, leads to vasoconstriction of the glomerular microcirculation. Cocaine has been associated with accelerated and malignant hypertension as well as implicated in hastening the progression of hypertensive nephrosclerosis to ESRD.[125,126]

In an autopsy study 40 kidneys from patients with cocaine-related deaths were compared with kidneys from 40 accident victims.[127] The ratio of the number of sclerotic glomeruli to the total number of glomeruli was 18-fold greater in cocaine users than in controls. In that study, they also found significant differences in the degree of periglomerular fibrosis, the degree of interstitial cellular infiltrate, and hyperplastic arteriolosclerosis in cocaine users compared to controls. Medial thickening, luminal narrowing, and vessel obstruction were absent in the control group. The patients with cocaine-related deaths were found to have advanced coronary atherosclerosis, more extensive than expected in normal populations older than 60 years of age.

In a case-control study performed to examine recreational drug use as a risk factor for ESRD, cocaine use was associated with a threefold increased risk for developing ESRD.[128] In a prospective cohort including 647 patients, followed for a 15-year period, there was a threefold increased risk of kidney functional decline associated with use of cocaine or crack as compared to nonusers.[129]

Although these studies demonstrate an association of cocaine use and progression to CKD and ESRD, prospective, epidemiologic studies are needed to clarify the relationship between cocaine use and the development of CKD.

ECSTASY AND OTHER AMPHETAMINES

Ecstasy (MDMA: 3,4-methylenedioxymethamphetamine), originally used as an appetite suppressant, became a commonly used recreational drug. MDMA is rapidly absorbed, reaching plasma peak levels in approximately 2 hours.[130] It is metabolized by the liver and excreted by the kidney.

The increased physical activity, overheated environments, and dehydration associated with its use can result in hyperthermia. It has been shown that MDMA can cause fever even in the absence of strenuous exercise. Associated side effects can be mild—nausea, vomiting, headaches, cramps—or serious—convulsions, hyperpyrexia, hepatic dysfunction, rhabdomyolysis, disseminated intravascular coagulation, and acute kidney injury.[131]

There is an increased risk of hyponatremia associated with ingestion of large quantities of water to prevent dehydration and inappropriate antidiuretic hormone secretion.[132] As a consequence, cases of cerebral edema have occurred after ecstasy abuse. Ecstasy has marked sympathomimetic effects and has been associated with cases of accelerated hypertension and acute kidney injury.

SOLVENTS

Fumes of toluene or toluene-containing compounds (spray paint, household and model glue, lacquer, and paint thinners) contain a number of volatile substances including n-hexane, methyl ketones, chlorohydrocarbons, and benzene. The deliberate inhalation of volatile solvents ("glue sniffing") first emerged as a form of substance abuse in the early 1960s. Solvents can rapidly cause hallucinations of short duration (15 to 30 minutes), and are associated with a variety of electrolyte and acid–base disturbances. In addition, serious cardiac, pulmonary, hepatic, neurologic, and renal complications may develop, as well as sudden death.

The nephrotoxic insult of volatile glues appears to be due principally to toluene. Various renal lesions have been associated with its abuse: microhematuria, pyuria and proteinuria, distal renal tubular acidosis and Fanconi syndrome, urinary calculi, glomerulonephritis, Goodpasture syndrome, ATN, hepatorenal syndrome, and acute and chronic interstitial nephritis.[101] Taher and coworkers first recognized non-anion gap hyperchloremic metabolic acidosis in association with toluene "sniffing" in two patients.[133] The tubular defect was documented by the presence of metabolic acidosis (pH, 7.2 to 7.3) with a normal anion gap, hyperchloremia (level of 118 to 120 mEq per L), and an inappropriately high urinary pH (>6.0). Numerous other cases of hyperchloremic metabolic acidosis, due to toluene inhalation, have subsequently been reported. In addition, transient congenital renal tubular dysfunction with hyperchloremic metabolic acidosis due to maternal toluene abuse have been described.[134]

Toluene, a hydrophobic compound, accumulates in lipoidal structures. After sniffing stops, this volatile chemical is excreted via the lungs. The conversion of toluene to organic acids occurs in the liver where toluene is metabolized by the cytochrome P-450 system. There is a spectrum of disorders that are responsible for the metabolic acidosis associated with toluene abuse, caused primarily by the conversion of toluene to hippuric acid, with the subsequent rapid excretion of hippurate in the urine.[135] Patients with a fast metabolism of toluene by the cytochrome P-450 system have a high rate of production of organic, hippuric, and benzoic acids. With a normal kidney function these patients will usually present a hyperchloremic acidosis and a wide anion gap, if the production exceeds the excretion of the anions. In the presence of impairment in the rate of excretion of ammonium, demonstrated in 20% of the patients, the net acid excretion is reduced and, hence, distal renal tubular acidosis is present.[135] In chronic toluene abuse increased concentrations of NH_3 in the interstitium can lead to complement activation. Thus, toluene abuse can cause chronic interstitial diseases, and ultimately impair the ammonium excretion causing distal renal tubular acidosis.[136]

In addition to the acid-base disorders, fluid and electrolyte abnormalities are also common in the clinical presentation of toluene abuse. In fact, nausea and generalized weakness, which may result from volume contraction and hypokalemia, are the symptoms that frequently lead the patient to seek medical attention. The excretion of hippurate increases the excretion of ammonium, sodium, and potassium. About one quarter of the patients present with severe hypokalemia (potassium <2mEq per L). The degree of hypokalemia may be underestimated by the metabolic acidosis, a factor that should be anticipated during the treatment. The presence of concomitant hypophosphatemia increases the risk of rhabdomyolysis.[137]

LITHIUM

Introduction

Lithium is currently a drug of choice for treating bipolar illness and is widely used in this population. Approximately 0.1% of the U.S. population is undergoing lithium treatment for psychiatric problems and 20% to 54% of these patients have urinary symptoms during and after lithium use.[138] Approximately 30% of patients taking lithium experience at least one episode of lithium toxicity.

Chronic lithium ingestion in patients with bipolar illness has been associated with several different forms of kidney injury. The narrow therapeutic window (1 to 1.5 mEq per L during acute therapy and 0.6–1.2 mEq per L during maintenance therapy) contributes substantially to the frequency of acute and chronic toxicity. Close monitoring of serum levels is important to prevent acute and chronic kidney failure. Prevention is important and patients should be instructed to drink 10 to 12 glasses of liquid every day during lithium therapy, and keep a regular (non-low) salt diet. Patients should be oriented to contact a health care provider if fever, diarrhea, or vomiting develops. Certain drugs, especially diuretics, should be avoided with lithium use if possible. Cyclosporine and nonsteroidal anti-inflammatory drugs (NSAIDs; except low dose aspirin) potentially increase serum lithium levels and should be avoided. There is no gender or ethnic predisposition to the development of lithium toxicity, although some studies suggest that women may require fewer drugs to achieve therapeutic serum levels than men.

The most common renal side effect of lithium therapy is nephrogenic diabetes insipidus (NDI). Chronic tubulointerstitial nephropathy is the most common form of CKD associated with lithium therapy.[139] Although the majority of studies show infrequent and relatively mild renal insufficiency attributable to lithium therapy, ESRD secondary to lithium-associated chronic tubulointerstitial nephropathy does occur in a small percentage of patients, although the incidence of long-term lithium nephropathy is still a matter of debate.[139]

Renal Effects of Lithium

Lithium is a univalent cation, completely absorbed by the gastrointestinal (GI) tract. The drug is not protein bound and is completely filtered at the glomerulus. Although the majority, up to 60%, of the filtered load is reabsorbed by the proximal tubule, a significant amount is also absorbed in the loop of Henle and the early distal nephron. Lithium can act as a substitute for sodium in several sodium channels, particularly the sodium-hydrogen exchanger in the proximal tubule (NHE_3), the sodium/potassium/2chloride exchanger in the thick ascending limb of the loop of Henle ($NKCC_2$), and the epithelial channel of the cortical collecting tubule (ENaC).

Lithium nephrotoxicity can occur as soon as a month after the onset of use of the drug. The most common symptoms, polyuria and polydipsia, are reversible but can become permanent with chronically maintained high serum levels of lithium. Lithium-associated natriuresis is caused by the impaired regulation of the expression of the epithelial sodium channel in the cortical collecting tubule.[140] Lithium use partially inhibits the ability of aldosterone to increase apical membrane ENaC expression, resulting in inappropriate sodium losses.[141]

Nephrogenic diabetes insipidus is a common side effect of lithium; up to 12% of patients develop frank diabetes insipidus. Lithium impairs the ADH stimulatory effect on adenylate cyclase, and decreases cAMP levels.[142] Studies suggest that the ability of lithium to produce nephrogenic diabetes insipidus may also have an independent effect from cAMP generation and be associated with a decreased AQP2 mRNA levels.[143] Lithium most likely impairs water permeability in the principal cells by inhibiting water channel delivery and, over a prolonged period of time, by suppressing channel production.[144]

In patients with urine-concentrating defects, the improvement usually take weeks to months to occur, and can persist for years in some cases. One case report describes patients who still had diabetes insipidus 8 years after cessation of therapy. In another report, of a small subset of patients, up to 63% had persistent defects 1 year after stopping lithium.[138] The prolonged time to recover the concentrating ability may be linked to underlying renal histologic damage and may be worse with neuroleptic use and prolonged lithium therapy. Boton and colleagues, in a review, documented a 54% correlation between impaired urine-concentrating ability and the duration and total dosage of lithium treatment.[145] Chronic lithium use is also associated with a distal tubular acidification defect, exerted from the luminal side of the cell. Lithium is not known to cause significant hyperkalemia.

Acute Kidney Injury

Lithium toxicities appear to be dose and concentration dependent. Serum concentrations between 1 and 1.5 mEq per L will most likely cause impaired concentration, lethargy, irritability, muscle weakness, tremor, slurred speech, and nausea. Plasma concentrations greater than 2.5 mEq per L are associated with kidney failure. The mechanism associated with lithium acute kidney injury involve volume depletion due to natriuresis and water diuresis accompanied by elevated lithium levels, altered mental status, and subsequent poor oral intake. Cases of acute kidney injury, as a result of lithium-induced neuroleptic malignant syndrome, have also been described.[146] Adequate and timely fluid replacement can rapidly restore kidney function. Loop diuretics can decrease the lithium reabsorption in the loop of Henle and increase lithium excretion. This approach can be used in case of lithium toxicity, if adequate intravascular volume is maintained. Acetazolamide combined with sodium bicarbonate can be used because acetazolamide inhibits the reabsorption of lithium by the proximal tubules. Adequate electrolyte supplements, especially sodium and potassium, are fundamental. Lithium is entirely dialyzable and hemodialysis is the most efficient way to decrease lithium levels when patients cannot be managed medically or when kidney function is severely impaired. Dialysis is usually necessary when lithium concentrations are above 4 mmol per L. Hemodialysis increases lithium clearance to 3.0 L per hour; however, following hemodialysis, a rebound effect in the lithium concentrations can be observed. The rebound is expected to occur as lithium has a slow rate of redistribution from the peripheral tissues into the central compartment or from release of lithium from bone stores. Therefore, hemodialysis should be extended or repeated at frequent intervals.

Chronic Kidney Disease

The debate around lithium-induced CKD is the result of conflicting cross-sectional and longitudinal epidemiologic studies.[147] Some of these studies show a low incidence of kidney dysfunction in lithium-treated patients. Data from 14 studies, including 1,172 patients, estimated that the prevalence of reduced GFR, measured by different methods, was 15%.[145] The development of lithium-induced chronic tubulointerstitial nephritis was first demonstrated by Hestbech et al. in 14 patients treated with lithium for about 2 to 15 years.[148] Markowitz et al. recently reemphasized the risk of often irreversible biopsy-proven lithium toxicity being responsible for combined glomerular and tubulointerstitial damage.[139] Other studies have demonstrated interstitial fibrosis, tubular atrophy, and glomerulosclerosis among the chronic histologic changes associated with lithium. These

studies suggested that lesions correlated with the duration of lithium. However, most of the biopsy samples were obtained from patients that had a history of acute lithium toxicity, and many of the histologic changes were also identified in the control group. The histologic changes associated with acute lithium include ATN with nonspecific changes such as distal tubular flattening, proximal tubular necrosis, and cytoplasmic vacuolation and cellular and nuclear polymorphism of the distal tubular epithelial cells. Although chronic tubulointerstitial nephropathy represents a somewhat nonspecific pattern of disease, the presence of tubular cysts is highly characteristic of lithium toxicity, having been reported in up to 40% of cases.[149] Markowitz et al. revealed a chronic tubulointerstitial nephropathy in all 24 patients with biopsy-proven lithium toxicity, with associated cortical and medullary tubular cysts (62.5%) or dilatation (33.3%). The degree of tubular atrophy and interstitial fibrosis was graded as severe in 58.3%, moderate in 37.5%, and mild in 4.2% of cases. There was a surprisingly high prevalence of focal segmental glomerulosclerosis (50%) and global glomerulosclerosis (100%), sometimes of equivalent severity to the chronic tubulointerstitial disease. Despite discontinuation of lithium, seven of nine patients with initial serum creatinine values >2.5 mg per dL progressed to ESRD.[139] In 2003 a French group studied 74 patients with lithium-induced kidney failure.[150] They showed that the creatinine clearance at referral and at last follow-up was inversely related to the duration of lithium therapy in both univariate and multivariate analyses adjusting for age, gender, hypertension, and proteinuria. In 29 patients with a kidney biopsy, the degree of interstitial fibrosis was related to the lithium duration and cumulative dose. They estimated the prevalence of lithium-related ESRD as two per 1,000 dialysis patients, and the average time between onset of lithium therapy and ESRD was 20 years.[150] Although a minimal increase in the protein excretion rate has been reported in some patients who were taking lithium for at least 2 years, overt proteinuria is not a common complication. A rare association between minimal-change nephrotic syndrome and lithium administration has also been described. Renal dysfunction is often irreversible despite lithium withdrawal, and early detection is essential to prevent progression to ESRD. Although the debate around the rate of progression to CKD and ESRD associated with the chronic use of lithium continues, regular monitoring of estimated creatinine clearance is mandatory in long-term lithium-treated patients.

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CHAPTER

35 Acute Tubulointerstitial Nephritis

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For most of its medical history, the kidney has been considered a tubular organ. Even after Marcello Malpighi (1628–1694) described his eponymous "corpuscles" and William Bowman (1816–1892) that of his "capsule," the kidney continued to be viewed as a secretory tubular organ. In fact, after the milestone report of dropsical albuminuric medical cases by Richard Bright (1789–1858), which launched the study of kidney disease, the inflammatory diseases of the kidney that he described were classified in 1858 by Rudolph Virchow (1821–1902) as a nephritis principally affecting the tubules ("parenchymatous" nephritis) or the interstitium ("interstitial" nephritis). It is only after the improved resolution of microscopes and refinements in tissue processing that in 1869 "glomerular" nephritis was added to the list of nephritides by Edwin Klebs (1834–1913). Still, most diseases of the kidney continued to be considered as tubulopathies rather than glomerulopathies well into the first decades of the 20th century. It is within this context that the pathologic diagnosis of acute interstitial nephritis (AIN) was described in 1898 by William Councilman (1854–1933). In his now classic paper, based on a review of the literature and his postmortem examination of 42 cases, he reported on a nonsuppurative inflammatory interstitial lesion of the kidney occurring predominantly in patients with diphtheria (24 cases), scarlet fever (10 cases), and less frequently in those with other infectious diseases. He described the pathologic changes as

> an acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; the exudation is not purulent in character, and the lesions may be both diffuse and focal

and identified the infiltrating cells as "plasma cells that had migrated from the blood vessels and multiplied locally by mitotic division." He localized the foci of cellular infiltrates to three sites: the boundary zone of the pyramids, the subcapsular region of the cortex, and around the glomeruli. These meticulous observations and the lucid description are just as valid today as they were then, and they encapsulate the lesions of this disease of the kidneys more succinctly and thoroughly than much of what has been written since they were first reported.[1,2]

Subsequent reports confirmed Councilman's observation that nonsuppurative lesions of the renal interstitium appeared after short but variable periods from the onset of acute infections that were primarily streptococcal in origin.[3,4] This concept of acute tubular and interstitial injury following an acute infection was so well accepted that initial reports of acute tubular necrosis (ATN), which appeared before the proper characterization of acute renal failure due to tubular necrosis during World War II, termed the new lesion acute hematogenous interstitial nephritis and argued for the similarity of the lesions of ATN to those of AIN.[5] The difficulty in the differential diagnosis of these two variants of tubulointerstitial disease persists to this day if the kidney tissue is not examined carefully and is interpreted without benefit of the clinical history.[1,6,7] The introduction of antibacterials during World War II and the eradication of serious and fatal streptococcal infections resulted in a loss of interest in the entity described by Councilman, whereas attention focused on ischemic or nephrotoxic ATN as the predominant cause of acute kidney injury (AKI). Therefore, it is ironic that interest in AIN was revived in the 1960s when the very antibiotics used to treat streptococcal infections were identified as a cause of AIN.[1,8,9] In fact, the bulk of current reports in the literature are on drug-induced AIN, and the number of drugs implicated as causing AIN continues to increase, as does that of the variations in their clinical and laboratory manifestations.[8–10] AIN has since come to define the clinicopathologic syndrome that develops in diverse conditions, including infections, and is characterized by an acute deterioration of kidney function, the pathologic features of which remain those first described by Councilman as "cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium . . . and the lesions may be both diffuse and focal."[2]

Although this clinicopathologic syndrome initially was referred to as acute interstitial nephritis,[2] the more inclusive

and precise descriptive terms acute tubulointerstitial nephritis (ATIN) and acute tubulointerstitial nephropathy are used now.[1,11] The preferential use of tubulointerstitial stems from the fact that although the dominant morphologic features are those evident in the interstitium, the tubules also are affected, albeit to a degree that may be difficult to detect in some cases on light microscopy. Importantly, and independent of the severity of their structural injury, the disorders of tubular function constitute a characteristic component of the disordered kidney function and differentiate this entity from other forms of AKI due to glomerular or vascular disease. In fact, tubular dysfunction is an invariable accompanying feature of the reduction in glomerular filtration rate (GFR) in ATIN and, as a rule, precedes any clinically evident decrease in GFR.[1] In addition, although the interstitial cellular infiltrates contribute significantly to the pathogenesis of the disease, there is increasing evidence that the tubules play an important role in the processing and presentation of the antigenic stimulus and the immunopathogenesis of the disease process.[12–15] Although ATIN may be suspected clinically, its diagnosis depends on the presence of characteristic morphologic changes of the renal parenchyma. The adjective acute refers to the sudden onset and rapid progression of the clinical features of this syndrome and not to its pathologic features, which are notable for mononuclear cell infiltration rather than the polymorphonuclear leukocytes characteristic of an acute inflammatory reaction.

PATHOLOGIC FEATURES

Independent of the causative agent or the clinical condition responsible for ATIN, the principal morphologic features that characterize it are an increase in interstitial volume, mainly because of edema; mononuclear cell infiltrates of varying degrees and distribution; tubular injury and degeneration of differing severity, which in general are localized to the sites of the greatest cellular infiltrates; and the transformation of tubular epithelial cells into migratory fibroblasts that cross the basement membrane (Fig. 35.1). The glomeruli are generally spared, but may show some degree of periglomerular infiltrates and ischemic change except in ATIN associated with nephrotic syndrome, where an effacement of the podocyte foot processes is seen on electron microscopy. Although the extent and severity of each of the tubulointerstitial lesions show some correlation with the level of reduction in GFR, the closest correlation appears to be with the infiltrative process. The interstitial infiltrative lesions may be

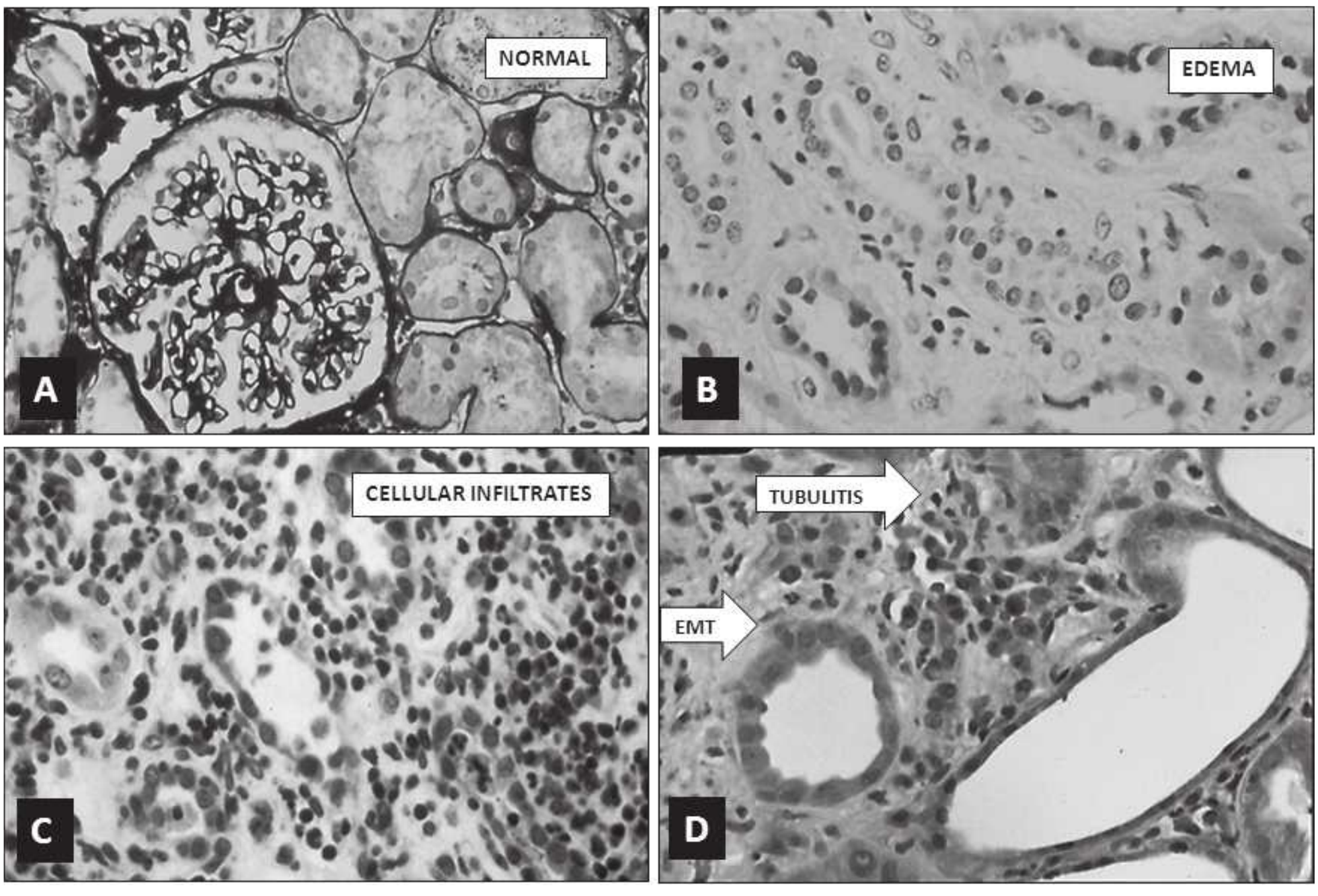

FIGURE 35.1 The pathologic features of a kidney biopsy in acute tubulointertitial nephritis. Compared to a normal kidney **(A),** there is increased interstitial space between the tubules showing edema **(B)**; interstitial infiltration by mononuclear inflammatory cells and bilobed eosinophils **(C)**; and variable manifestations of tubular injury **(D)** showing peritubular infiltrating cells disrupting the tubular basement membrane or "tubulitis" and epithelial-mesenchymal transition (EMT).

diffuse but usually are patchy in distribution and are most evident in the cortex and corticomedullary junction, and less commonly in the outer medulla of the kidney. When the infiltrating cells are meager, they show a focal peritubular distribution that is most evident in the corticomedullary region; when profuse, they are more prominent at certain foci but literally obscure the normal appearance of the tubules and extend to the periglomerular space (Fig. 35.1C). Diffuse infiltrative lesions are associated with higher levels of serum creatinine and a poorer prognosis for recovery than patchy infiltrative ones.[1,8,9]

The infiltrating cells are composed mostly of mononuclear cells, lymphocytes, and plasma cells, and rarely, polymorphonuclear cells and macrophages. The prognosis appears to be less favorable when 1% to 6% of the interstitial infiltrating cells are composed of neutrophilic granulocytes.[1,9] By the same token, an increase in the number of macrophages (usually <10%) and the presence of granulomatous reactions are associated with a prolonged course of AKI and varying degrees of residual impairment of kidney function.[1,9,16] When present, eosinophils are sparse and constitute only a small component of the infiltrating cells, except in occasional cases of antibiotic-induced ATIN, where they may be marked. The presence of infiltrating eosinophils shows no relation to the increased eosinophilia or the presence of urinary eosinophils that also occur in drug-induced ATIN. A role for a specific eosinophil chemoattractant cytokine, eotaxin, which is expressed in the kidney, has been proposed as a mechanism for the tissue eosinophilia of ATIN.[17,18]

An immunologic characterization and an analysis of the interstitial cellular infiltrates reveals that most (up to 80%) of the mononuclear cells are activated T lymphocytes. B lymphocytes also are present but constitute a much smaller portion of the cellular infiltrates, being highest in cases due to nonsteroidal anti-inflammatory drugs (NSAIDs).[1,8] The infiltrating T cells are predominantly CD4+ and CD8+ cells. In most cases, the CD4 variety predominates over the CD8 variety.[1,19] Natural killer lymphocytes are rare; when present, they constitute only a small proportion of the infiltrating cells.[18] A slight prevalence (just over 50%) of the CD8+ subset has been reported in cases of ATIN associated with massive proteinuria after exposure to NSAIDs.[8,9] The relevance of this observation to massive proteinuria as a feature of ATIN is unknown.[1,19] Convincing evidence has been advanced that the infiltrating cells are antigenically activated and immunologically engaged in the pathogenesis of the lesions. However, no diagnostic pattern of markers or cell types has come to be identified with the lesions associated with any specific form of ATIN.[1,20]

Variable degrees of tubular injury usually are present, but tubular atrophy is absent. The tubular lesions are most evident at the site of the greatest concentration of infiltrating cells. They usually are focal and are more severe in the proximity of infiltrating cells. A distinguishing lesion that results from these localized peritubular infiltrates is the disruption of the tubular basement membrane (TBM) and its epithelial cell lining, so-called "tubulitis," which is characteristic of ATIN (Fig. 35.1D). Granulomatous reactions, multinucleated epithelioid cells, and polymorphonuclear leukocytes are detected in cases with marked tubular injury that display tubulitis. Another feature of tubular injury is the phenotypic change of their lining epithelial cells into migrating fibroblasts, so-called epithelial-mesenchymal transition (EMT) (Fig. 35.1D), an event considered to herald the onset of fibrosis.[15] Even in the absence of evident epithelial cell injury on light microscopy, an electron microscopy reveals structural cell changes, the loss of the brush border, and the fragmentation or lamination of the TBM. As a rule, immunofluorescent studies are not revealing. Scant and nonspecific granular staining for immunoglobulins, usually without complement, may be detected along the TBM. In rare drug-induced cases, linear deposits of immunoglobulin G (IgG) and complement (C3) may be present, indicating antibodies directed against tubular membrane antigens.[1,8,9]

There is increasing evidence for a participatory role of the epithelial cells in the pathogenesis of ATIN.[8,9,13–15] The enhanced expression of the human leukocyte antigen (HLA) class II antigens (HLA-DR) of injured tubular epithelial cells has been demonstrated, but shows no correlation with the intensity or phenotype of the infiltrating interstitial cells.[20] The overall profile of immunocompetent cells identified in ATIN suggests an important pathogenetic role for cell-mediated immunity in which the epithelial, vascular, and infiltrating cells are active and participating components.[8,9,21–24]

PATHOGENESIS

The immune response implicated in the pathogenesis of ATIN can be categorized into three phases: an antigen expression and recognition phase, an integrative or regulatory phase, and an effector or mediator phase (Fig. 35.2). In the first phase, either the resident peritubular interstitial cells or the injured tubular epithelial cells, which express major histocompatibility complex (MHC) class II, may function as antigen presenters.[1,25]

The subsequent integrative or regulatory phase may suppress or intensify the reaction to the stimulus provided by the antigen-presenting cells. This is a rather complicated phase of the immune response, in which T cells and antibodies directed at the presented antigen play a central role. Locally produced cytokines implicate a participatory role for the resident interstitial, tubular epithelial, and vascular pericytes and endothelial cells in this regulatory phase.[13,14,24,25] The exposure of tubular epithelial cells (TECs) to selected insults or toxins results in the activation of the TECs and their expression of various growth factors and chemokines, such as interleukins (IL), monocyte chemoattractant proteins (MCP), MHC, vascular endothelial growth factor (VEGF), transforming growth factor (TGF), and regulated on activation, normal T expressed and secreted (RANTES).[26,27] In this phase, the subsequent bidirectional cross-talk between the

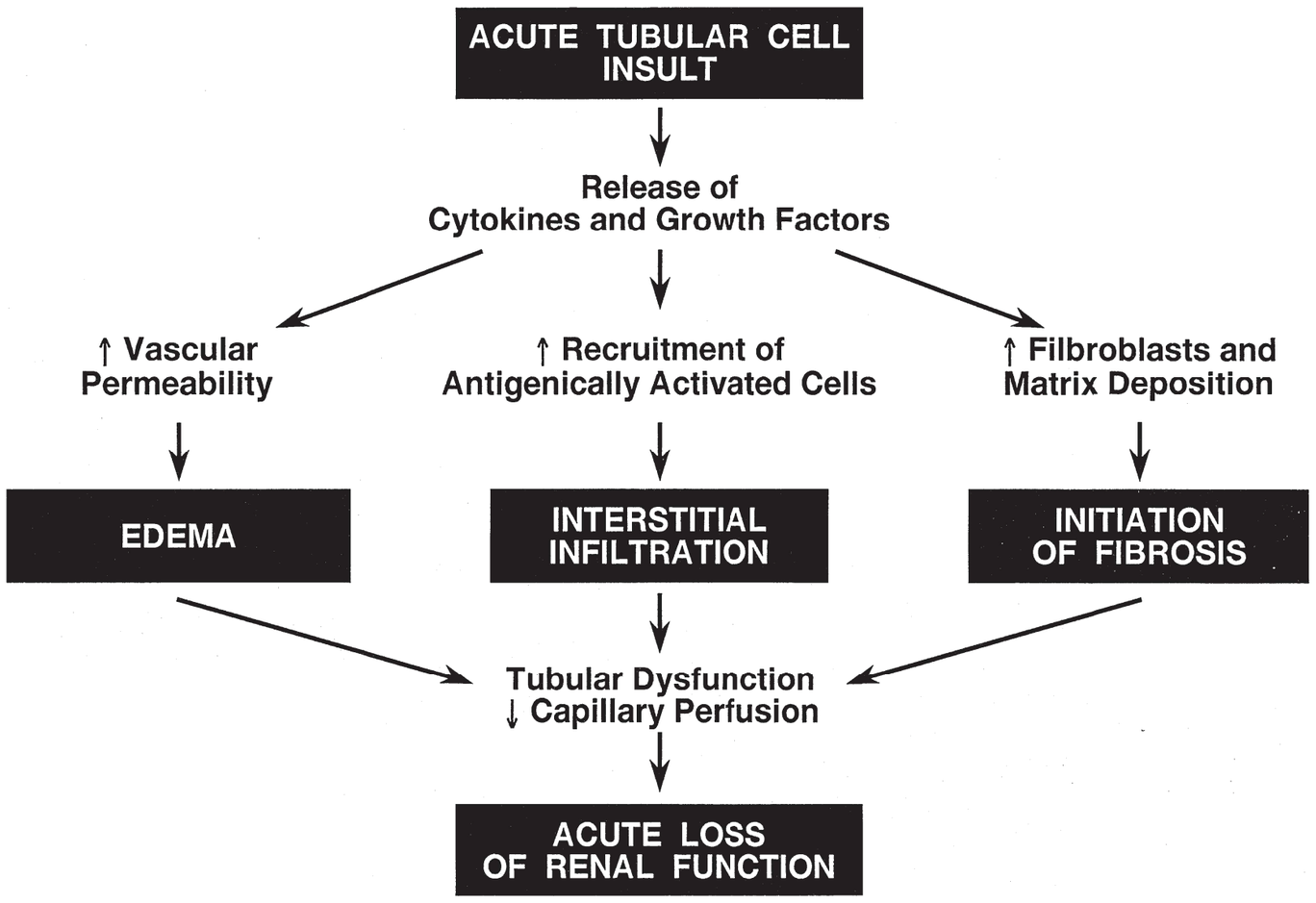

FIGURE 35.2 The pathogenic pathways implicated in acute tubulointerstitial nephritis.

TECs and the recruited infiltrating inflammatory cells, either by soluble factors or by direct contact, ultimately modulates the course of renal involvement and its potential for reversibility.

The final effector or mediator phase is mediated primarily by humoral factors released by the infiltrating cells, with the TECs and vascular pericytes playing a participatory role. The cytotoxic cells may induce injury by the release of proteases, and the inducer cells may do so by the release of lymphokines, which in addition to a direct detrimental action, augment the role of the macrophages. In turn, the release of collagenases, elastases, and reactive oxygen species by the macrophages magnifies the injury initiated by the lymphocytes. Lymphokines also promote fibroblast proliferation and alter the balance in favor of increased matrix synthesis rather than removal.[8,13,14,26]

In the final analysis, it is the presence of a rather wide range of activated mononuclear cells and their interaction with each other and with renal parenchymal cells during the integrative and regulatory phases that is potentially damaging to the kidney (Table 35.1). Several of the cellular (epithelial, endothelial, lymphocytes, macrophages) signals have varying, often overlapping, functions that interact to modulate or amplify the inflammatory reaction of ATIN. Some of those that have been identified include cell surface markers that have either antigenic (MHC, HLA class II, secreted protein acidic and rich in protein [SPARC]) or adhesive (intercellular [ICAM-1] and vascular [VCAM-1] adhesion molecules, integrins, selectins) properties. Others are chemoattractant MCP-1; osteopontin; macrophage inflammatory protein-1 (MIP-1); eotaxin; RANTES, proinflammatory (interleukin [IL]-6 and IL-8; TGF-β; platelet-derived growth factor-β [PDGF-β]; granulocyte–monocyte colony-stimulating factor [GM-CSF]; tumor necrosis factor-α [TNF-α]), vasoactive (adenosine; nitrous oxide [NO]; endothelin-1 [ET-1]), cytotoxic (metalloproteinases [MP]; tissue inhibitor metalloproteinases [TIMP-1]; reactive oxygen radicals; ferric ion), or fibrogenic [TGF-β, PDGF, IL-1, IL-6, TNF, plasminogen activator inhibitor (PAI)]. Evidence also exists for possible protective cytokines that can favorably modify the proinflammatory sequence of events.[27] The resultant integration, during the regulatory and effector phases, either suppresses the effector phase, as in mild forms of ATIN, or amplifies it, as in severe forms of ATIN. Ultimately, feedback mechanisms and the removal of the inciting agent restore the response to injury to its baseline dormant state with consequent recovery of normal kidney function or residual permanent damage.[1,18,24,28–31]

The bulk of the available information about the phases of the immune response and the interstitial inflammatory reaction in ATIN derives from studies in experimental animals, particularly in models of recombinant rats and mice.[9,26,29,32] Unfortunately, there are no animal models that correspond to the reaction that occurs in human ATIN. A principal limitation of human studies is that the data derived from kidney biopsies provide information only at fixed moments in the course of a lesion that is an evolving process. The same limitation applies to the serologic data provided in clinical studies that are obtained at a fixed period, usually when the lesion is well established and often at its worst, with limited or no data available on the onset or resolution phases of the lesion. These limitations notwithstanding, considerable evidence has accrued for a role of the principal immunologic mechanisms—cell-mediated injury, anti-TBM antibodies, immune complex deposition—in the pathogenesis of ATIN. Although there is evidence that all three mechanisms may be operative to varying degrees in different patients, the bulk of the available information favors a predominant role for cell-mediated injury.[8,9,25]

Contrary to the extensive experimental data in support of anti-TBM disease from animal models of interstitial disease, anti-TBM antibodies have been detected only rarely

35.1 Immunomodulatory Signals Involved in the Pathogenesis of Acute Tubulointerstitial Nephritis

Antigenic cell surface markers
Major histocompatibility complex (MHC)
HLA class II
Secreted protein acidic and rich in protein (SPARC)
Adhesive cell surface makers
Intracellular adhesion molecule 1 (ICAM-1)
Vascular adhesion molecule 1 (VCAM-1)
Integrins
Selectins
Chemoattractants
Monocyte chemoattractant protein 1 (MCP-1)
Regulated on activation, normal T-cell expressed and secreted (RANTES)
Macrophage inflammatory protein-1 (MIP-1)
Eotaxin
Proinflammatory cytokines
Interleukin-6 (IL-6)
IL-8
Transforming growth factor α (TGF-α)
Granulocyte monocyte-colony stimulating factor (GM-CSF)
Platelet derived growth factor β (PDGF-β)
Tumor necrosis factor α (TNF-α)
Vasoactive substances
Adenosine
Nitric oxide (NO)
Endothelin 1 (ET-1)
Cytotoxic substances
Metalloproteinases (MP)
Tissue inhibitor metalloproteinases (TIMP-1)
Reactive oxygen species (ROS)
Ferric ion
Profibrotic cytokines
TGF-β
PDGF
IL-1
IL-6
TNF
Plasminogen activator inhibitor (PAI)

in human ATIN.[1] The dearth of evidence for anti–TBM-mediated disease is far from convincing for an important role, if any, of this mechanism in human ATIN. The same limitations apply for a role of immune complex–mediated ATIN. The cases in which granular deposits of IgG have been detected have been in patients with Sjögren syndrome[32] and systemic lupus erythematosus,[29,30] the underlying disease mechanism of which accounts for the deposition of immune complexes in the kidneys as well as other body organs. Granular deposits in other, more common forms of ATIN are rare and never a dominant feature.

By contrast, the evidence in favor of cell-mediated disease is overwhelming. The infiltrating cells are antigenically activated. T-cell reactivity has been demonstrated from in vitro studies of lymphocyte stimulation.[19,22,32–34] Comparative studies of ATIN due to NSAIDs or antibiotics have failed to reveal significant differences in the overall percentage composition of T cells, B cells, and monocytes.[20] The reported differences in infiltrating cell subtypes can be due to individual genetic background, the nature of the insult, and the point in time during the course of the disease when biopsies were obtained. Nevertheless, it is clear that where sought, activated antigens have been demonstrated on the surface of the infiltrating cells, and plasma cells containing IgE, IgA, IgG, and IgM have been detected. However, no diagnostic pattern of markers or infiltrating cells has emerged.[1,20]

FUNCTIONAL MANIFESTATIONS

The principal manifestations of ATIN are those of tubular dysfunction, which is one reason that the term tubulointerstitial nephritis was introduced in preference to the initial appellation of interstitial nephritis. As a rule, tubular dysfunction precedes the onset of azotemia, which in turn precedes that of oliguria. As such, vigilance to clinically evident abnormalities of tubular function, including polyuria, is essential to the early detection of ATIN during its initial reversible stages.

Because of the focal nature of the lesions and the segmental nature of normal tubular function, the pattern of tubular dysfunction that results varies depending on the major site of injury, whereas the extent of damage determines the severity of tubular dysfunction (Fig. 35.3). The principal hallmarks of glomerular disease, such as salt retention, edema, and hypertension, characteristically are absent. However, massive proteinuria does occur in ATIN as a result of NSAIDs use and, occasionally, antibiotic use.[8–10,34] In addition to the direct injury to epithelial cells that may account for tubular dysfunction, changes in the interstitial volume and composition contribute to the functional abnormalities that develop. A major part of the reabsorbed or secreted tubular fluid has to traverse a true interstitial space.[35] The structure, composition, and permeability characteristics of the interstitial space must, out of necessity, exert an effect on such an exchange. A change in any of these parameters of the interstitium or those of the epithelial cell tight junctions, by delaying equilibration or exchange processes, could account for the functional alterations of the renal tubule that develop in ATIN (Fig. 35.3). It also is possible that changes in the pressure of the supporting interstitium, which are transmitted to the periarterial space, could affect the blood

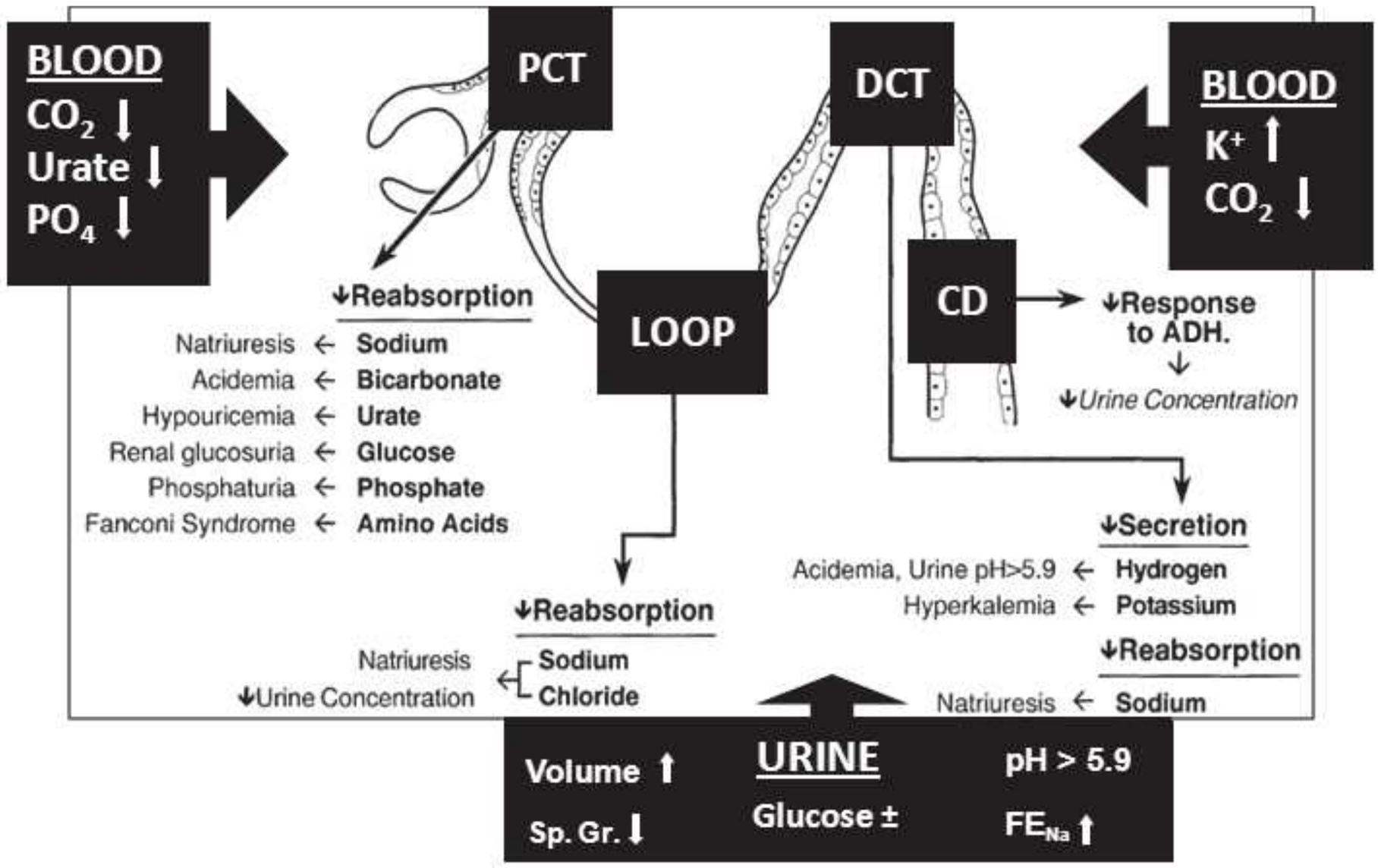

FIGURE 35.3 A schematic representation of the sites of tubular dysfunction in acute tubulointerstitial nephritis. The abnormalities are shown in bold lettering and their clinical manifestations are shown in regular lettering. The *boxed arrows* indicate the principal changes reflected in blood and urine tests. The *arrows* indicate the directional changes. *PCT*, proximal convoluted tubule; *DCT*, distal convoluted tubule; *Loop*, loop of Henle; *CD*, collecting duct; *Sp. Gr.*, specific gravity; FE_{Na}, fractional excretion of sodium; PO_4, phosphate; CO_2, carbon dioxide content.

flow to the adjacent tubule and thereby cause further tubular dysfunction. Tubular alterations also may be reflected in reductions of the GFR through tubuloglomerular feedback as the function of the affected proximal tubular segments becomes compromised and both the rate and the load of solute delivery to the macula densa are altered.[1] Furthermore, the increase in hydrostatic pressure of the edematous interstitium, as has been demonstrated in allograft rejections, may adversely affect the intra-arteriolar pressure and may cause a reduction of the hydrostatic pressure transmitted to the glomerular capillaries. In addition, a vasoactive effect of the cytokines, eicosanoids, and reactive oxygen species elaborated by the interstitial infiltrating cells and injured epithelial cells may affect renal hemodynamics through the increased production of vasoconstrictive substances, such as thromboxane A_2 and leukotrienes, and through the decreased production of vasodilators, such as nitric oxide, by the injured proximal tubule cells.[36–38] Evidence for a role for cytokines released by the infiltrating cells in the reduction of renal blood flow and GFR has been advanced from studies of an experimental model of acute obstructive nephropathy that is characterized by acute mononuclear cell infiltrates.[39]

The tubulointerstitial lesions usually are principally localized in the cortex and corticomedullary junction. Cortical lesions predominantly affect either the proximal tubule or the distal tubule. Corticomedullary lesions affect the loop of Henle and the medullary collecting duct. The change in the normal function of each of the affected segments then determines the manifestations of tubular dysfunction (Table 35.2 and Fig 35.3). Lesions principally affecting the proximal tubule result in bicarbonaturia (proximal renal tubular acidosis), glucosuria (renal glucosuria), aminoaciduria, β_2-microglobinuria, phosphaturia, and uricosuria.[1] The latter two events can be valuable in suggesting the possibility of tubulointerstitial disease when the serum phosphate and urate levels are lower than expected in any patient with reduced kidney function. The presence of glucosuria when blood sugar levels are normal should always lead to a consideration of ATIN. The distal nephron segment secretes hydrogen and potassium and regulates the final amount of sodium chloride excreted. Lesions primarily affecting the distal tubule will result in the distal form of renal tubular acidosis, hyperkalemia, and salt wasting. Lesions that primarily involve the corticomedullary junction disproportionately affect the loops of Henle, the collecting ducts, and other medullary structures essential to achieving and maintaining medullary hypertonicity, resulting in different degrees of nephrogenic diabetes insipidus, polyuria, and nocturia. Because of the reduced proximal tubular reabsorption and the disrupted medullary function, a polyuric phase almost always precedes the onset of oliguria in ATIN.[1]

Although this general framework is useful in localizing the site of injury, considerable overlap is encountered

35.2 Causes, Principal Sites of Injury, and Patterns of Tubular Dysfunction in Acute Tubulointerstitial Nephropathies

Site of Injury	Causes	Tubular Dysfunction	Clinical Manifestation
Cortex			
Proximal tubule	Antibiotics Multiple myeloma Immunologic diseases Neoplastic diseases Idiopathic	↓ Reabsorption: Na+, glucose, HCO_3^-, urate, PO_4, amino acids	Glucosuria, hypouricemia, hypophosphatemia, aminoaciduria, alkaline urine, acidemia
Distal tubule	Antibiotics Immunologic diseases NSAIDs Idiopathic	↓ Secretion: H+, K+ ↓ Reabsorption Na+	Alkaline urine, acidemia, hyperkalemia, inability to preserve sodium
Medulla and papilla	Infections Analgesics NSAIDs Disorders of uric acid, calcium, oxalate Immunologic diseases Idiopathic	↓ Reabsorption: Na+ ↓ Concentrating ability	Polyuria, nocturia, inability to preserve sodium

NSAIDs, nonsteroidal anti-inflammatory drugs.

clinically with different degrees of proximal, distal, and medullary dysfunction present in any one patient, all of which usually precede any clinically detectable changes in GFR. In most cases, however, the reduction in GFR and consequent azotemia are the presenting clinical abnormalities calling attention to the acute kidney injury. Preceding or coexistent evidence of tubular dysfunction, unless specifically sought, may go undetected and may either delay the diagnosis or result in the wrong clinical diagnosis. One can only speculate on the number of cases of ATIN that go undetected clinically because of the presence of tubular dysfunction alone in the absence of significant azotemia or oliguria—hence the importance of monitoring for tubular dysfunction in patients exposed to agents known to be associated with ATIN, and the necessity of documenting tubular dysfunction in those with mild azotemia to marshal evidence for the diagnosis of ATIN, before its progression to kidney failure (Table 35.2). Importantly, it is at this early stage of the disease that the lesions of ATIN are potentially reversible and responsive to therapy.[8,9,40]

CLINICAL FEATURES

The clinical presentation of individual cases of ATIN is diverse and varies to some degree depending on the causative factor (Table 35.3). The manifestations best characterized in methicillin-induced ATIN can be considered the prototypically classic clinical manifestations of a hypersensitivity reaction, around which general variation occurs depending on the causative agent and individual variation occurs depending on the particular case encountered clinically.[8,9,21,25]

In most cases, symptoms develop several days or even weeks after exposure to the inciting agent, which in the case of drugs, is not dose dependent. The classic triad of low-grade fever (35% to 70%), fleeting skin rash (25% to 40%), and mild eosinophilia (35% to 60%) described with methicillin-induced ATIN is not always present, and certainly it is less common for all three to occur together. Their documentation depends to some degree on the vigilance with which they are sought because they may be mild and transient. The full triad was noted to be present in approximately 20% of cases of methicillin-induced ATIN, but the triad was noted only in less than 10% of cases of ATIN induced by other drugs in which any one of them (fever, rash, or eosinophilia) is less likely to occur than with methicillin.[8,9,11,41] The skin rash consists of erythematous maculopapular lesions, which often are pruritic, and preferentially affect the trunk and the proximal portion of the extremities. Flank pain or a sense of fullness, reflecting edema-induced distention of the renal capsule, may be present in over one-third of the cases when queried and may be the presenting symptom in some cases.[42] Gross hematuria, another presenting feature, may be present in 5% to 15% of drug-induced ATIN cases. A transient

35.3 Prevalence of Clinical Manifestations of Acute Tubulointerstitial Nephropathies

Systemic		Urinary	
Fever	35%–70%	Gross hematuria	5%–15%
Rash	25%–40%	Micro hematuria	70%–90%
Eosinophilia	30%-60%	Proteinuria (<2 g/d)	70%–85%
Flank pain	25%–40%	Pyuria	70%–80%
Arthralgias	25%–40%	FE_{Na} >1%	~100%
Hypertension	10%–20%	Eosinophiluria	? %

FE_{Na}, fractional excretion of sodium.

eosinophilia, often in methillin-induced ATIN (~60%) and less commonly with other drugs (~30%), is present, but may go undetected unless specifically sought. A history of arthralgia may be elicited in more than one-fourth of cases.[8,9,21]

A urinalysis can be helpful in the diagnosis (Table 35.3). Proteinuria, hematuria, and pyuria are present in most cases. The proteinuria is mild, seldom exceeds 2 g per day, and only rarely is in the nephrotic range, except in those cases due to NSAIDs.[8,9,11] Microscopic hematuria is present in 70% to 90% of cases; rarely, red blood cell casts may be detected. The pyuria is nonspecific except when eosinophils are detected in appropriately prepared and carefully examined urinary sediment.[1,8,9] White blood cell casts occur and are fairly characteristic when they contain eosinophils.[43] Although eosinophils may be observed with a Wright stain of well spun urinary sediment, the use of a Hansel stain on the sediment of an alkalinized urine sample is superior in detecting eosinophils. The mere detection of eosinophiluria is not specific for ATIN.[1,44] The sensitivity of eosinophiluria for the diagnosis of ATIN has been estimated to be about 60% and its specificity has been estimated to be 85%, with a positive predictive value of 38%. These are figures derived from retrospective chart reviews of cases of clinically suspected ATIN but which were not established by a kidney biopsy or definite etiology.[44,45] Eosinophiluria is present in approximately 15% of hospitalized patients, in whom it usually is caused by a variety of other inflammatory diseases of the urinary tract, and in only 14% of those with eosinophiluria is it due to ATIN.[44,45] The presence of eosinophiluria is a better predictor of ATIN when more than 5% of the urinary leukocytes are eosinophils, in which case 40% of the eosinophiluric patients have been estimated to have ATIN, as opposed to an incidence of 3.5% in those in whom less than 1% are eosinophils.[44,45] Other conditions in which an eosinophiluria exceeding 5% may be present are urinary tract obstruction, cystitis, contrast-induced AKI, IgA nephropathy, and cholesterol emboli. Thus, the detection of eosinophiluria, although useful, is neither necessary nor sufficient for the diagnosis of ATIN. It is regrettable that compared to urine examinations for eosinophils in the diagnosis of ATIN, it is rare that attempts are made for the detection of specific tubular dysfunctions characteristic of ATIN such as renal glucosuria, increased fractional excretion of uric acid, an inability to concentrate the urine maximally, and to conserve sodium.[1]

The impairment in kidney function varies, ranging from discrete selective abnormalities of tubular function to frank kidney failure, with or without oliguria.[1,8,9,11] As a rule, increments in blood urea nitrogen and serum creatinine develop after tubular dysfunction is detectable and while the patient is still nonoliguric or even polyuric. Oliguria develops if early features of ATIN, modest azotemia, decrements in estimated GFR, and evidence of tubular dysfunction go undetected and exposure to the offending agent continues. Kidney injury is more likely to occur in older patients and is more severe in those who become oliguric. The duration of the oliguric period is variable, ranging from a few days to several weeks. Supportive renal replacement therapy may be required in approximately one-third of those patients. Reversal of kidney injury and a return to baseline kidney function is the rule in the majority of cases (about 60% to 65%). Irreversible kidney injury can occur but is rare (about 5% to 10%), whereas partial recovery with a persistent impairment of kidney function is relatively more common (10% to 20%), especially in cases where interstitial fibrosis and granulomas are present in biopsy specimens.[8,9,11,21]

Increased kidney size on ultrasonography, reflecting an interstitial edema, is common but nondiagnostic. Radioactive gallium uptake by the kidney, reflecting interstitial cellular infiltration, can be detected in one-third of cases, but lacks diagnostic specificity.[46] This modality may be particularly useful when a kidney biopsy is contraindicated or is difficult to perform in severely ill patients.[46] The lymphocyte stimulation

test can be valuable in the diagnosis of drug-induced ATIN, especially in determining the responsible agent in cases of multiple-drug therapy.[29,30] The serum level of IgE can be elevated and IgE-containing plasma cells have been demonstrated among the renal interstitial cellular infiltrates, providing further evidence for a hypersensitivity reaction.[25]

INCIDENCE AND DIAGNOSIS

The frequency with which ATIN accounts for cases of clinically encountered AKI is difficult to establish. The diagnosis of ATIN is based on the finding of characteristic morphologic features on a kidney biopsy and on the identification of the causative factor. Both of these requirements for a correct diagnosis are fraught with difficulties and limitations, leading to the overall underdiagnosis of ATIN.

Part of the difficulty associated with diagnosing ATIN stems from the relatively low index of suspicion with which its possibility is considered clinically and the general reluctance to perform a kidney biopsy in cases of AKI in general, and in cases of ATIN in particular when symptoms subside after the discontinuation of the causative medications.[7] Almost all cases of ATIN that have been biopsied have been in renal failure. Even if a biopsy is performed, there are difficulties in differentiating the lesions of ATIN from those of ATN, which is a distinct possibility in most cases of AKI in which the prevailing clinical condition could be conducive to either ATIN or ATN.[1,6] An essential differential feature between the two entities is the magnitude of interstitial edema and cellular infiltrates, which are more prominent in ATIN, and the magnitude of tubular cell injury, which is more prominent in ATN (Fig. 35.4). However, given the variable degree to which each of these features may be present in each entity, there is sufficient overlap between the extent of edema and the severity of tubular injury in ATIN and ATN to make it difficult to differentiate among them on morphologic features alone, at least in some cases.[6,45] There also are difficulties associated with identifying the causative factor. Clinically, the removal of a suspected agent followed by the reversal of the lesion strongly suggests the diagnosis of ATIN. This can be particularly convincing in the presence of systemic manifestations of a hypersensitivity reaction, such as fever, skin rash, and eosinophilia in addition to AKI, all of which subside on the removal of the inciting factor or agent. Difficulties arise when the only manifestation is AKI and when a number of corrective measures are instituted simultaneously. Moreover, most patients are on several drugs, and there is the expected tendency to incriminate the most common and better known agents that have been associated with ATIN.[8] The diagnostic limitations of removing agents wrongly surmised as a cause of ATIN have been shown by studies in which drug-induced lymphocyte stimulation testing (DLST) revealed causative agents that had not been suspected clinically.[34]

Most of the figures quoted in the literature on the incidence of ATIN stem from retrospective studies based on kidney biopsies. A review of unselected kidney biopsy specimens reveals a low incidence of ATIN. A review of biopsy specimens from patients with unexplained AKI reveals a frequency that ranges from 8% to 22%,[7,47–50] which generally is higher in the elderly population.[51] Thus, given the very small number of patients with AKI who are subjected to a kidney biopsy, the necessity of a kidney biopsy to diagnose ATIN, and the failure to suspect the diagnosis of ATIN clinically all render conclusions derived from biopsy reports of limited value in estimating the true incidence of ATIN.[7] Furthermore, because of the variable and often subjective reasons for which kidney biopsies are done in different centers, any attempt to compare or combine results from different reports would not be useful. In cases of clinically evident AKI, ATIN probably accounts for approximately 15% to 20% of them.[7–9]

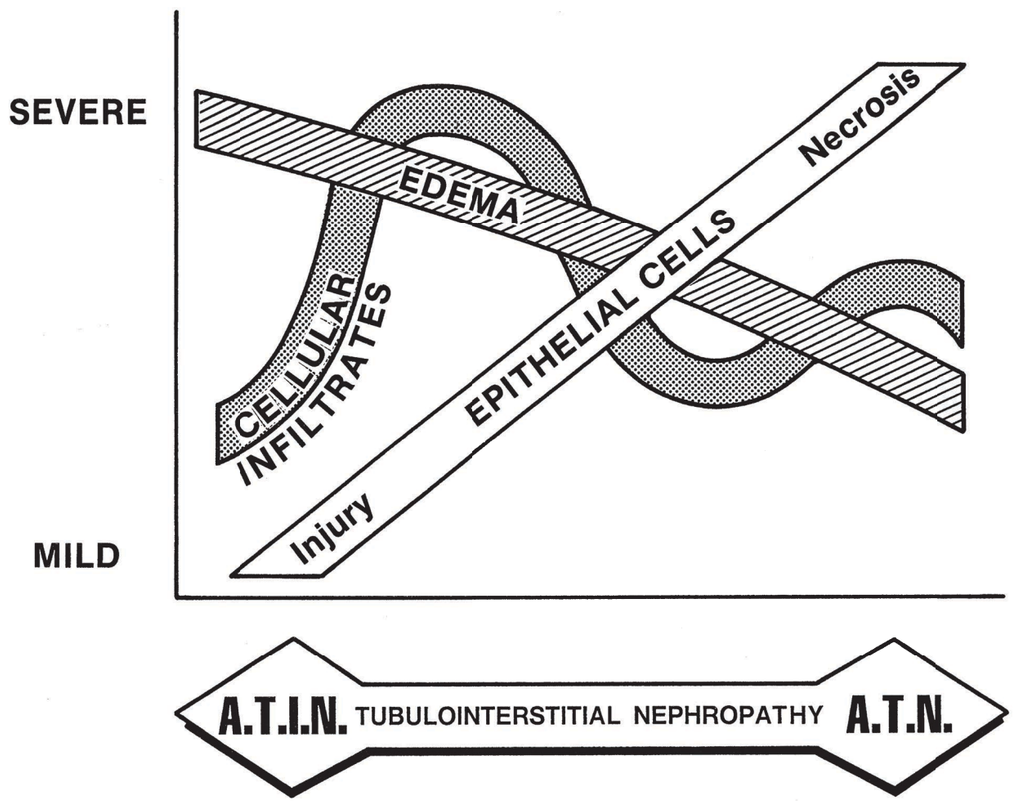

FIGURE 35.4 A schematic representation of the spectrum of structural changes associated with cases of acute renal failure due to acute tubulointerstitial nephritis (ATIN) and acute tubular necrosis (ATN). (Reproduced from: Eknoyan G. Acute renal failure associated with tubulointerstitial nephropathies. In: Brenner BM, Lazarus JM, eds. *Acute Renal Failure,* 2nd ed. New York: Churchill Livingstone; 1988:491, with permission.)

TREATMENT

The primary treatment of ATIN is that of the underlying cause if an infection or a systemic disease and the discontinuation of the suspected offending agent if it is drug-induced. In addition to supportive measures, including dialysis where indicated, it is important to inform the patient in order to ensure the prevention of reexposure to the incriminated drug.

As a hypersensitivity reaction with systemic manifestations in which steroids are often used, patients with ATIN have been treated with corticosteroids.[25] From the outset of reported studies in the 1960s, there has been suggestive evidence that a short course of treatment with steroids improves the kidney lesions and expedites recovery from drug-induced ATIN; however, this is not a uniformly observed response and the use of steroids has been controversial.[46,50]

Unfortunately, no controlled clinical trials are available; all reported studies are retrospective in nature, contributing to the controversy over the use of steroids. In a retrospective evaluation of biopsy-proven cases of ATIN in 2004, the routine use of steroids was not associated with a statistical difference in kidney function or outcome.[47] Recent retrospective studies report a more rapid and complete recovery when steroids are administered early, within 7 to 14 days of developing ATIN, at a time that there is absence or only minimal interstitial fibrosis on a kidney biopsy.[16,21] Nevertheless, the use of steroids should be considered in all patients with persistent kidney injury after the inciting agent has been discontinued, and in those whose biopsy reveals granulomatous lesions that are associated with an increased risk of permanent kidney injury.[1,8,10,21] Reversals of apparently permanent kidney injury after long-term steroid therapy in patients on maintenance hemodialysis have been reported.[1,52–54]

If steroids are used, a response usually is evident relatively early after their initiation. The course of treatment should be brief, and the steroids should be discontinued if no response is observed after 3 to 4 weeks of therapy.[1,16,52] If kidney function improves, steroid therapy should be maintained for 4 to 6 weeks with a slow dosage taper. For patients who fail to respond to steroids or are intolerant of them, a beneficial effect has been suggested from treatment with mycophenolate mofetil.[53] In this report of eight cases of biopsy-proven ATIN, all of whom had failed to respond to 6 months or longer of steroid therapy, treatment with mycophenolate for up to 2 years resulted in an average decline of creatinine levels by 0.3 mg per deciliter. However, given the long duration of the disease in these patients, it is questionable that they can actually be considered as cases of true ATIN. Whether mycophenolate is equal or superior to steroids as an initial therapy for ATIN remains to be addressed.[54]

Among the causes of ATIN, infections and drugs are the most common (Table 35.4).

35.4 Principal Conditions Associated with Acute Tubulointerstitial Nephropathy

Infections
Invasive of renal parenchyma
Reactive to systemic infections
Drugs
Antibiotics
Sulfonamides
Nonsteroidal anti-inflammatory drugs
Other drugs
Systemic diseases
Metabolic disorders
Immune diseases
Neoplastic disease
Idiopathic with or without uveitis

INFECTIONS

Infectious causes of ATIN often are overlooked; as a consequence, their diagnosis is missed and their incidence as a cause of ATIN is underestimated. This occurs for several reasons. First, the introduction of antibiotics led to the eradication of serious streptococcal infections that were first associated with ATIN. Subsequently, the recognition that these antibiotics could also result in ATIN has led to the preferential consideration of anti-infective drugs as the principal cause of ATIN. Second, the hemodynamic changes associated with severe infections and the potentially nephrotoxic antibiotics used for their treatment are usually implicated as a cause of any deterioration of kidney function without an adequate investigation into ATIN. Third is the common but questionable notion that postinfectious acute glomerulonephritis is the most common kidney lesion associated with infections. Finally, modest reductions in kidney function and a return to baseline as the infection is treated limit the clinical consideration given to changes in kidney function. In conclusion, unless the possibility of infection-induced ATIN is considered seriously and the diagnosis is pursued actively, cases of infection-induced ATIN will go undetected, and its true incidence will remain underestimated. Nevertheless, several new causes of infection-induced ATIN have come to be identified since Councilman described their association.

Tubulointerstitial lesions may develop in the kidney either because of direct invasion of the kidney parenchyma by the infective microorganisms or because of reactive changes to a nonrenal systemic infection.

Invasive Infections

The classic and by far the most common example of ATIN is due to direct bacterial invasion of the kidney, although fungal, viral, and parasitic infections also may account for it. The usual forms of pyelonephritis are considered in Chapter 23 and are not detailed here, except to highlight their differential features from ATIN reactive to a systemic infection. Apart from the classic local and systemic manifestations of acute pyelonephritis that clinically differentiate it from reactive ATIN, there are distinct morphologic differences between the two entities that deserve emphasis. Acute bacterial pyelonephritis is a focal rather than a diffuse lesion, which usually is limited to individual pyramids and rarely affects all the pyramids of the kidney. The areas of infection are characteristically wedge-shaped, with the apex directed toward the medulla. The infected foci are focal and sharply demarcated from the adjoining uninfected parenchyma, and lack the tendency to spread laterally.[55] Contrast-enhanced computed tomography scans reveal the typical wedge-shaped, nodular, hypodense areas corresponding to the distribution of these

typical lesions and heavy cellular infiltrates in pyelonephritis, which are quite distinct from the generalized and diffuse changes of ATIN.[46] In the absence of an obstruction, the infection tends to be confined to the originally affected lobule and to resolve gradually over a period of weeks. An acute reduction in kidney function is rare except in severe cases and in those with an obstruction, or when the infection is superimposed on a preexisting chronic kidney disease.[56] As recovery occurs, the initial polymorphonuclear leukocytic infiltrate is replaced by mononuclear cells and the development of streaks of fibrous tissue, which account for the cortical scars encountered in such cases.[57]

Occasional cases of ATIN have been reported in association with otherwise invasive infections such as mycoplasmal pneumonia,[1] toxoplasmosis,[1] salmonellosis,[58] cytomegalovirus infection,[59] infectious mononucleosis,[60] polyomavirus infection,[1] hantavirus infection,[61] Rocky Mountain spotted fever,[1] leptospirosis,[62] Legionnaires' disease,[63] candidiasis,[1] cryptococcus,[64] Yersinia pseudotuberculosis,[65] Ascaris lumbricoides,[66] hepatitis A virus,[67] and adenoviral infection.[68] A greater number of such cases are now encountered clinically and recorded in the literature because of the increasing number of immunocompromised hosts such as patients with AIDS, transplant recipients, and the elderly. Although passage of the implicated organisms through the renal parenchyma and their isolation from the urine has been demonstrated, their localization in the kidney is less well substantiated. The interstitial cellular infiltrates are mononuclear cells rather than polymorphonuclear cells, and evidence has been advanced for an immunologic mechanism responsible for the renal reaction elicited by some of them.[69] Antigenic material has been demonstrated in the renal parenchyma of humans with ATIN associated with some of these infectious agents, such as Mycoplasma, polyomavirus, the rickettsiae responsible for Rocky Mountain spotted fever, and leptospira.[1,70] The complex nature of the lesions encountered in some of these infections can be better appreciated from a specific consideration of some of them.

Leptospirosis

Acute kidney injury occurs in almost half the cases of leptospirosis, and the degree of kidney involvement often determines the gravity of the disease. In fact, in endemic areas of the disease, leptospirosis is one of the most common causes of AKI.[1] Although there might be evidence of renal involvement in the preicteric phase of the disease, it is in its icteric phase that ATIN develops in conjunction with a rapid deterioration of kidney function. The renal lesions of leptospirosis are limited exclusively to the tubules and the interstitium. They are focal in distribution and are most pronounced at the corticomedullary junction, a structural feature characteristic of ATIN lesions that are reactive to infections. Initially, interstitial edema and cellular infiltration are minimal but ultimately become prominent, such that the whole kidney becomes enlarged, congested, and soft. The infiltrates consist of mononuclear cells, plasma cells, and eosinophils. The interstitial lesions may be present even in the absence of clinically detectable kidney injury. The tubular lesions occur during leptospiral migration through the renal parenchyma, and during this stage the spirochetes can be detected in the urine. Thus, whereas direct tubular injury by Leptospira may account for some of the renal lesions, evidence exists for a leptospiral antigen-mediated immunologic mechanisms of injury.[70–72] Leptospiral outer membrane proteins and lipoproteins have been implicated in causing tubular dysfunction and initiating immunologic injury by inhibiting Na^+-K^+-Cl^- cotransporter activity and messenger RNA (mRNA) synthesis in the thick ascending limb.[71,72] This could account for the polyuria and hypokalemia frequently seen in these cases. Furthermore, the experimental exposure of cultured proximal and thick ascending limb cells to the leptospiral outer membrane proteins results in the activation of a proinflammatory cascade, with increased induction gene expression of nuclear factor-kappaB (NF-κB), iNOS, MCP-1, and TNF-α.[70,71] The superimposition of shock and volume depletion in severe cases of the disease may further contribute to the tubular lesions, and the structural changes observed may represent the cumulative effect of both direct invasion and ischemia, possibly coupled with an immune injury.[1]

Although the interstitial changes may persist for several months in an occasional patient, usually with persistent spirochetes in the urine, in most cases the renal changes subside within a few weeks after the resolution of the disease with appropriate antibiotic therapy. In these usual cases, kidney function returns to normal, whereas in those who develop a chronic carrier state the leptospira localize in the kidney, fibrosis and tubular atrophy develop, and permanent impairment of kidney function ensues.[1,73]

Hantavirus Infection

The once mysterious entities of "hemorrhagic fever with renal syndrome" in Asia and "nephropathia epidemica" in Europe are now recognized as due to infection with one of the related RNA viruses of the Hantavirus genus, which belong to the family of Bunyviridae. The availability of serologic tests for the specific antibodies, which appear early in the course of the infection and may persist indefinitely, now allows for the easier diagnosis of this once enigmatic disease that mimics several of the features of leptospirosis.[74,75]

The renal lesions consist of the classic changes of ATIN. They are most prominent in the outer medulla and the corticomedullary junction, and together with the hallmarks of Hantavirus nephropathy include vascular congestion and interstitial hemorrhage.[74] This accounts for the microhematuria and, because of the enlarged kidneys, for the back pain present in most cases. The transient heavy proteinuria (>2 g per day) noted at the onset of the disease correlates with the urinary excretion of IL-6.[76] The severity of the lesions and the frequency of renal injury are higher and the prognosis is worse in the Asian than in the European and American varieties of the infection.[74,77]

Polyoma Virus

Polyoma is a double-stranded DNA virus family, one strain of which, the BK virus, commonly causes ATIN. Typically, the virus is acquired in childhood but remains latent—over 70% of adults are seropositive.[78] However, when the immune system is compromised, as in AIDS patients and transplant recipients, the virus reemerges and causes ATIN.[79,80] Its incidence in kidney transplant recipients is reported to be 3% to 5%, with an associated 10% to 50% graft loss among those affected.[81,82] It is considered to result from overimmunosuppression; thus its incidence has increased with the use of newer, more potent immunosuppresants. Decreasing immunosuppression has been effective in the management of some cases but is associated with increased episodes of acute rejection.[81,82]

The diagnosis of BK virus nephropathy (BKVN) is based on findings on a kidney biopsy that are distinct from those of acute rejection. This includes tubulitis with an abnormal chromatin pattern in the tubular epithelium and intranuclear viral particles on an electron microscopy.[82] The finding in the urine of cells that have viral inclusions, so-called decoy cells, are indicative of viral replication in the urinary tract. Quantifying viral DNA load is potentially useful for the detection of significant viral reactivation, and can serve as a quantifiable surrogate marker of the course of the infection.[81] Biopsy-proven BKVN is always associated with greater than 10^4 viral copies per milliliter.[82] There are reports of another polyoma virus (the JC virus) associated with ATIN in renal transplant recipients; however, this strain is considered less virulent than the BK virus.[82]

Candidiasis

Disseminated candidiasis almost always affects the kidney. Candida albicans causes two types of lesions in the kidneys. The first type, which is relevant to this chapter, occurs during the candidemic phase with the initial seeding of the kidney, which rapidly elicits an inflammatory reaction with polymorphonuclear infiltration limited to the renal cortex in 52% to 82% of cases.[83] Depending on the severity and the extent of the lesions, pyuria and candiduria may be the only findings, with varying degrees of renal insufficiency in approximately half the cases. When multiplication extends to the tubular lumen, they may invade the interstitial tissue and elicit a severe interstitial inflammatory reaction in the medulla.[1]

The second type of lesion develops in the more chronic form of the infection and results from the multiplication of the organisms in the urinary tract, where no limiting inflammatory reaction can be elicited. This can result in the proliferation of the organisms into mycelial filaments that form occluding bezoars of Candida in the renal pelvis and calyces resulting in obstructive nephropathy, hydronephrosis, and chronic tubulointerstitial nephritis.[84]

Hepatitis A Virus

Acute hepatitis A (AHA) is one of the most common infectious diseases worldwide; it is usually self-limiting and primarily affects the liver.[69,85] Extrahepatic manifestations, especially kidney involvement, is rare but has been reported with fulminant or nonfulminant AHA. In one series, 7.2% of patients with AHA presented with AKI, and 33% of this group underwent kidney biopsy. The common histopathologic lesions included acute tubular necrosis and ATIN.[85] This is far different from the well-described immunologically mediated glomerular lesions of hepatitis B and C. The mechanism of AHA-associated AKI is unknown, but has been attributed to bile salt nephrotoxicity. In most reports, the clinical course is benign, although renal recovery can be substantially delayed.[69,85]

HIV

Patients with AIDS constitute a special challenge in the diagnosis of ATIN, not only because of their propensity to contract unusual infections,[86] but also because of the number of drugs they consume, such as protease inhibitors and sulfamethoxazole, which can cause ATIN.[87,88] Acute tubulointerstitial nephritis without other features of HIV-associated nephropathy is reported in 11% of HIV-positive patients who undergo a kidney biopsy.[85] Sometimes, this lesion is encountered in the setting of immune reconstitution inflammatory syndrome (IRIS), a multiorgan system disorder occurring at the initiation of antiretroviral therapy.[89] Tubulointerstitial nephritis in HIV-positive patients commonly presents with normotension, pyuria, and minimal proteinuria.[87,88] The renal lesion consists of a diffuse lymphocytic interstitial inflammation and tubulitis, with sparing of the glomeruli.[90] Prior to the availability of antiretroviral therapy, HIV-positive patients with AKI were often treated with corticosteroids. In those with an improved renal function after steroids, repeat biopsies showed the resolution of concurrent interstitial nephritis.[91]

Noninvasive Infections

Acute tubulointerstitial nephritis may complicate infections in the absence of bacteremia and parenchymal invasion of the kidneys. Before the availability and widespread use of antibiotics, ATIN was a relatively common complication observed at the autopsies of cases of β-hemolytic streptococcal infections.[1,3,4] Although the lesions of ATIN were noted to be more common in cases of scarlet fever,[3] the more severe destructive lesions occurred in cases of diphtheria.[4] The availability of specific vaccines has eradicated diphtheria, whereas specific antibiotic therapy has ameliorated the severity of streptococcal infections such that, although renal lesions continue to occur, their clinical outcome is now more favorable. Unlike the relatively later renal changes of streptococcal infections, which occur in the 2nd or 3rd week after the onset of the infection and cause typical postinfectious acute proliferative glomerulonephritis, the lesions of ATIN usually occur early in the course of the infection, often between the 9th and 12th day of the disease, and in the absence of edema or hypertension.[1,3,4]

In contrast to the detailed morphologic changes reported in the early literature describing this entity, the clinical

information provided is sparse. Where noted, proteinuria and pyuria were said to be common.[4] In a careful study of the urinary sediment of 14 children with scarlet fever, abnormal sediment was reported to be present in all cases. In larger series, the urinary sediment is noted as being abnormal in only 50% to 60% of the cases.[1]

The lesions described by Councilman[2] are much less rarely noted in the current literature, and most of the available information on them dates back to the preantibiotic or early antibiotic periods, although they continue to be encountered, particularly in children,[92] and must be considered in streptococcal infections with AKI in the absence of hypertension and edema, and with normal complement levels.[93]

Drugs

Drugs have emerged as the most common cause of ATIN. Drug-induced ATIN has been observed in 6.5% to 27% of all renal biopsy samples when only cases of unexplained AKI are considered.[7,21] First described in conjunction with the sulfonamides used in the 1940s, the lesion was next reported to occur during penicillin therapy in the 1950s, and was ultimately best characterized in patients treated with methicillin in the 1960s.[8,9] Since then, the number of drugs implicated as a cause of ATIN continues to increase. The most common causes of drug-induced ATIN are antibiotics and NSAIDs.[8,94] Although the implicated mechanisms and the general morphologic feature on a kidney biopsy are the same as those described with methicillin, the clinical presentation of drug-induced ATIN is variable with other drugs. Symptoms range from asymptomatic to constitutional (fevers, chills), flank pain, or arthralgias. The presentation is usually nonoliguric, and rarely with the triad of eosinophilia, fever, or skin rash.[21] The blood may show elevated IgE or an increased sedimentation rate, and the urine may contain red blood cells or white blood cell casts.[12,13,21]

The association of ATIN with most of the incriminated drugs is quite rare and often based on individual case reports. The evidence for a causative role of the drug is merely circumstantial, particularly in the absence of a kidney biopsy. Even in cases where a kidney biopsy is available, the possibility of ATIN due to the underlying infection or of ATN due to the hemodynamic instability of infected patients could make an interpretation of the biopsy difficult and the attribution of ATIN to the implicated drug suspect, especially in instances where only a single case is reported in the literature. In cases where a kidney biopsy is not available for documentation, the acute deterioration of kidney function and abnormal urinary sediment, even in the presence of the eosinophiluria used to implicate ATIN, may be due to any of the other possible causes, such as preexisting kidney disease, acute tubular necrosis, postinfectious glomerulonephritis, or a direct nephrotoxic effect of the implicated agent or one of the coadministered drugs. The latter possibility is particularly confounding because any one of the multiple agents used in the treatment of most patients can be responsible for ATIN, even when least suspected.[1,34]

Drug-induced ATIN is an idiosyncratic hypersensitivity reaction, as evidenced by several facts: It occurs in only a small number of people exposed to the drugs; it is not dose-related; it often is associated with other systemic manifestations of hypersensitivity such as fever, skin rash, eosinophilia, and arthralgia; the reaction recurs on reexposure to the same drug or one of its congeners; circulating antibodies to the incriminated drugs have been demonstrated in some instances; and laboratory evidence for a hypersensitivity reaction has been documented in some cases. In addition, the structural features of individual drugs (propionic acid derivatives) or those common to some groups of drugs (β-lactams) do seem to predispose one to the development of ATIN. Histologically, drug-induced ATIN can be discriminated from other types of AKI due to the presence of a T lymphocyte rich interstitial infiltrate.[95] The immunogenicity of drugs is thought to depend on their ability to form covalent bonds with larger proteins to form a hapten-carrier complex, which is an immunogenic molecule that stimulates a T- and B-cell immune response.[25,95]

Antibiotics

Of the several antibiotics that have been incriminated as a cause of ATIN, most share the β-lactam structural ring—penicillin, its derivatives, and the cephalosporins.

Methicillin

Methicillin is the agent with the highest incidence of drug-induced ATIN and the one in which the clinical features of this side effect of antibiotics are best characterized.[1,8,11] The mean period of drug administration before the renal lesions occur is approximately 15 days, with a reported range of 10 to 45 days. The incidence of ATIN has been estimated to be approximately 2% of those exposed to the drug and increases to 15% in those who are exposed to it for over 2 weeks.[96] In those who experience a reaction, subsequent exposure to methicillin results in the recurrence after a much shorter period of exposure and at lower doses of the drug. With the reduced clinical use of methicillin, there has been a reduction in the number of cases encountered clinically and reported in the literature.

Systemic manifestations of a hypersensitivity reaction are a dominant feature of methicillin-induced ATIN, and are more evident than in most other drug-induced ATIN (Table 35.3). Characteristically, drug-induced fever appears after the initial fever caused by the infection has subsided following the initiation of methicillin therapy. The recurrent episode of fever usually is accompanied by a skin rash, eosinophilia, and arthralgias. The skin rash is fleeting and consists of erythematous, often pruritic, maculopapular lesions that are present in 30% to 50% of cases. Eosinophilia is common (60%) but transient, as is the eosinophiliuria. Arthralgias are nonspecific and less common (10% to 20%) than other systemic manifestations.[1,8,9,21]

As a rule, patients are polyuric as kidney function begins to deteriorate, and when the AKI is first detected, it is nonoliguric in two-thirds of patients. Independent of

the severity of the AKI, the outcome is favorable after the withdrawal of methicillin in the majority of cases. The initial improvement, which is rapid over the first few weeks of discontinuing methicillin, is followed by a slower return of kidney function to normal in most (greater than two-thirds of cases), but only to near normal in some 20% of cases.[11,21] Instances of persistent kidney injury are rare (<10%), and are more likely in those whose kidney biopsy reveals diffuse infiltrates, interstitial fibrosis, granulomatous reaction, and severe tubular injury.[97,98] Recovery is faster and more likely in those whose dominant kidney lesions consist of interstitial edema rather than cellular infiltrates, and when the cellular infiltrates are focal rather than diffuse.

A metabolic derivative of methicillin, dimethoxyphenylpenicilloyl, has been implicated as the inciting antigen on the basis of its demonstration by immunofluorescence techniques along the TBM and the detection of circulating antibodies to dimethoxyphenylpenicilloyl in cases of ATIN.[99] However, neither of these findings appears to be specific to the incidence of ATIN because both have been shown to be present in methicillin-treated cases with no evidence of acute kidney injury.[1]

Rifampin

Since the introduction of rifampin (rifampicin) as an antituberculous agent in 1976, over 150 cases of ATIN have been ascribed to it in the literature. As a rule, AKI occurs during intermittent administration of the drug (once or twice weekly), and usually appears several months after the institution of therapy. A more abrupt onset occurs with rifampin readministration after a hiatus in its previous daily, uneventful use. ATIN during continuous daily rifampin therapy also has been noted. However, most cases reported have occurred during intermittent therapy, and the number of cases noted has decreased since the intermittent use of the drug has been abandoned.[100,101]

Circulating antibodies to rifampin have been detected in most, but not all, affected patients, as well as in the serum of those without any adverse reaction to the drug. The suggestion has been made that the kidney lesions develop in response to a critical level of antigen–antibody complexes that accumulate during intermittent rifampin therapy.[102] However, immunofluorescence staining of kidney biopsy specimens for deposits of antibodies to rifampin has been negative in most cases, although it has been positive in an occasional case. A role for cell-mediated immune injury appears more likely based on in vitro studies of lymphocyte activation on exposure to rifampin.[103]

A direct nephrotoxic effect that is slower in onset has been reported with rifampin. A kidney biopsy in such cases reveals marked proximal tubular injury but very little interstitial infiltration.[100,101] Actually, the majority of lesions of rifampin-induced AKI are those of ATIN. Occasionally, glomerular lesions have also been reported. Certainly, the accompanying systemic manifestations—eosinophilia, recurrence on challenge, the reported humoral studies, as well as the morphologic features seen on kidney biopsy—are strongly supportive of ATIN due to an allergic hypersensitivity reaction to the drug as the most common lesion of rifampin-induced AKI. The severity of AKI due to ATIN is variable. Dialysis may be required in some patients in whom prolonged oliguria develops. The course of renal injury usually is favorable, with the return of kidney function to normal in most cases after the withdrawal of rifampin, although a residual impairment of renal function may occur in some. The role of steroids in accelerating recovery from rifampin-induced ATIN is undetermined. The lesion has occurred in a patient who was receiving steroids for other reasons.[1]

Penicillin and Congeners

Acute tubulointerstitial nephritis that is clinically and pathologically undifferentiable from that described with methicillin also occurs with penicillin and its congeners, but is much less common than with methicillin. The dose of penicillin used has been fairly large, 12 to 60 million U per day, although ATIN has been reported at lower doses and even after a single dose.[104]

Other penicillin derivatives that have been associated with the lesions of ATIN are, in descending order of frequency, ampicillin, oxacillin, carbenicillin, nafcillin, and oxacillin and amoxicillin.[102,105]

Cephalosporins

Acute tubulointerstitial nephritis also has been noted with the cephalosporins. It is more common with cephalothin, followed in descending order of frequency by cephalexin, cephradine, cefoxitin, cefazolin, cefaclor, cefotaxime, cefotetan, cefoperazone, cephaloridine, cefamandole, ceftriaxone, and cephapirin.[8,106–109]

Linezolid

Linezolid is the first member of the oxazolidinone class and is a generally well-tolerated oral agent active against most methicillin-resistant gram-positive bacteria. It has been linked with an increasing number of hypersensitivity reactions, including ATIN. In reported cases, ATIN developed within 2 weeks of initiating the drug, often with systemic manifestations (rash, fever, eosinophilia, arthralgias), and with renal function recovery with corticosteroids.[110] Most linezolid-associated systemic hypersensitivity adverse effects correlate with the duration of treatment.[111]

Sulfonamides

The introduction of sulfonamides in the 1930s heralded a new and welcome era in the specific treatment of infectious diseases. Despite their usefulness and general safety, severe adverse reactions were quite common with these agents and often necessitated cessation of their use. The introduction of antibiotics during the following decade led to a consistent decline in the use of sulfonamides until the introduction of trimethoprim–sulfamethoxazole (TMP/SMX),[112] when a resurgence of adverse reactions attributed to sulfonamides

was observed, necessitating modification of the warnings in the labeling of TMP/SMX. The advent of AIDS with the associated prophylactic use of TMP/SMX,[112] as well as its use in transplant recipients,[113] has led to the increased incidence of these side effects.[114,115]

The renal effects observed with the initially available, less soluble sulfonamides (sulfathiazole, sulfapyridine, sulfadiazine) were due to the precipitation of sulfa crystals in the renal tubules, with consequent hematuria, obstructive uropathy, and kidney injury. The renal lesions consisted of focal tubular necrosis, sometimes with sulfa crystals noted in the lumen, and with peritubular interstitial infiltration and edema in the affected segments.[116] The introduction of more soluble sulfonamides (sulfisoxazole, sulfamethoxazole, sulfamerazine, sulfasalazine) made this complication less common, but by no means eliminated it.[117,118] An idiosyncratic hypersensitivity reaction as a cause of the acute renal injury, which was recognized from the outset, now is the principal cause of the renal adverse effects of sulfonamides. The symptoms develop at a mean of 14 days after the initiating therapy. The onset of AKI is generally preceded by fever and skin rashes of varying severity. Eosinophilia is an accompanying finding in most cases. Unlike antibiotic-induced ATIN, there may be evidence of multiple organ involvement (heart, liver, lungs) in patients who have sulfonamide-induced ATIN. The kidney injury is reversible after the withdrawal of the drug and the institution of steroid therapy, although residual fibrosis and tubular atrophy with persistent impairment of renal function may occur.[117,118] The predisposition of patients to idiosyncratic reactions has been attributed to individual differences in the metabolism of sulfonamides. Those who develop idiosyncratic reactions are slow acetylators of sulfonamide, which leads to the accumulation of drug metabolites that can covalently bind to cell macromolecules and can cause cell injury, or can elicit a secondary immunologic phenomenon.[25] Acute kidney injury due to a hypersensitivity reaction also occurs with TMP/SMX. The increase in serum creatinine that occurs with this agent does not necessarily indicate renal injury because trimethoprim interferes with the tubular secretion of creatinine, with a consequent increase in the serum creatinine level, without necessarily affecting true glomerular filtration rate. The clinical and morphologic features of ATIN induced by TMP/SMX are identical to those induced by other sulfonamides and are due to the sulfamethoxazole content. Most of the reported cases of TMP/SMX-induced ATIN have been in patients with reduced kidney function or in transplant recipients. The propensity of patients with reduced kidney function to ATIN has been attributed to the accumulation of a toxic metabolite, which predisposes them to the adverse effects of the drug, much like that noted in the genetically predisposed slow acetylators. Crystalluria occurs with the use of high doses of TMP/SMX. In patients with severe renal insufficiency, this could cause further deterioration of kidney function without necessarily eliciting an allergic reaction.[1,114,115]

Other Antibiotics

The incidence of ATIN with other antibiotics is much less than that encountered with methicillin, penicillin congeners, and rifampin, except for ciprofloxacin, with which an increasing number of cases continue to be reported.[119–121] Isolated instances of ATIN have been reported with minocycline, doxycycline, gentamicin, polymyxin, vancomycin, lincomycin, mezlocillin, chloramphenicol, erythromycin, flurithromycin, netilmicin, norfloxacin, tetracycline, ethambutol, telithromycin, moxifloxacin, and levofloxacin.[1,8,9,121,122]

Nonsteroidal Anti-Inflammatory Drugs

NSAIDs provide a relatively effective approach to the treatment of musculoskeletal pain and inflammation, which are among the most common complaints of an aging population. As a result, NSAIDs have become one of the most widely prescribed category of drugs. Recent reports of an increased relative risk for cardiac death associated with NSAIDs are bound to have a dampening effect on their widespread use.[123,124]

The most serious untoward effects of NSAID use have been noted in the gastrointestinal tract and the kidney. The renal effects include a reduction in GFR and renal blood flow, acute and chronic renal injury, and abnormalities of sodium and potassium homeostasis.[123,125] By far the most common of these side effects is the hemodynamically mediated, reversible AKI related to the prostaglandin-inhibitory action of these agents, which occurs principally in patients with congestive heart failure, cirrhosis of the liver, nephrotic syndrome, septicemia, and volume depletion from any cause. Less common has been the development of AKI due to tubulointerstitial nephritis, which, unlike other drug-induced ATINs, is frequently associated with massive proteinuria. A necrotizing vasculitis also has been reported in a few cases in association with fenoprofen, indomethacin, and diclofenac.[1,126]

The duration of exposure to NSAIDs before the onset of ATIN is variable, ranging from 2 weeks to 18 months; as a rule, ATIN develops after prolonged exposure (mean: 5.4 months) to the incriminated agent.[8,9,21] The propionic acid derivatives fenoprofen, ibuprofen, and naproxen account for approximately three-fourths of the cases reported.[124,126,127] Fenoprofen alone has been incriminated for over one-half of the reported cases. The lesions have been noted after the self-administration of over-the-counter ibuprofen, of mefenamate in drug mixtures, and to recur after exposure to different NSAIDs of the same group or on reexposure to the same agent.[128] Other NSAIDs that have been incriminated include, in decreasing order of frequency, tolmetin, zomepirac, indomethacin, diclofenac, and diflunisal.[1,8] Isolated cases have been reported with the use of phenylbutazone, mefenamate, phenazone, sulindac, noramidopyrine, flurbiprofen, and piroxicam.[8] Contrary to initial expectations, they also occur with the selective cyclooxygenase-2–inhibitory agents such as celecoxib, rofecoxib, and nimesulide.[9,21]

The proteinuria and clinical manifestations of the nephrotic syndrome usually are of insidious onset and precede the

onset of azotemia. Nephrotic syndrome, without renal insufficiency, may be the only manifestation in approximately 10% of cases. On the other hand, kidney injury without nephrotic syndrome occurs in approximately 15% of cases.[8,9,11,126] The variation in clinical presentation has been attributed to the severity of the allergic hypersensitivity reaction, with a more rapid course presenting with reduced kidney function and nephrotic syndrome in hyperreactors.[127] The clinical features of hypersensitivity reaction (fever, rash, eosinophilia) occur in less than 20% of cases, compared with an incidence of over 80% in patients with methicillin-induced ATIN. The serum complement and IgE levels usually are normal, and anti-TBM antibodies are absent. Most of the patients reported have been older than 60 years of age, which is more a reflection of the greater use of NSAIDs in this age group than of age as a predisposing factor.[124] In the diagnosis of proteinuria due to NSAIDs, it should be kept in mind that tolmetin can produce pseudoproteinuria, due to the effect of its dicarboxylic metabolite on the detection of proteinuria by the acid precipitation (sulfosalicylic acid and trichloroacetic acid) methods. In such cases, no proteinuria is detected with dye-impregnated reagent strips, electrophoresis, or nephelometry.[1]

A histologic examination of the kidney reveals the characteristic changes of ATIN. In some cases, the proportion of infiltrating B lymphocytes and CD8+ cells has been more than that noted in other forms of ATIN. Eosinophils have been observed in one-third of the cases reported and may constitute up to 20% of the infiltrating cells, even when eosinophiluria is absent. The glomeruli are minimally altered, if at all, except for the effacement of the podocyte foot processes consistent with minimal-change disease. Mesangial electron-dense deposits have been noted in rare instances in association with modest membranous and subendothelial deposits. Membranous glomerulonephritis, reversible after drug withdrawal, has been reported with sulindac and other NSAIDs. Immunofluorescent microscopy is negative, although a slight, nonspecific focal immunofluorescence of the glomeruli and TBM occasionally has been noted. Granulomatous reactions occur in severe cases.[8,9,21,127]

The kidney injury, although insidious in onset, can be severe enough on clinical presentation to require supportive dialytic therapy in approximately a third of the patients. The severity of the kidney injury may stem from the additive effects of ATIN, reduced renal blood flow due to the inhibition of prostaglandin, altered glomerular permeability, and an absence of systemic effects, the latter of which leads to delayed diagnosis early in the course of the disease process until patients finally present with nephrotic syndrome and AKI. The response to discontinuation of the offending NSAIDs has been favorable in most cases, with improvement of kidney failure occurring within a few days, but with a slower subsidence of proteinuria over weeks or months. Cases of persistent kidney injury have been reported. Progression of the kidney lesion to focal glomerular sclerosis has been noted on repeat kidney biopsies in some patients.[1,127] Steroids have been used in the treatment of severe cases. The response to steroid therapy, if any, appears to be slow, and the value of corticosteroids in this entity remains questionable.[1,8]

The pathogenesis of NSAID-associated ATIN remains undetermined. The rarity of the lesion despite the widespread use of these agents (estimated at 1 in 5,300 patient-years of treatment with fenoprofen) supports an idiosyncratic reaction.[124]

Protease Inhibitors

Since 1995, nine protease inhibitors have been approved for the treatment of HIV infection: saquinavir, ritonavir, tindinavir, nelfinavir, lopinavir, atazanavir, fosamprenavir, tipranavir, and darunavir. ATIN has been reported with two of these agents: indinavir and atazanavir. The former is primarily known for its association with crystal nephropathy. It is possible that the crystalluria (even if asymptomatic) triggers an inflammatory response significant enough to cause ATIN.[88]

Atazanivir is a newer protease inhibitor, which has been linked to some 10 cases of ATIN since its approval in 2004.[129] In one biopsy series of 22 HIV positive patients on antiretroviral therapy, 3 of the 4 cases of ATIN were associated with use of atazanavir. All 3 cases resolved after the discontinuation of this agent without the use of steroids.[130]

Phenindione

An increasing number of hypersensitivity reactions were noted with the anticoagulant phenindione after its introduction in 1947. The more common reactions were skin rash, fever, diarrhea, blood dyscrasias, stomatitis, and hepatitis, in descending order of frequency. The reactions appeared after 3 to 5 weeks of therapy and continued to progress even after discontinuation of the drug, proving fatal in one-half of the cases. AKI caused by ATIN, which was granulomatous in some cases, also was reported in approximately 30 cases, usually coincident with the development of jaundice and hepatotoxicity. ATIN was accompanied by the classic triad of skin rash, fever, and eosinophilia, usually with a prolonged period of oliguria and death in one-third of the cases reported, chronic kidney disease in some, and massive proteinuria in others.[8,9]

Because of the high incidence (10%) of these adverse side effects and their severity, this agent was prescribed much less over the years, leading to the cessation of its manufacture in 1983 for economic reasons. It is, however, still available in some countries and as an investigational drug in the United States. Phenindione is an inanedione derivative, or a vitamin K antagonist. Newer agents from this class (fluindione, anisoindione, diphenadione) remain in use and have been linked with ATIN.[131]

Allopurinol

Severe hypersensitivity reactions to allopurinol have been reported since its introduction in 1963. They have the features of a diffuse vasculitis involving multiple organs.[132] The symptoms appear approximately 4 weeks after the initiation

of therapy and consist of fever, rather severe generalized skin rashes that may be desquamative, eosinophilia, hepatic failure, and prolonged oliguric kidney injury.[1,132]

The structural changes in the kidney are characteristic of ATIN, including those of a granulomatous reaction in severe cases. Although renal lesions have been reported in patients with normal kidney function, most cases have occurred in either patients with preexisting kidney disease or in those receiving other drugs that might themselves cause ATIN. The decreased excretion of a toxic metabolite of the drug, possibly oxypurinol, has been implicated as the inciting agent in those with reduced kidney function. A predisposing role of thiazides, furosemide, and ampicillin has been implicated in those who have experienced a reaction while receiving other drugs.[1,21,132]

H_2-Antagonists and Proton Pump Inhibitors

H_2-Antagonists, including cimetidine and ranitidine, have been used to treat acid-related gastrointestinal disorders since 1976. Proton pump inhibitors (PPIs) are also used to treat acid reflux and have been in use since 1989; this class of drug is now more widely used because of its demonstrated superiority. Both classes are associated with drug-induced ATIN.[8,21]

The renal lesions reported with cimetidine are patchy, with focal areas of tubule cell injury and interstitial inflammatory infiltrates consisting of lymphocytes and a few eosinophils.[133] In one carefully studied case report, the presence of infiltrating IgE-producing plasma cells strongly implicates cimetidine in a cell-mediated immune reaction.[134] ATIN has also been reported with other H_2-antagonists such as ranitidine.[41,133,135,136]

Omeprazole was the first PPI to be introduced, and now six different PPIs are available. ATIN has been reported with five of them: lansoprazole,[137] omeprazole,[48,138–140] rabeprazole,[141] esmoprazole,[142] and pantoprazole.[143] The first case of PPI-induced AIN was described in 1992, and now over 100 cases have been ascribed to this class of drugs.[9,21] Fewer than 10% of PPI-induced ATIN present with the classic symptoms of the hypersensitivity syndrome (fever, rash, eosinophilia); most patients report nonspecific complaints such as weakness, fatigue, malaise, and anorexia.[122,141,142] Eosinophils are identified in up to 83% of the renal biopsy samples.[21,141] In one series of 18 biopsy-proven cases of PPI-induced ATIN, reduced kidney function remained present at 6 months.[144]

Phenytoin

One of the adverse side effects associated with the anticonvulsant phenytoin, formerly known as diphenylhydantoin, is a delayed hypersensitivity reaction characterized by a systemic reaction and ATIN. The systemic manifestations include fever, hepatitis, myositis, lymphadenopathy, skin rashes that may be exfoliative, eosinophilia, and anemia. Nephrotic syndrome has been observed in some patients. The lesions are reversible on withdrawal of the drug; steroid treatment has been successfully used in most reported cases.[145,146] Circulating antibodies to human TBM were detected, and linear deposits of anti-TBM antibodies demonstrated along the renal TBM in two cases.[147]

Diuretics

An acute reduction in kidney function often is associated with the use of diuretics. The renal insufficiency that results usually is prerenal in origin and is a result of the compensatory mechanisms mediated by extracellular fluid volume depletion induced by the diuretics. Less commonly, the deterioration of kidney function is the result of other mechanisms, including direct renal toxicity, such as with mercurial diuretics; obstruction, such as with triamterene nephrolithiasis; necrotizing vasculitis, such as with thiazides; and occasionally, ATIN that has been noted to occur with furosemide, thiazides, triamterene, chlorthalidone, ethacrynic acid, tienilic acid, and indapamide.[8,148] Most of the reported cases have been associated with the use of thiazides and furosemide, both of which are structurally related to sulfonamides, another group of drugs associated with ATIN. A potentiating role of triamterene used in conjunction with thiazides or furosemide has been suggested.[149]

Several of the patients with diuretic-induced ATIN had preexisting kidney disease, although the lesions also are noted in patients with normal kidney function. The deterioration of kidney function usually is insidious in onset but may be rapid and lead to oliguric AKI. Systemic symptoms of drug-related hypersensitivity reaction (fever, skin rash, and eosinophilia) were present in almost all the patients reported. The kidney biopsy findings were those of classic ATIN, with a granulomatous reaction in an occasional case. Abatement of symptoms and a return of kidney function to baseline have occurred in all patients reported after the removal of the incriminated diuretic and the administration of steroids to some patients.[1,8,148]

Given the frequency with which diuretics are prescribed, the fact that less than 20 cases of ATIN have been reported with their use reflects the relative rarity of diuretic-induced ATIN.

Alternative Medicines

Alternative or traditional medicines are used by a growing number of patients for preventive and palliative care. Due to a lack of professional surveillance, specific data on systemic and kidney toxicity are not easily available. Furthermore, the use of alternative medications is not always disclosed to the physician. The alternative medicines reported to cause ATIN include: cape aloes, uno degatta, taxus celebrica, Tung shueh, ephedra, glycyrrhiza, hypericum, and ledum. Most associations are via case reports or case series with fewer than 10 patients. It is possible that the cause of AKI is adulteration of these drugs with known nephrotoxins, such as NSAIDs or aristolochic acid. Use of alternative medications is more common in East Asia or Africa where it is said to be a more common and underreported cause of end-stage renal disease (ESRD).[150]

Antineoplastic Agents

Antineoplastic agents represent one of the fastest growing pharmaceutical markets. Most agents are linked to isolated tubular injury or a reduction in the glomerular filtration rate. For example, interstitial nephritis is absent in most cases of the archetypal nephrotoxin in this class, cisplatin.[151] Yet, as novel agents receive approval, their association with AKI and ATIN will continue to grow.[152]

In gemcitabine-induced nephritis, crystal deposits within the tubular lumen and subsequent crystal-induced epithelial damage likely instigates ATIN.[153] In a patient receiving lenalidomide for multiple myeloma, the diagnosis was suspected when ATIN recurred on rechallenge, prompting a change in chemotherapy.[154] The tyrosine kinase inhibitors (sunitinib and sorafenib) are generally well tolerated, but hypersensitivity reactions, including ATIN, have been reported with them.[155]

Other Drugs

The list of other drugs associated with ATIN continues to expand. Most of these have been isolated, single case reports of patients who often were using more than one drug. By far the most common among these to be associated with ATIN are the analgesics glafenin, and its derivatives, antrafenin and floctafenin.[8,21] Although direct drug toxicity with lesions of ATN has been incriminated as the cause of AKI in some of these patients, clinical and laboratory evidence of an immunologically mediated hypersensitivity reaction causing ATIN has been sufficiently well documented to justify incriminating these agents as a cause of ATIN.[8,10]

Other drugs that have been implicated are sulfinpyrazone, aminopyrine, azathioprine, quinine, clofibrate, propylthiouracil, carbamazepine, amidopyrine, amphetamine, phenobarbital, p-aminosalicylic acid, captopril, diltiazem, methyldopa, acyclovir, interferon, indinavir, griseofulvin, ifosfamide, intravenous immunoglobulin, phentermine, phendimetrazine, foscarnet, interleukin, rosiglitazone, etanercept, and even aspirin.[1,8,155–157]

Snake and Insect Bites

The kidney is prone to injury due to snake venom toxicity; however, the spectrum of renal involvement varies widely.[158] Snakebite-associated toxicities are classified as hemotoxic or myotoxic, with the incidence of postbite AKI ranging from 5% to 29%. Degeneration of the tubular epithelial cells has been observed with both types of snake venom.[158] There are several case reports linking the Russell's viper bite to ATIN, which is attributed to the metalloproteases present in the venom that bind to renal integrins and disrupt the cell matrix and cellular adhesion.[158] Apart from specific antivenoms, treatment is generally supportive and may include plasmapheresis or blood exchange.

Insect bites, particularly of the Hymenoptera order (bees, wasps, etc.) are linked with anaphylactoid and hypersensitivity reactions, including ATIN.[159,160] Wasp envenomation has been associated with ATN, ATIN, and pigment nephropathy.[161] In the reports of wasp bite–associated ATIN, eosinophiluria and eosinophilic infiltration of the renal parenchyma are common.

SYSTEMIC DISEASES

Although most cases of ATIN are due to a hypersensitivity reaction to drugs or infections, an acute deterioration of kidney function with the structural changes of ATIN also occurs in a variety of systemic diseases. In these, the renal involvement is due either to the metabolic disturbances associated with the underlying disease, the immunologic basis of the primary disease process, or the infiltrative nature of the disease (Table 35.4).

Metabolic Disorders

The metabolic disorders in which ATIN develop are those due to abnormalities in the metabolism of urate, oxalate, calcium, potassium, and heavy metals.[1,162]

Uric Acid

The kidney, as the major organ responsible for uric acid excretion, becomes the principal target organ affected by disorders of urate metabolism. Renal involvement results from the precipitation of uric acid in the renal parenchyma and the urine outflow tract. Depending on the load of uric acid presented to the kidney and the duration of the exposure, the parenchymal lesions that develop are those of either an acute or a chronic urate nephropathy. In the acute form of urate nephropathy, the renal changes are those of an ATIN.[162] The most common condition in which this is encountered is in AKI associated with the tumor lysis syndrome and in massive soft tissue destruction such as rhabdomyolysis.[163]

Oxalate

The increased metabolic production or intestinal absorption of oxalate and its consequent excretion by the kidney almost invariably results in the precipitation of calcium oxalate in the urine outflow tract. Microcrystallization first occurs in the proximal tubule, where oxalate is secreted. However, the lesions that develop are more prominent in the medulla, where the urine is concentrated and acidified. This results in injury of the epithelial cells with interstitial edema and inflammatory cell infiltration around the injured tubular segments, which is characteristic of ATIN.[163,164]

When the hyperoxaluria is sudden and massive, such as after ethylene glycol ingestion, methoxyflurane anesthesia, or the ingestion of star fruit, the acute deterioration of kidney function occurs, the morphologic features of which are those of ATIN.[165,166] Otherwise, when the hyperoxaluria is modest and chronic, such as in inflammatory bowel disease, the onset of kidney injury is insidious and the lesions are those of chronic tubulointerstitial nephritis, although AKI associated with enteric hyperoxaluria can occur.[164,167,168]

Immune Diseases

Diseases due to an immune mechanism primarily affect the glomeruli, with secondary involvement of the tubules and the interstitium. Primary tubulointerstitial nephritis mediated by an underlying altered immune mechanism is extremely rare in humans. The two conditions in which an immune mechanism may result in AKI primarily due to tubulointerstitial lesions, with only limited glomerular involvement, are a transplanted kidney and systemic lupus erythematosus.[1,9]

The diagnosis of ATIN in kidney transplant recipients can be challenging.[169] Despite the numerous medications these patients receive, drug-induced ATIN is an uncommon cause of AKI in them. Drug-induced ATIN lesions can resemble acute cellular rejection morphologically, and both entities can show improvement after steroid therapy. Although infiltrating eosinophils can be seen in acute cellular rejection, their presence and localization at the corticomedullary junction and the presence of granulomatous lesions are suggestive of drug-induced ATIN, whereas that of tubulitis is a defining feature of an allograft rejection.[170,171] Clinically, the diagnosis is compounded by the fact that allograft recipients are prone to infection-induced ATIN, such as cytomegalic virus,[172] adenovirus,[173] and polyoma virus.[174]

In systemic lupus erythematosus, the glomerular lesions are the main renal complication. There are fewer than 20 cases reported demonstrating predominant tubulointerstitial lupus nephritis with only minimal glomerular abnormalities.[175]

AKI with prominent tubulointerstitial lesions may occur in other diseases due to altered immune mechanisms, but again, only rarely in the absence of glomerular lesions. These diseases are Sjögren syndrome, mixed cryoglobulinemia, anti-neutrophil cytoplasmic antibody–associated vasculitis, crescentic glomerulonephritis, sarcoidosis, IgA nephropathy, primary biliary cirrhosis, and autoimmune pancreatitis.[176–179] ATIN is also associated with inflammatory bowel disease that is unrelated to calcium oxalate deposition or 5-aminosalicylic acid use.[180,181]

Neoplastic Diseases

An acute deterioration of kidney function may occur in any neoplastic disease, either because of direct invasion of the kidney or the urinary tract by malignant cells or because of the metabolic disorders (hypercalcemia, hyperuricemia) that may be caused by the malignancy. The institution of antineoplastic therapy, which may itself be nephrotoxic or can magnify the existing tumor-related metabolic disorder (hyperuricemia), is another mechanism of the acute renal injury experienced by these patients.[182] Lymphoproliferative disorders and plasma cell dyscrasias are the two neoplastic diseases in which ATIN due to neoplastic cellular infiltrates is a principal cause of the AKI.[1,183]

Lymphoproliferative Disorders

The kidney is one of the most common extranodal sites of metastatic lymphomas, although a primary lymphoma in the kidneys without evidence of extrarenal lesions also can occur.[183,184] Infiltration of the kidney is more common in non-Hodgkin lymphomas and acute lymphoblastic leukemia than in Hodgkin disease.[183] Leukemic infiltration of the kidney has been noted in two-thirds of patients with leukemia, usually in those with the acute form of the disease.[1] In one large autopsy case series, lymphomatous infiltrates were present in 34% of cases but was diagnosed in only 14% before the time of death.[184,185] Renal infiltrates usually are bilateral and symmetrical and may go undetected unless extensive parenchymal infiltration occurs, in which case flank pain, palpable, tender kidneys, gross hematuria, and AKI are the presenting findings.[184,185] The infiltrates are localized to the interstitium and compress the tubules but sparing the glomeruli, thereby mimicking the classic morphologic features of an ATIN. Irradiation of the kidneys or systemic chemotherapy can result in a dramatic reversal of the structural and functional abnormalities.[185]

Plasma Cell Dyscrasias

The renal complications of plasma cell dyscrasias are a major cause of morbidity and mortality in patients with multiple myeloma. AKI occurs in approximately 10% of these patients, and chronic kidney disease is present in more than two-thirds of them.[1,186]

The pathogenesis of AKI is multifactorial.[186,187] The acute lesions directly related to multiple myeloma, the so-called myeloma cast nephropathy, are those that result from the excessive production of light chains and their precipitation as dimers in the distal tubules. As a result, varying degrees of injury, necrosis, and regeneration of the tubular epithelial cells occur, with interstitial edema and inflammatory cellular infiltration. Immunofluorescent study of the kidney tissue can provide the definitive diagnosis when fluorescence is positive for specific sera for either κ or λ light chains.[187]

IDIOPATHIC ACUTE TUBULOINTERSTITIAL NEPHRITIS

Since the entity of idiopathic ATIN was first recorded in the literature in 1972, an increasing number of cases of ATIN in the absence of exposure to drugs, infections, or any of the other conditions usually associated with ATIN have been reported.[8,9,21]

The only common feature of these cases has been the presence of reversible AKI and the finding of edema and a mononuclear inflammatory infiltration of the interstitium on a kidney biopsy. Although certain laboratory features of a hypersensitivity reaction (elevated IgE levels, eosinophilic infiltrate, and eosinophiluria) are present in some of the cases, the systemic manifestations of a hypersensitivity reaction (fever, arthralgia, rash) are characteristically absent. Linear deposits of IgG, C3, and anti-TBM antibodies have been detected on immunofluorescent studies of kidney biopsies in some cases and granular deposits of C3 and IgG were seen

in others, but no deposits were present in the majority.[188,189] A subtype of idiopathic ATIN with a prominent lymphoplasmacytic infiltration of IgG4-positive plasma cells has been reported; the presence of this infiltrate portends a better response to corticosteroids.[190]

The clinical presentation of most cases has been nonspecific, except for the acute deterioration of kidney function. The AKI has been severe enough in a third of reported cases to necessitate supportive dialytic therapy. The outcome has been favorable in most cases, with recovery occurring spontaneously or after the institution of steroid therapy. There also have been cases of irreversible kidney injury that have failed to respond to steroid therapy.[1,22] Clinically, the nonspecific presentation of idiopathic ATIN coupled with its favorable prognosis and response to steroids, at least in some cases, mandates that its possibility be considered in the differential diagnosis of every patient with AKI in whom the cause of the kidney dysfunction is uncertain.

Idiopathic ATIN constitutes more than 15% of cases of AKI that are sampled for a biopsy.[50] Systemic diseases, such as chronic active hepatitis or ulcerative colitis, have been present in the occasional case. Uveitis, on the other hand, has been a feature of several patients with idiopathic ATIN. This group constitutes a variant that deserves separate consideration.

Idiopathic Acute Tubulointerstitial Nephritis with Uveitis

An acute eosinophilic interstitial nephritis that occurred in association with an anterior uveitis of unknown cause was first described in 1975 in two patients who also had bone marrow and lymph node granulomas.[191] The syndrome of ATIN with uveitis has since been noted to occur rarely in other granulomatous conditions, but most reported cases have been in patients with no bone marrow granulomas.[192] In reported cases, tubulointerstitial nephritis and uveitis (TINU) is more common in adolescents with a 3:1 female to male predominance, although the lesion may also occur in adults and the elderly. Familial occurrence and an association with certain HLA serotypes have been suggested, with a strong association with the HLA-DQ and HLA-DR alleles.[193] Anorexia, asthenia, nocturnal fever, and weight loss may be present for several months before the onset of the ocular complaints. A moderate anemia is common, and hyperglobulinemia usually is present.[194] There also is evidence of tubular dysfunction, such as glucosuria, proteinuria, aminoaciduria, β_2-microglobulinuria, impaired urinary concentrating ability, and azotemia.[194] Uveitis may occur as a manifestation of a variety of systemic diseases but rarely in association with ATIN, except in sarcoidosis and Sjögren syndrome.[1] Hence, the occurrence of uveitis and ATIN is termed renal ocular syndrome or tubulointerstitial nephritis and uveitis (TINU) syndrome.[1,195]

More than 200 cases of TINU syndrome have been described worldwide, primarily in the ophthalmology and pediatric medical literature.[195] The cause remains undefined but has been presumed to be the result of an autoimmune process in genetically predisposed individuals or a reaction to an as yet undetermined viral infection. It has been described in individual cases of toxoplasmosis, tuberculosis, giardiasis, chlamydiosis, and Epstein-Barr virus infection.[195–197] The demonstration of circulating immune complexes in some cases has led to the suggestion of a role for an immune-mediated process. Urinary β_2-microglobulin and serum Krebs von den Lunge–6 (KL-6) protein have been reported as potential diagnostic markers.[195] The occurrence of hyperthyroidism with TINU syndrome suggests that thyroid function should be measured in all TINU patients.[198,199] Both the ocular and renal changes respond to a brief course of steroid treatment, but can relapse. Spontaneous remission without steroid treatment has been reported, primarily in children; hence, the reluctance to use steroids in this age group.[194–197] Uveitis may be asymptomatic, indicating the need for ophthalmologic examination in all cases of idiopathic ATIN.[200] Uveitis can precede the onset of ATIN or a relapse without any renal manifestations, in which instances it responds to topical steroid or cycloplegic treatment.[195] In children and adolescents, the long-term prognosis is good, with the recovery of kidney function and no documented visual loss. In adults, the prognosis is less favorable and kidney injury requiring dialysis may develop, particularly if steroid therapy is withheld.

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CHAPTER

36 Acute Kidney Injury Associated with Pigmenturia or Crystal Deposits

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ACUTE KIDNEY INJURY RESULTING FROM HEME PIGMENTS

Myoglobinuric Acute Kidney Injury

Rhabdomyolysis is a potentially life-threatening syndrome involving damage and the breakdown of skeletal muscle causing myoglobin and other intracellular proteins and electrolytes to be released into the circulation. This syndrome is a relatively common cause of acute kidney injury (AKI)* and accounts for 7% to 10% of AKI in the United States.[1] Perhaps the first historical suggestion of rhabdomyolysis goes back to biblical times where it is related that the Israelites became ill and died after eating large quantities of quail, which had probably fed on hemlock seeds. In modern times, the initial description of the consequences of traumatic muscle injury on kidney function is attributed to Bywaters and Beall,[2] who vividly documented the brown-black granular casts, a reduction in urinary output, hyperkalemia, and ultimately, death in the victims of crush injuries at the time of the London bombing during World War II. In addition, Bywaters et al.[3,4] were the first to establish a definite pathophysiologic relationship between crush injury, myoglobinuria, and acute tubular necrosis.

Causes of Rhabdomyolysis and Myoglobinuria

A variety of conditions and diseases can lead to rhabdomyolysis and AKI, and the list of causes is constantly being expanded with new case reports (Table 36.1). Although the list is long, it can be divided into eight basic categories: (1) direct muscle injury, (2) drugs and toxins, (3) genetic disorders, (4) infections, (5) excessive muscular activity, (6) ischemia, (7) electrolyte and endocrine/metabolic disturbances, and (8) immunologic diseases. The common denominator for all the causes is a disruption of normal skeletal muscle cell structure or metabolism leading to derangements in Ca^{2+} homeostasis. Adenosine triphosphate (ATP) depletion further interferes with Ca^{2+} sequestration, leading to lethal intracellular Ca^{2+} overload that activates a number of autolytic enzymes, causing myofibril and membrane damage.[5] The subsequent death and lysis of skeletal muscle cells results in the release of intracellular contents into the circulation. In the United States, the three most common causes of rhabdomyolysis are drug abuse (with a substantial percentage related to ethanol use), muscle compression, and seizures.[1]

Crush injuries[1,6,7] and prolonged compression of the limbs can lead to massive rhabdomyolysis and its sequelae, including AKI. Significant volume and electrolyte imbalance may ensue due to a massive influx of extracellular fluid and solutes into and efflux of major intracellular ions such as potassium and phosphate out of the damaged cells.[1,7]

Drugs and toxins have also been implicated in causing rhabdomyolysis.[8,9] Several mechanisms underlie drug- and toxin-induced rhabdomyolysis, including (1) drug-induced coma leading to compression of a limb; (2) excessive muscular activity (e.g., phencyclidine, LSD, hemlock); (3) drug-induced hyperthermia; (4) drug-induced vasoconstriction with muscle ischemia (e.g., cocaine); (5) impaired ATP formation (e.g., cyanide, salicylates); (6) the induction of potassium or phosphorus depletion (e.g., diuretics); (7) a hypersensitivity reaction resulting in myositis; (8) a direct toxic effect on skeletal muscle cells (e.g., ethanol); and (9) drugs whose mechanism of toxicity is still controversial (e.g., statins).[10,11] Although certain drugs, such as heroin[12] or ethanol,[13] may have a direct toxic effect on skeletal muscle cells, a more important factor in causing rhabdomyolysis is the occurrence of a coma after their use leading to muscle compression and ischemia. In addition, drug use may be associated with other conditions that predispose one to rhabdomyolysis. For example, in the alcoholic patient, concomitant hypokalemia,[14] hypophosphatemia,[15] and starvation[16] may contribute to rhabdomyolysis. The presence of multiple etiologic factors may be a common scenario, as noted in a large clinical series by Gabow et al.,[17] in which more than

*In recent years, the term acute kidney injury (AKI) has replaced acute renal failure (ARF), because AKI denotes the entire clinical spectrum from mild increases in serum creatinine to overt renal failure (Molitoris et al. J Am Soc Nephrol. 2007;18:1992–1994).

36.1 Causes of Rhabdomyolysis

- Traumatic muscle injury
  - Crush injuries
  - Compression/pressure necrosis
  - Severe burns
  - Contact sports
  - Direct muscle trauma
- Drugs and toxins (partial list)
  - Ethanol
  - Heroin
  - Barbiturates
  - Cocaine
  - Amphetamines
  - Benzodiazepines
  - Phencyclidine
  - HMG-CoA reductase inhibitors (statins)
  - Fibric acid derivatives (clofibrate, gemfibrozil)
  - Hemlock
  - Salicylates
  - Carbon monoxide
  - Ethylene glycol
  - Isopropyl alcohol
  - Snake and insect venoms
  - Succinylcholine
  - Colchicine
  - Propofol
  - Paraphenylenediamine
  - Colchicum autumnale (autumn crocus)
  - Monensin
- Genetic disorders
  - Phosphorylase deficiency (McArdle disease)
  - Phosphofructokinase deficiency
  - α-Glucosidase deficiency
  - Carnitine palmityltransferase deficiency
  - Amylo-1,6-glucosidase deficiency
  - Phosphohexoseisomerase deficiency
- Infections (partial list)
  - Influenza
  - Tetanus
  - Gas gangrene
  - Legionnaires' disease
  - Shigellosis and salmonellosis
  - Coxsackievirus
  - Leptospirosis
  - Streptococcus
  - HIV
- Excessive muscular activity
  - Vigorous exercise
  - Seizures/status epilepticus
  - Delirium tremens
  - Status asthmaticus
  - Psychotic muscle contractions
  - Tetany
- Ischemia
  - Arterial occlusion
  - Compression
- Electrolyte and endocrine/metabolic disorders
  - Hypokalemia
  - Hypophosphatemia
  - Hypothyroidism
  - Diabetic ketoacidosis
  - Diabetic hyperosmolar nonketotic coma
  - Hypothermia and hyperthermia
- Immunologic disease
  - Polymyositis
  - Dermatomyositis

HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

one factor capable of injuring muscles was present in 51 of 87 episodes of rhabdomyolysis. One such etiologic factor demonstrating the variable causes of rhabdomyolysis is fire ant bites. In a recent case report, a patient was described who developed AKI because of rhabdomyolysis after extensive red fire ant bites.[18] It was suggested that formic acid, an important constituent of fire ant venom, was the underlying mechanism for rhabdomyolysis. In small doses, formic acid is an antibiotic, but in larger doses it acts as an inhibitor of the mitochondrial cytochrome oxidase complex causing tissue suffocation and, consequently, cell death.[18]

Various hereditary enzyme deficiencies and defects have been associated with rhabdomyolysis and myoglobinuria. These are divided into groups of patients with hereditary deficiency of enzyme(s) in (1) glycolytic/glycogenolytic pathways; (2) the fatty acid oxidation pathway; (3) the Krebs cycle; (4) the pentose phosphate pathway; (5) the purine nucleotide cycle; (6) the mitochondrial respiratory chain; (7) patients prone to malignant hyperthermia; and (8) other miscellaneous causes such as sarcoplasmic Ca^{2+}-ATPase deficiency.[19]

The myositis occasionally associated with infectious diseases such as influenza, HIV, and leptospirosis can lead to a

disruption of skeletal muscle cells and thus rhabdomyolysis and myoglobinuria.[19,20] In addition, infections like gas gangrene produce a clostridial toxin that is directly myotoxic.[20]

Excessive muscular activity has been increasingly recognized as a common and preventable cause of rhabdomyolysis.[21,22] Strenuous and exhaustive exercise, especially in deconditioned men (so-called "white collar" rhabdomyolysis), can result in serious rhabdomyolysis.[23] Contributing factors to this syndrome include exercising in a hot or humid environment, volume depletion, fasting, eccentric muscle contractions (e.g., running downhill), preexistent muscle injury (e.g., alcoholic myopathy), and male sex.[23] Intense muscle contractions deplete energy reserves, thus disrupting normal cellular transport processes and permitting calcium to accumulate in the cell, resulting in the activation of proteolytic enzymes and cell death. Based on a number of studies,[23] physical training raises the threshold and induces a degree of resistance to the development of exertional rhabdomyolysis. Training may induce this adaptation by increasing the number of collateral blood vessels, hence improving oxygen delivery, fuel storage, and use. Other conditions associated with excessive muscle contractions and significant rhabdomyolysis include seizures, tetanus, delirium tremens, electrical shock injury, and extensive burns.

Severe potassium deficiency can lead to rhabdomyolysis, myoglobinuria, and AKI. Hypophosphatemia, especially in the setting of severe alcoholism, has been associated with muscle cell injury and rhabdomyolysis.[15] Other metabolic conditions that have been reported to cause rhabdomyolysis include hyperaldosteronism, ketoacidosis, hypothyroidism, and deranged core body temperature.[19]

Myoglobin Metabolism

Myoglobin is composed of a folded polypeptide portion (globin) and a prosthetic group, heme, which contains an atom of iron.[24,25] Based on tracer studies, the half-life of myoglobin in the circulation varies from 1 to 3 hours; after 6 hours, it disappears completely.[24,25] Small quantities of myoglobin (milligram amounts) released during normal conditions are probably cleared by the reticuloendothelial system. Because of its relatively small molecular weight and size, larger quantities of myoglobin released from the muscle in states of injury or disease are readily filtered at the glomerulus and thus can be cleared by renal mechanisms.

In human circulation, myoglobin appears to be bound to an α_2-globulin that has a binding capacity of 23 mg per deciliter. Because myoglobin is loosely bound to α_2-globulin at concentrations below 23 mg per deciliter, approximately 15% to 50% of the myoglobin is in an unbound state and is filtered (fractional clearance relative to inulin, 0.75) and excreted in the urine. This interesting kinetic relationship between myoglobin and its binding protein probably explains why myoglobin is detected in the urine when plasma levels are less than 23 mg per deciliter.[24,25] According to Kagen,[26] the effective renal threshold for myoglobin occurs when the plasma concentration exceeds 0.5 to 1.5 mg per deciliter. Based on a distribution volume of myoglobin of 28.5 L and a muscle myoglobin content of 4 mg per gram, Knochel[27] has calculated that injury of approximately 102 g of muscle would be required to exceed a renal threshold of 1.5 mg per deciliter. Beyond this threshold, the factors that determine the urinary concentration and the excretion rate of myoglobin include (1) the plasma concentration of myoglobin, (2) the extent of myoglobin binding in plasma, (3) glomerular filtration rate (GFR), and (4) urine flow rate.

Myoglobin is visible in plasma or urine to the unaided eye when the concentration exceeds 100 mg per deciliter. Because of the relatively rapid renal clearance of myoglobin, visible plasma levels of myoglobin have never been reported. Knochel[27] has estimated that a visible plasma level of myoglobin would require the destruction of 7.1 kg of muscle in an anephric patient. In contrast, because myoglobin is cleared rapidly in patients with a normal renal function, visible myoglobinuria is achieved with far less muscle necrosis. For example, necrosis of only 178 g of muscle, achieving a plasma myoglobin level of only 2.5 mg per deciliter, is sufficient to produce visible myoglobinuria in a patient with normal renal function excreting concentrated urine.[27] However, reduced renal function or a high urine flow rate decreases the concentration of myoglobin in urine, thus diminishing the use of a visual inspection of the urine to detect myoglobinuria for a given amount of muscle necrosis. In these situations, benzidine, guaiac, or orthotoluidine (dipstick) tests detect levels as low as 0.5 mg per 100 mL. These tests, however, do not distinguish between myoglobin and hemoglobin. This can be accomplished by immunodiffusion.[28] Multiple alternative methods of detection are now available, including hemagglutination inhibition, radioimmunoassay, and complement fixation.[19]

Given the rapid renal clearance of myoglobin (1 to 6 hours), its presence in the blood or urine may not be the most sensitive method to detect rhabdomyolysis. In contrast, creatine kinase, an intracellular muscle enzyme, appears to be a more sensitive plasma marker for rhabdomyolysis because of its slower clearance (serum half-life, 1.5 days), and therefore is the preferred diagnostic modality.[29]

Pathophysiology of Myoglobinuric and Hemoglobinuric Acute Kidney Injury

Given the biochemical similarity between myoglobin and hemoglobin, the general consensus that they share a common pathogenetic pathway, and the fact that the classical animal models used to study pigment-induced oliguric AKI share intravascular hemolysis (hemoglobinuria) and rhabdomyolysis (myoglobinuria), the pathogenesis of these two pigments are presented together in this discussion.

The proposed mechanisms by which myoglobinuria or hemoglobinuria cause AKI include (1) hypovolemia and renal ischemia, (2) direct tubular toxicity of myoglobin/hemoglobin, (3) tubular obstruction from heme pigment casts, and (4) glomerular fibrin deposition. As in many clinical syndromes, it is probably the interplay of these proposed

FIGURE 36.1 The pathophysiologic processes involved in myoglobinuric and hemoglobinuric acute kidney injury. (Modified from Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. *Kidney Int*. 1996;49:317, with permission.)

mechanisms that results in AKI rather than any one single factor. These interactions are schematized in Figure 36.1.

Hypovolemia and Renal Ischemia

During the initial phase of glycerol-induced AKI (an animal model for rhabdomyolysis), there is a marked reduction in cardiac output (36%), renal blood flow (RBF) (20%), and an increase in renal vascular resistance.[30] Subcutaneous or intramuscular (but not intravenous) glycerol not only produces muscle injury but also causes sequestration of fluid into the injection site.[31] Thus, the hemodynamic changes are due, in part, to the migration of plasma water into the site of injury with consequent severe intravascular volume contraction occurring in this model of myohemoglobinuric AKI. Comparable hemodynamic changes occur in clinical settings that damage and cause necrosis of the skeletal muscle such as crush syndrome.[7] Moreover, the conditions that predispose one to rhabdomyolysis, such as drug-induced coma with accompanying poor oral intake, or excessive insensible fluid losses from exhaustive exercise or burns, contribute to intravascular volume depletion and compromise of renal function.

In the initial phases of glycerol-induced AKI, the reduction in RBF is associated with a redistribution of regional blood flow from the outer to the inner cortex[32] and vasoconstriction of the afferent and efferent arteriole. The proposed mediators of this initial renal vasoconstriction include (1) increased sympathetic nerve activity, (2) augmented activity of the renin–angiotensin system, (3) reduced nitric oxide production and availability, (4) suppressed renal prostaglandin production, (5) increased plasma vasopressin concentration, and (6) glomerular microthrombi.[33] The reduction in nitric oxide may be due to the fact that heme proteins can scavenge this important endogenous vasodilator. Nitric oxide synthase inhibition worsens and nitric oxide supplementation protects against glycerol-induced AKI. This lends support to the protective effects of nitric oxide in the pathogenesis of myoglobin-induced AKI.[34]

The critical role that intravascular volume depletion plays in the pathogenesis of myohemoglobinuric AKI is demonstrated by studies in which volume status is manipulated in the glycerol-treated rat. In initial studies, Oken et al.[35,36] noted that renal damage was ameliorated if the rats ingested adequate quantities of water before the administration of glycerol. Similarly, Hsu et al.[30] found that the reduction in RBF and function in response to the administration of glycerol was attenuated in rats chronically drinking saline compared with rats drinking water. Reineck et al.[37] provided a better understanding of the important temporal relationship

between volume expansion and improvement of renal function in the glycerol-treated rat. Like other investigators, they noted a significant reduction in RBF and GFR after the administration of glycerol. These variables could be restored to normal levels by volume expansion with Ringer's solution at 3 and 6 hours, but not at 18 hours after the administration of glycerol. They concluded that the initial decrease in GFR and the low fractional excretion of sodium was due to a decrease in RBF (renal hypoperfusion), whereas other events (e.g., tubular necrosis) accounted for decreased GFR at later time points. These preclinical studies support the clinical observation that, initially, patients with myoglobinuric AKI have features of prerenal azotemia, including a low urinary fractional excretion of sodium.[38] In addition, they provide the rationale for early use of volume expansion in patients with rhabdomyolysis and hemoglobinuria.

Myoglobin and Hemoglobin Nephrotoxicity. Bywaters and coworkers[2,3] expanded on their original description of the clinical syndrome of rhabdomyolysis-induced AKI to examine the role of myoglobin as a direct nephrotoxin. They noted that rabbits ingesting an acid diet with a urine pH below 6.0 had AKI after the infusion of human myoglobin, whereas rabbits ingesting a normal diet were spared from renal injury.[3] Other investigators[39,40] have confirmed this observation that intravenous infusions of myoglobin are relatively benign but can become highly nephrotoxic in the setting of acidemia/aciduria and volume depletion. Vetterlein et al.[41] demonstrated that infusions of myoglobin had no effect on RBF in normal rats, but worsened RBF in hypotensive animals. Thus, it appears that heme proteins can intensify the degree of vasoconstriction in the setting of hypovolemia. This may explain the clinical observation that the mere presence of myoglobinuria or a markedly elevated creatine kinase at the time of hospital admission had little predictive value in determining who experiences AKI.[17] These observations suggest that other conditions (i.e., volume depletion, acidemia) are required for renal injury to occur.

To address the question of why heme pigments are nephrotoxic only in certain metabolic conditions, Braun et al.[31] investigated the effect of breakdown products of heme pigments on renal tubular transport. First, they noted that 4 hours after a subcutaneous glycerol administration to rats there was both swelling and pallor of the proximal tubule and depression of normal tubular uptake of hippurate and tetraethylammonium. The investigators measured the uptake of hippurate in renal cortical slices incubated with various specific heme proteins or their derivatives and found that incubation with hemoglobin did not depress uptake if the pH of the medium was kept at 7.4. However, uptake was depressed when the pH was lowered to 5.4 or during hypoxic conditions. In an acidic medium (pH $<$5.6), both myoglobin and hemoglobin dissociate into ferrihemate (hematin; molecular weight, 670 Da) and their respective globin moieties.[42] Incubation with ferrihemate, regardless of the pH of the medium, depressed the uptake of hippurate in the renal cortical slices, whereas incubation with either globin or albumin alone had no significant effect on transport. The inhibitory action of ferrihemate on hippurate transport could be mitigated if the incubation medium also contained albumin, which presumably bound the ferrihemate. Intravenous injection of ferrihemate has been shown to cause glomerular and tubular damage in the dog.[43] Therefore, it has been proposed that after filtration by the glomerulus, myoglobin or hemoglobin is converted to ferrihemate in the presence of an acid tubular fluid, or after exposure to the acid pH of cellular lysosomes, and it is this metabolite that is directly nephrotoxic.

These and other studies implicate the heme moiety as a potent pro-oxidant molecule.[44,45] It is well established that free heme can facilitate the production of reactive oxygen species via Fenton/Haber-Weiss reactions. Under physiologic conditions, free heme is sequestered by heme binding proteins, and oxidative stress can cause the release of heme, thereby increasing free heme levels. In addition, evidence suggests that the iron component of heme is the culprit of heme-induced oxidative damage.[44,45] The central role of iron has been substantiated by a number of studies demonstrating amelioration of both myoglobinuric and hemoglobinuric AKI and lipid peroxidation by the iron chelator, deferoxamine.[46] On the other hand, Zager[47] has also shown that deferoxamine attenuates renal damage in the glycerol-induced model of AKI, but concluded that iron toxicity is mediated by factors other than free radical generation. For example, it has been suggested that heme protein endocytosis in the proximal tubule sensitizes the tubular cell membranes to the damaging effects of phospholipase A_2.[48] In addition, heme proteins appear to deplete cellular ATP stores and, thus, have an adverse effect on cellular energetics.[5] Iron toxicity may be due to redox cycling of the heme moiety from ferrous to ferric and to ferryl oxidation states.[49]

In order to contend with the pro-oxidant heme moiety, the kidney induces antioxidant defensive machinery, including heme oxygenase-1 (HO-1).[44,45] HO-1 catalyzes the rate-limiting step in the oxidative degradation of heme liberating equimolar amounts of iron, carbon monoxide, and biliverdin. Iron in turn induces the expression of ferritin. HO-1 is known to have important antioxidant, anti-inflammatory, and antiapoptotic functions that have been attributed to one or more of its byproducts.[44,45] Nath et al.[50] have demonstrated that the renal induction of both HO-1 and ferritin is increased in the glycerol-induced model of myohemoglobinuric AKI. Prior induction of HO-1 coupled with increased ferritin synthesis attenuated renal damage, whereas pharmacologic inhibition of the enzyme or its gene deletion worsened renal function.[50,51] This increased activity of HO-1, or possibly a broad-based proximal tubular cytoresistance in the kidney, may explain the experimental observation that after induction of myohemoglobinuric AKI rechallenging the animals with a second dose of glycerol does not result in AKI.[52] One speculation is that in the setting of clinical myoglobin-induced AKI, there may be factors contributing to the inhibition of HO-1 and ferritin synthesis, or a

diminution in proximal tubular resistance, resulting in both an accumulation of nephrotoxic iron and in tubular necrosis.

Tubular Obstruction. Filling of the tubular lumen by pigmented casts that become inspissated and obstruct urinary flow with subsequent injury to tubular epithelium is one of the earliest mechanisms proposed to explain the nephrotoxicity of the heme pigments.[53] In their original clinical description of rhabdomyolysis-induced AKI, Bywaters and Beall[2] described the prominent histologic features, including the appearance of tubular obstruction by cellular debris and pigmented casts. It has been suggested that hypovolemia and academia, and the concomitant acidic concentrated urine, facilitate the precipitation of filtered myoglobin or hemoglobin leading to obstructive cast formation.[54] The presence of the Tamm-Horsfall protein in the tubular lumen is critical for heme protein cast formation in the distal nephron. Moreover, an obstructing cast induces urinary stasis, providing for an extended time for proximal tubular heme reabsorption and its attendant tubular toxicity, as noted previously.[55]

Tubular obstruction can decrease GFR either by increasing the tubular pressure and thus decreasing the glomerular transcapillary hydraulic pressure, or by inducing the release of factors (e.g., thromboxane) that cause renal vasoconstriction, thereby reducing glomerular blood flow. The importance of tubular obstruction as a possible mechanism of heme pigment–induced AKI is suggested by the studies of Zager[47] that explored the reasons why mannitol exerts a protective effect against this syndrome. The major beneficial effect of mannitol was attributed to its diuretic effect, which presumably decreased cast formation and proximal tubular uptake of heme proteins. Similarly, alkalinization of the urine may mitigate against myoglobinuric AKI by increasing the solubility of myoglobin (reduced cast formation) and inducing a solute diuresis.[54]

Although there is evidence that tubular obstruction may be a factor in the pathogenesis of the AKI, it probably is not the primary cause of the initial decrease in GFR in myoglobinuric AKI. Rather than high intratubular pressures from obstructing casts, intratubular pressures were found to be low in the glycerol-induced model of AKI.[35] This observation was interpreted to indicate that the presence of casts is the result, rather than the cause, of the decrease in GFR and urine flow. Instead of causing the initial decrease in renal function, cast formation may play a role in the maintenance of the renal failure once it develops.[56]

Glomerular Fibrin Deposition. Because of the liberation of tissue factors, both rhabdomyolysis and intravascular hemolysis can initiate disseminated intravascular coagulation (DIC).[19] Fibrin strands have been demonstrated in glomeruli from patients[57] and experimental animals[58] with rhabdomyolysis-induced AKI. Intravenous infusion of a muscle extract in rabbits resulted in DIC, renal dysfunction, and glomerular microthrombi, whereas an intravenous infusion of pure myoglobin had no untoward effect.[59] This led to the conclusion that myoglobin, per se, is not the primary cause of the coagulation cascade activation in the crush syndrome, but rather it is the release of other muscle constituents that induces DIC and the subsequent deposition of glomerular microthrombi that are responsible for rhabdomyolysis-induced AKI.

Clinical and Laboratory Features of Rhabdomyolysis and AKI

The diagnosis of myoglobinuria can be suspected from a history and physical examination. However, the clinical features of rhabdomyolysis are nonspecific and the course of the syndrome is quite variable depending on the underlying cause and the general condition of the patient. The syndrome has local as well as systemic features and early or late complications may occur. Because the prompt recognition of rhabdomyolysis is critical to preventing late complications, all suspected cases must undergo a complete clinical inquiry, observation, and laboratory follow-up.

Risk Factors for Acute Kidney Injury. The frequency of AKI in the setting of rhabdomyolysis is unknown, and reports of frequency have ranged from 13% to 50%.[1] Gabow and colleagues[17] emphasized that no single laboratory value could predict which patients are at high risk for the development of AKI. However, using discriminant analysis, patients could be separated into high- and low-risk groups, with the high-risk group (elevated serum potassium and creatinine and reduced serum albumin concentrations) having a 41% prevalence of AKI.

Based on a large historical cohort (157 patients), Ward[60] identified clinical and laboratory differences between those patients in whom renal failure did or did not develop, and factors predictive of progression to renal failure. As shown in Table 36.2, patients with rhabdomyolysis and renal failure were older, had a higher incidence of hypertension, and were more hypotensive and volume depleted. A significantly greater proportion of them had a creatine kinase level greater than 16,000 IU per liter, although elevations to this degree were seen in 10.7% of patients in whom renal failure did not develop (Table 36.3). The renal failure group also had significantly higher serum potassium and phosphorus levels and lower serum calcium and albumin concentrations, and was more acidemic with a concomitant lower urinary pH. Sepsis, burns, and drug ingestion were the causes of rhabdomyolysis more closely associated with the development of renal failure. Using multiple logistic regression analysis, a scoring system was developed predicting the risk of renal failure in patients with rhabdomyolysis based on the variables of serum phosphorus, potassium, albumin, and creatine kinase concentrations, and the presence of volume depletion and sepsis. A point score of 7 or higher predicted a greater than 50% likelihood for the development of renal failure. In a multivariate analysis of 72 consecutive patients with rhabdomyolysis due to illicit

36.2 Univariate Analysis of Clinical Variables in Patients with Rhabdomyolysis Developing and Not Developing Renal Failure

Variables	Group		
	Renal Failure (N = 26)	Nonrenal Failure (N = 131)	P[a]
Age, year (SD)	53.7 ± 20.6	41.4 ± 18.1	0.002
Male (%)	69.2	61.1	0.418
Hypertension (%)	46.2	22.9	0.026
Diabetes mellitus (%)	11.5	7.6	0.562
Previous renal disease (%)	19.2	3.8	0.051
Dehydration (%)	38.5	4.6	0.001
Hypotension (%)	34.6	14.5	0.040
Nephrotoxin exposure (%)	19.2	39.7	0.020
Diuretic use (%)	30.8	16.8	0.147
Nonsteroid drug use (%)	19.2	6.1	0.101
IV hydration (%)[b]	80.7	54.2	0.289
Osmotic treatment (%)	26.9	22.9	0.674
Bicarbonate treatment (%)	50.0	12.2	0.001

[a]The P value for difference in means or proportions between renal failure and nonrenal failure groups.
[b]Greater than 150 mL per hour averaged over the first 24 hours after admission. SD, standard deviation; IV, intravenous.

drug use, patients with a creatine kinase level greater than 25,000 IU per liter, hypotension, and leukocytosis were at a greater risk of developing AKI, whereas hyperthermia (temperature >38.5°C) was associated with a reduced risk.[61] This association does not indicate that hyperthermia is protective against rhabdomyolysis, rather it is most likely due to earlier presentation to, or evaluation or fluid resuscitation in the emergency department.

Urinalysis. Examination of the urine provides the first laboratory clue to the presence of myoglobinuria. Classically, the initial urine is dark (Table 36.4) and usually with an acid pH; the benzidine or orthotoluidine reagent gives a positive reaction for blood (3+ to 4+), but microscopic examination of the urinary sediment fails to reveal any red blood cells (RBCs). Specific tests for urine myoglobin determination are available in some clinical laboratories but, as noted earlier, urine myoglobin levels are not the most sensitive clinical markers for rhabdomyolysis. Although the strongest clinical clue for myoglobinuria is the presence of strongly heme-positive urine and the absence of RBCs, in one major series[17] hematuria was present in 32% and the dipstick was heme negative in 18% of the patients with rhabdomyolysis. In addition, proteinuria was detected by dipstick in 45% of patients,[17] which may be attributed to altered glomerular permeability or tubular transport of small proteins.[62] The urinary sediment demonstrates brown "debris" and, with the evolution of renal injury, pigmented brown granular casts and renal tubular epithelial cells are seen.

Serum Potassium Concentration. The most life-threatening consequence of rhabdomyolysis is the release of large amounts of intracellular potassium into the circulation. Given the crucial role that potassium plays in maintaining the homeostasis of resting membrane potential, it is evident that vital organs such as the heart are at greatest risk to sustain arrhythmogenic activity. This implies that an electrocardiographic follow-up is mandatory to monitor for potentially grave arrhythmias. Because more than 98% of total body potassium resides in cells, and skeletal muscle represents 60% to 70% of the total cellular mass, breakdown of even a small area of skeletal muscle releases a considerable potassium load. The presence of

36.3 Univariate Analysis of Laboratory Variables in Patients with Rhabdomyolysis Developing and Not Developing Renal Failure

Variables	Group		
	Renal Failure (N = 20)	Nonrenal Failure (N = 131)	p
Peak creatine kinase >16,000 IU/L, %	57.7	10.7	<0.001
Serum bicarbonate (mmol/L)	21.4 ± 7.2	23.7 ± 4.0	0.1306
Serum potassium (mmol/L)	4.73 ± 1.2	3.92 ± 0.6	0.0018
Serum phosphorus (mmol/L)	1.85 ± 1.08	0.06 ± 0.35	0.0006
Serum calcium (mmol/L)	2.02 ± 0.4	2.14 ± 0.2	0.1452
Serum albumin (g/L)	30.8 ± 10.0	35.9 ± 8.0	0.0107
Arterial pH	7.33 ± 0.10	7.38 ± 0.11	0.0495
Urinary pH	5.19 ± 0.06	5.75 ± 1.0	0.0009

All values are mean standard deviation except peak creatine kinase.

acidosis may shift more potassium extracellularly and worsen the hyperkalemia. As noted in the previous section on Risk Factors for AKI, admission serum potassium levels tend to be higher in patients who go on to experience AKI.[60] Approximately half of an acute potassium load is handled by renal excretion[63]; therefore, in AKI, serious hyperkalemia can result and is usually the major indication for dialysis.

Creatine Kinase. The classic laboratory finding of rhabdomyolysis is an elevated serum creatine kinase of at least five

36.4 Differential Diagnosis of Pigmenturia

Factors	Myoglobinuria	Hemoglobinuria	Porphyria
Urine color	Brown	Reddish brown	Dark red
Serum color	Clear	Pink	Clear
Orthotoluidine reaction	Positive	Positive	Negative
Watson-Schwartz porphobilinogen	Negative	Negative	Positive
Muscle pain/tenderness	Present	Absent	Absent
Serum creatine kinase level	Elevated	Normal	Normal
Serum haptoglobin	Normal	Decreased	Normal

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times the normal value, where the striated muscle isoenzyme (CK-MM) is predominately found. The serum half-life of creatine kinase (~36 hours) is much longer than myoglobin, which makes it a more reliable tool for diagnosis. Normal creatine kinase levels are 45 to 260 IU per liter. Following muscle injury, the level rises within 12 hours, peaks in 1 to 3 days, and declines 3 to 5 days after the cessation of muscle injury.[29] Although no correlation has been established between the absolute level of the creatine kinase and the risk for development of AKI, creatine kinase levels are significantly higher in patients in whom renal failure develops.[19,29] Following admission, changes in creatine kinase concentrations provide some insight into whether the rhabdomyolysis is worsening or resolving, and following levels is essential to observe for the "second wave" phenomenon (described later in this chapter).

Acid–Base Balance. The conditions that cause rhabdomyolysis involve tissue trauma or ischemia and predispose one to an augmented acid load. In a study by Ward,[60] patients with rhabdomyolysis who progressed to renal failure tended to be more acidemic. An elevated serum anion gap is usual in patients with rhabdomyolysis and due to the impaired renal excretion of intracellular organic acids released from damaged muscles, as well as a retention of inorganic anions such as phosphate.[64]

Uric Acid. Due to the release of intracellular purines from damaged myocytes, hyperuricemia is expected in patients with rhabdomyolysis, especially when the muscle injury is due to strenuous exercise or exertion.

Blood Urea Nitrogen: Creatinine Ratio. Both AKI and the increased release of creatine from damaged myocytes increase the serum concentrations of blood urea nitrogen (BUN) and creatinine. However, the rise in creatinine is more pronounced and, in turn, alters the normal 10:1 ratio of BUN to creatinine to a ratio of 6:1 or less. Based on creatine:creatinine kinetics and their respective concentrations in skeletal muscle, Oh[65] challenged this conventional view. He pointed out that the patient population in which rhabdomyolysis develops tends to have a larger percentage of younger men with a greater muscle mass, whereas other forms of AKI are more often associated with older and more cachectic patients who have less muscle mass and thus reduced creatinine production rates.

Calcium–Phosphorus Metabolism. The perturbations of calcium and phosphorus metabolism usually seen in most types of AKI appear to be exaggerated in rhabdomyolysis-induced AKI.[19,66] Following the destruction of muscle cells, the release of inorganic phosphorus into the plasma causes hyperphosphatemia[19,64] and subsequent hypocalcemia through the deposition of calcium phosphate in the destroyed muscle cells (dystrophic calcification) and other tissues. Hypocalcemia may be accentuated by the inhibition of renal vitamin D 1α-hydroxylase, which results in the downregulation of the production of the active form of vitamin D ($1,25[OH]_2D_3$). This observation may be explained by hyperphosphatemia, which is known to decrease synthesis of $1,25(OH)_2D_3$ and to stimulate the production of the parathyroid hormone.[64,67] A recent case report described elevated FGF23 levels in rhabdomyolysis-induced AKI and may provide a mechanism for the inhibition of renal 1α-hydroxylase.[68] Regardless of the mechanism, in the absence of frank tetany, hypocalcemia usually does not require treatment. In fact, correction of the hypocalcemia with vigorous intravenous calcium replacement may increase both dystrophic (calcium deposition in damaged muscle) and metastatic calcification due to the high serum PO_4 and the $Ca \times PO_4$ product.

Approximately 20% to 30% of patients with myoglobinuric AKI experience transient hypercalcemia during the recovery (diuretic) phase.[19,64] Early studies[69,70] suggested that hypercalcemia was due to the normal remobilization of calcium deposits in the injured muscle that occurs during the recovery phase of AKI. Alternatively, it has been proposed that as renal function improves, the combination of a decreasing serum phosphorus concentration and the ambient secondary hyperparathyroidism, secondary to hypocalcemia, stimulates the synthesis of $1,25(OH)_2D_3$ resulting in an "overshoot" hypercalcemia.[64] This augmented $1,25(OH)_2D_3$ production may be due, in part, to the release of vitamin D from damaged muscle tissue.[64,71]

Urinary Sodium Excretion. Impaired renal tubular reabsorption of sodium is typically seen in most types of oliguric AKI as manifested by a high fractional excretion of sodium. However, in both myoglobinuric and hemoglobinuric AKI, a low fractional excretion of sodium (<1%) has been observed[38] that resembles a prerenal azotemia during the early course. As noted earlier in this chapter, this phenomenon is most likely due to hypovolemia and vasoconstriction, which lead to renal hypoperfusion.

Disseminated Intravascular Coagulation. DIC is commonly present in patients with rhabdomyolysis and may be due to the release of intracellular thromboplastins that activate the clotting cascade.[63,64] Moreover, DIC may be an important factor in the pathogenesis of the AKI (see section on Glomerular Fibrin Deposition, previously).

Differential Diagnosis

Myoglobin-induced AKI should be suspected in patients with trauma presenting with the classic triad of heme-positive urine, an elevated serum creatine kinase level, and dark (pigmented) urine containing dirty-brown granular casts without RBCs. More subtle cases, usually associated with diffuse nontraumatic rhabdomyolysis, may be more difficult to detect. The differential diagnosis of pigmenturia is limited (Table 36.4). Although certain drugs may impart an orange, red, or brown hue to the urine such as rifampin and nitrofurantoin, they do not react with the benzidine or orthotoluidine reagent on the urine dipstick. Porphyrins also color the urine brown

but do not react to give a positive test for occult blood. The most difficult challenge is to discriminate myoglobin from hemoglobin in the urine. Because these are heme proteins, they both react with the benzidine or orthotoluidine reagent and both are associated with the absence of RBCs in the urine sediment. One helpful clue may be the color of the serum in these two conditions. Because myoglobin is relatively rapidly cleared by the kidney, serum levels of myoglobin are not sufficiently elevated to alter the color of the serum in patients with rhabdomyolysis. In contrast, because of its much larger size and its avid binding to haptoglobin, hemoglobin is not as rapidly cleared by the kidney and serum levels may be high enough to result in a pink discoloration of the serum in patients with hemoglobinuria.

Clinical Course and Complications of Myoglobinuric Acute Kidney Injury

Myoglobinuric AKI can run a course ranging from mild renal dysfunction with only transient oliguria and rapid recovery to a much more catastrophic disease requiring frequent dialysis for periods of 2 or 3 weeks. Typically, the duration of oliguria is 7 to 10 days; during this interval a period of anuria may exist for up to 3 days. Resumption of more normal urine formation heralds the recovery of renal function as patients enter the diuretic phase with a subsequent clearing of azotemia and the cessation of the requirement for hemodialysis.

In addition to muscle injury and AKI, patients with rhabdomyolysis may have peripheral neuropathies. These can result from compartment syndromes in which involved muscles become edematous in confined tissue spaces with compromise of blood supply to both muscle and nerves in the area.[69] Measurement of tissue pressure has been advocated as a tool in identifying those areas of damaged muscle at risk, and a surgical fasciotomy may be required to avoid this complication.[64] Swelling of the muscles can lead to impairment in the blood supply of the muscles, resulting in a recurrence or "second wave" of muscle necrosis, as reflected by a second rise in the serum creatine kinase concentration. Neuropathy also can result from traction if rhabdomyolysis is caused by prolonged coma, as from drug overdose.[64]

Prevention and Treatment of Myoglobinuric Acute Kidney Injury

Understanding the possible mechanisms by which rhabdomyolysis causes AKI can provide the basis for the various therapies advocated for this disorder. If possible, treatment of the underlying condition is a priority. Given the pathogenic nature of hypovolemia, renal hypoperfusion and, based on experimental and clinical data, early intravascular volume expansion by intravenous administration of NaCl 0.9% is essential to restore RBF, maintain GFR, and ultimately prevent AKI.[64,72] Because myoglobin is more nephrotoxic at an acid pH, most groups advocate alkalinization of the urine with sodium bicarbonate.[64,72,73] By correcting cellular acidosis, bicarbonate therapy may reduce renal tubular epithelial swelling and attenuate renal tubular and vascular collapse.[74] There is a theoretical concern that inducing a metabolic alkalosis with such treatment may enhance metastatic calcification, but the salutary benefit of bicarbonate therapy probably outweighs any untoward effect.

Following the repletion of volume and the production of urine within an acceptable range, the patient could undergo forced diuresis. Mannitol has long been recognized to be an effective agent in the prophylaxis against the development of experimental and clinical AKI, in particular when there is suspicion of compartment syndrome. However, recent studies have shown that the administration of NaCl 0.9% in combination with mannitol is not more effective in the prevention of AKI than the administration of NaCl 0.9% alone. Furthermore, mannitol can cause AKI and should be used with caution.[75]

Furosemide, a loop diuretic, has the theoretic advantage of inhibiting sodium transport in the thick ascending limb of the Henle loop. Oxygen consumption is dictated primarily by the rate of sodium transport, and a precarious balance exists in this segment between the rate of oxygen delivery and its consumption.[76] By inhibiting sodium transport, furosemide may reduce oxygen consumption in the face of limited delivery and thereby preserve cell viability. In addition, the augmented urinary flow induced by the diuretic may reduce the risk of tubular obstruction. However, loop diuretics can cause increasing acidification of the urine, worsening intravascular volume depletion, and can induce ototoxicity, and thus the use of these agents has not been generally recommended.[77]

Although there are no controlled trials to show a direct benefit of a "mannitol-bicarbonate cocktail" in the prevention of AKI in rhabdomyolysis, there are case reports suggesting such therapy was instrumental in averting renal injury.[78,79] Adequate fluid hydration and bicarbonate therapy, however, did not ameliorate the development of renal failure in a large retrospective study.[60] Moreover, in a retrospective evaluation of 382 intensive care unit trauma admissions with a creatine kinase of >5,000 IU per liter, the use of bicarbonate and mannitol in 40% of this group had no effect on rates of renal failure, the need for dialysis, and mortality, although there was a trend to a lower mortality rate in patients with creatine kinase greater than 30,000 IU per liter who were treated with bicarbonate and mannitol.[80] This may provide a window of therapeutic potential for patients with extremely high levels of creatine kinase. Initially, the optimization of intravascular fluid volume deficits should be carried out with dispatch using isotonic crystalloid solutions, usually normal saline. Variables useful in following this course of therapy include a physical examination of the state of the circulation and hematocrit, and the recording of external fluid balance. If the clinical assessment suggests that a euvolemic state has been achieved but no improvement in oliguria has occurred, a decision must be made about further intervention. Usually by this time, laboratory results offer further support for the diagnosis of myoglobinuria and acute renal insufficiency, and we recommend the prompt infusion of a mannitol–bicarbonate solution. This is made by adding two ampules, each containing 12.5 g mannitol in 50 mL, and two ampules of 50 mEq $NaHCO_3$ in 50 mL to 800 mL of 5% dextrose

in water (D5W) for intravenous infusion. This reconstituted liter is roughly isosmotic with plasma once the glucose is metabolized and contains both mannitol and 100 mEq $NaHCO_3$. It should be infused at 250 mL per hour; urine flow rate should increase by the end of the 4-hour infusion if the treatment is successful. If this is the case, the solution should continue to be administered at a rate equal to urine output and sufficient to achieve a urine pH greater than 6.5 until such time as azotemia has started to clear and all evidence of myoglobinuria has disappeared. If urine flow does not increase after the 4-hour infusion, the patient has entered the established phase of oliguric renal failure and should be treated conservatively until dialysis can be arranged based on conventional indications. This approach corrected oliguria, hastened the clearing of azotemia, and avoided the need for dialysis in roughly half of patients with myoglobinuric AKI.[79] As a group, these patients had somewhat lower indices of muscle damage and somewhat better preservation of renal function than the half that did not respond. Whether this reflects the earlier intervention or a less severe degree of muscle injury, or both, is not known, and it is also possible that vigorous volume expansion with normal saline alone might have caused the same result in some patients. Given that complications from the mannitol–bicarbonate infusion are few, even in those patients who do not respond, its use should be seriously entertained in patients with myoglobin-induced AKI.

When AKI has become established, dialysis must be used. Early and intensive hemodialysis may be associated with significantly lower morbidity and mortality rates.[64,81] Experience with peritoneal dialysis indicated that solute clearance using this modality was inadequate to keep pace with the rapid rate of solute appearance in these highly catabolic patients,[82] and thus, hemodialysis should be the modality of treatment. Even so, daily hemodialysis often is required for the first several days until the consequences of extensive muscle injury have abated and rates of urea and potassium accumulation have fallen. Thereafter, a schedule of thrice-weekly dialysis usually is adequate unless other factors, such as continued catabolism from infection or surgical wound débridement, or volume overload from parenteral nutritional therapy demand more frequent treatments. Although the overall prognosis for the renal failure is favorable, the ultimate prognosis for the patient probably depends more on other coexisting conditions such as sepsis, bleeding, and respiratory failure.

Hemoglobinuric Acute Kidney Injury

During most types of extravascular hemolysis, the released hemoglobin is quickly taken up by the reticuloendothelial system and metabolized to bilirubin. Thus, extravascular hemolysis rarely results in AKI. On the other hand, due to the binding capacity of haptoglobin, which effectively sequesters free hemoglobin, and advances in blood bank technology that prevent massive intravascular hemolysis from mismatched blood transfusions, the frequency of hemoglobinuric AKI is an uncommon event compared to myoglobinuric AKI.

Causes of Hemoglobinuria

Hemoglobinuria results from the filtration of free hemoglobin in plasma, due almost exclusively to intravascular hemolysis, which occurs in a variety of conditions (Table 36.5). Although each of the listed causes may be associated with acute renal dysfunction, hemoglobinuria is more likely

36.5 Causes of Hemoglobinuria and Acute Kidney Injury

Genetic defects Glucose-6-phosphate dehydrogenase deficiency Paroxysmal cold hemoglobinuria March hemoglobinuria
Infection Malaria Clostridia
Transfusion reactions
Chemical agents Arsine copper sulfate poisoning Glycerol Quinine sulfate Analine Benzene Hydralazine Fava beans Cresol Sodium chlorate Methyl chloride Coal tar products
Venoms Rattlesnake, copperhead, water moccasin, coral snake Tarantula Brown recluse spider
Traumatic/mechanical destruction Prosthetic valves Disseminated intravascular coagulation Extracorporeal circulation
Miscellaneous
Heat stroke
White phosphorus
Hemoglobin infusions

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to occur in only a few settings. These include hemolytic transfusion reactions, DIC, march hemoglobinuria, glucose-6-phosphate dehydrogenase deficiency, and infections with clostridia and Plasmodium falciparum malaria, the latter causing blackwater fever.[83]

Hemoglobin Physiology and Metabolism

Hemoglobin has a molecular weight of 68,000 Da and is a tetramer of two α and two β globin chains surrounding a ferrihemate core. As noted earlier, free hemoglobin in plasma is tightly bound to haptoglobin, and the hemoglobin–haptoglobin complex is too large to be filtered by the glomerulus. Thus, free hemoglobin appears in the urine only after the plasma concentration of hemoglobin exceeds the maximum binding capacity of haptoglobin, which is approximately 100 mg per deciliter (in contrast to 1.5 mg per deciliter for myoglobin). In the setting of intravascular hemolysis, the relatively low renal clearance of hemoglobin (fractional clearance relative to inulin, 0.03) results in an increase in plasma hemoglobin levels sufficient to be visible to the naked eye as pink-colored plasma, whereas with rhabdomyolysis, the rapid renal clearance of myoglobin (fractional clearance, 0.75) prevents myoglobin retention in the plasma, and the plasma color is not visibly altered. The color of the plasma is an important "bedside" clue that helps to distinguish between these two forms of pigmenturia.

Clinical and Laboratory Features of Hemoglobinuric Acute Kidney Injury

Because both myoglobin and hemoglobin are heme-containing proteins, and the heme moiety has been implicated as a major factor in inducing renal injury, it is generally accepted that the mechanisms by which they both cause nephrotoxicity are similar. Moreover, in the clinical settings, most commonly associated with it other pathogenetic mechanisms have been proposed to account for the AKI. For example, with hemolytic transfusion reactions, the interaction of antigens on the red cell stroma with preformed antibodies may be responsible for adverse effects on kidney function.[84] In DIC, afferent arteriole and glomerular capillary fibrin deposition are the events most directly related to AKI.[85] March hemoglobinuria occurs from the traumatic hemolysis of RBCs, most likely in people with a genetic susceptibility[83]; AKI in this setting results from a volume depletion as well as hemoglobinuria. In blackwater fever, hemolysis is caused by the abrupt release of P. falciparum trophozoites and perhaps also from the quinine used to treat it.[86] These patients are dehydrated, volume depleted from sweating, and have high fevers. Clostridial sepsis also has multiple effects on renal function including hypotension, acidosis, and DIC, as well as hemolysis.[87]

Laboratory features of intravascular hemolysis and hemoglobinuria include (1) increased serum lactate dehydrogenase (LDH) levels, (2) low serum haptoglobin levels, (3) increased unconjugated (indirect) serum bilirubin, (4) increased reticulocyte count, and (5) hyperkalemia.[83] As with myoglobinuria, hemoglobinuria and hemoglobinuric AKI are associated with pigmented urine casts. The differential diagnosis and clinical course of hemoglobin-induced AKI are similar to those described for myoglobinuria.

Prevention and Treatment of Hemoglobinuric Acute Kidney Injury

The prevention of hemoglobinuric AKI involves many of the same preventive measures for myoglobinuric AKI, such as correcting the volume depletion and the administration of bicarbonate. In fact, Bywaters' therapy to treat crush injuries using saline and bicarbonate in the 1940s was based on earlier reports demonstrating such therapy was beneficial in preventing renal failure in mismatched blood transfusion reactions.[88] Interestingly, in an experimental animal model of hemoglobinuric AKI, the simultaneous administration of the amino acid, lysine, prevented the development of AKI. This was attributed to the ability of lysine to inhibit proximal tubular reabsorption of hemoglobin or its heme moiety.[89] The clinical use of such therapy remains to be determined.

The management of sustained AKI usually requires hemodialysis. These patients, in general, are less catabolic than patients with rhabdomyolysis. The AKI usually lasts 1 to 2 weeks, but full recovery of renal function is often the case.

CRYSTAL-INDUCED ACUTE KIDNEY INJURY

Uric Acid Nephropathy

Acute uric acid nephropathy is the term given to the development of AKI caused by renal tubular obstruction by urate and uric acid crystals. The main clinical setting in which uric acid nephropathy occurs is the treatment of malignancy, especially of leukemia and lymphoma. Treatment of these malignancies results in cell death and the release of large amounts of uric acid precursors. Some patients with these malignancies also have renal insufficiency and high serum uric acid levels before chemotherapy, possibly because of early uric acid nephropathy and the rapidly dividing cell population.[90]

Properties of Uric Acid

The final breakdown product of purine degradation in humans is uric acid (Fig. 36.2). Most other mammals degrade purines to the soluble end product allantoin, but humans lack the enzyme uricase. Uric acid (2,6,8-trioxypurine) is a weak acid with a pK_a of 5.75. Urates are the ionized form of uric acid, and at a physiologic pH of 7.4, over 95% of uric acid dissociates into urates, with 98% existing as monosodium urate. However, uric acid predominates in acidic urine. Although initial in vitro and in vivo studies had shown urate binding to plasma proteins, urate binding to human serum proteins probably is not significant.[90]

At a temperature of 37°C and a plasma pH of 7.40, the saturation point of urate is at a concentration of 8.8 mg per deciliter, which is only slightly above the normal physiologic range

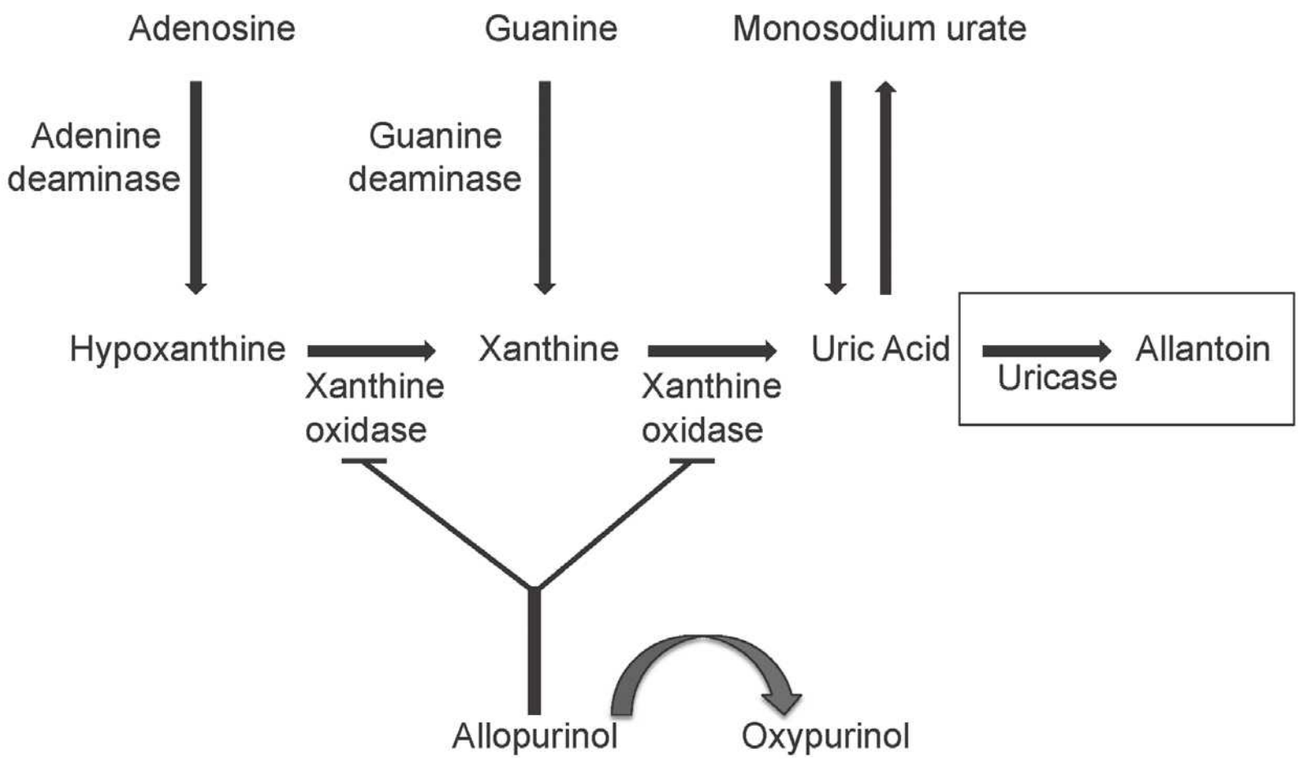

FIGURE 36.2 The pathway of purine degradation showing the competitive inhibition of urate formation by allopurinol and the site of action of rasburicase. The conversion of uric acid to allantoin by uricase (urate oxidase) does not occur in humans.

in humans.[91] However, urate crystal precipitation in the bloodstream does not occur even with concentrations much higher than the saturation point. On the other hand, the precipitation of urate occurs in extracellular fluid when the solubility concentration is exceeded. The most important factor affecting the solubility of uric acid is pH. For example, in a buffer medium at a pH of 5.0, saturation with uric acid occurs at a concentration below 10 mg per deciliter, whereas at a pH above 7.0, saturation occurs at a concentration above 150 mg per deciliter.[91]

There are four components to the renal handling of urate (Fig. 36.3). First, urate is filtered freely at the glomerulus. Virtually all of this filtered urate is then reabsorbed in the proximal tubule. An amount equal to 50% is then secreted, and after further absorption, 10% is finally excreted.[92] In humans, there is net reabsorption of urate with the fractional excretion of urate being approximately 10%.[93] Animal micropuncture studies have localized the primary nephron site of urate absorption and secretion to the proximal tubule. An anion exchanger and a voltage-dependent pathway seem to be the mechanisms involved in urate transport.[93]

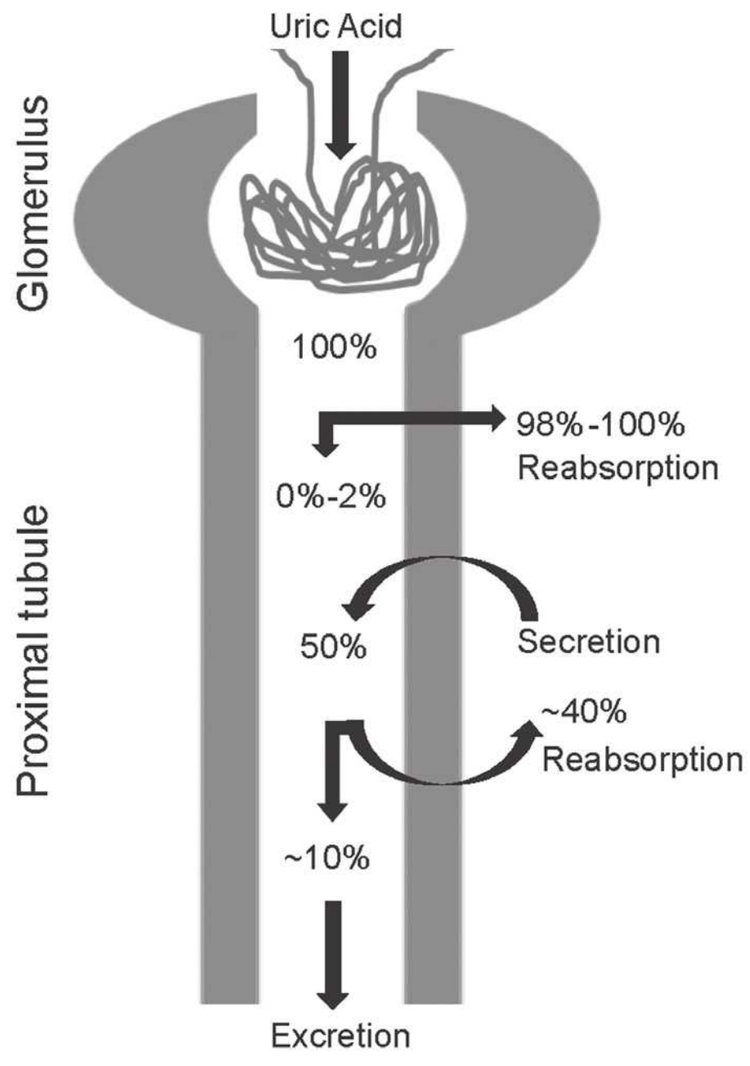

FIGURE 36.3 The renal elimination of uric acid. A four-component model of renal uric acid handling.

Pathogenesis of Uric Acid Nephropathy

Experimental animal models of uric acid nephropathy are characterized by hyperuricemia, hyperuricosuria, and uric acid deposits in and dilation of the kidney tubules, as observed in the clinical entity.[93] Along with the presence of extensive distal tubule deposits of uric acid and urate, micropuncture studies in the rat have shown increased proximal and distal tubular pressures. The vasa recti also show deposits, and efferent arteriolar and peritubular capillary pressures also are increased.[94]

Humans with malignancies and hyperuricemia have an increased urinary excretion rate and urinary concentration of uric acid both in the presence and absence of renal insufficiency.[90] Autopsy studies have documented the presence of uric acid crystals in patients with leukemia. The uric acid crystals were found only within the lumens of renal tubules, in contrast to patients with gout, in whom no uric acid crystals were found in tubular lumens.[95,96] In addition, internal hydronephrosis has been described in association with the intraluminal uric acid crystals, but the glomeruli and tubules usually are intact.[97] Supporting the concept that mechanical intraluminal obstruction causes uric acid nephropathy are the observations that the renal failure reverses after a short time and that there is earlier and greater depression of inulin clearance compared with p-aminohippurate clearance.[97] This evidence is consistent with the concept that uric acid nephropathy occurs because of uric acid crystals obstructing the renal tubular segments with maximum acidifying and concentrating abilities, namely, the distal tubule and the collecting duct. The obstruction then leads to increased intraluminal pressure, decreased filtration pressure, and a reduction in GFR.

Clinical and Laboratory Manifestations

The initial reports of uric acid nephropathy focused on patients treated for acute lymphoblastic leukemia, 10% of whom had uric acid nephropathy.[90,97] In these early patient series, risk factors for uric acid nephropathy included urine pH less than 5.0, dehydration, rapid response to chemotherapy, elevated serum uric acid, increased urinary excretion of uric acid, and preexisting renal insufficiency.[97] Tumor lysis syndrome and acute uric acid nephropathy develop primarily during the treatment of leukemia and lymphoma, but can also occur in association with the treatment of other types of malignancies or in other situations associated with elevated plasma levels and the urinary excretion of uric acid. Multiple other nonhematopoietic neoplasms have been reported to cause acute uric acid nephropathy and tumor lysis syndrome.[90] Hyperuricemic AKI also has been reported after epileptic seizures, during pregnancy, following heat stress, and in the setting of cyclosporine use and renal transplantation.[98–100]

Uric acid nephropathy during tumor lysis syndrome is characterized by elevations in serum urea nitrogen, creatinine, potassium, uric acid, and phosphate concentrations, and by a decrease in the serum calcium concentration. Hyperuricemia before chemotherapy occurs in 30% to 50% of patients with leukemia and lymphoma, and renal insufficiency seems to be more common in patients with hyperuricemia before chemotherapy.[90] The uric acid levels are now routinely normalized with allopurinol, alkalinization, and diuresis prior to the initiation of chemotherapy.[90] Patients receiving chemotherapy are at risk for other forms of renal failure in addition to acute uric acid nephropathy, and the urinary uric acid-to-creatinine ratio is a useful test to differentiate these various forms of renal failure. A ratio greater than 1 is consistent with acute uric acid nephropathy.[90] This is supported by the observation that both the serum uric acid concentration and the urinary excretion of uric acid are elevated in acute uric acid nephropathy as opposed to other forms of renal failure, where serum uric acid concentrations may be high but urinary excretion is not elevated.[101] The urinary uric acid-to-creatinine ratio is more helpful in the diagnosis of uric acid nephropathy than urinalysis, which usually is nondiagnostic. The urine sediment may be normal or may occasionally reveal amorphous material containing uric acid crystals.[90] Uric acid crystals appear as needle-shaped, negative birefringent crystals or as microcrystallites (Fig. 36.4A,B).

Elevations in BUN and serum creatinine typically develop 2 days after the initiation of chemotherapy, with a return to baseline after 7 to 10 days. Prior renal insufficiency seems to predispose one to the development of uric acid nephropathy.[102] The AKI is usually of the oliguric variety, even when treated with diuretics.[102] When indicated, one to four dialysis treatments are usually sufficient before the spontaneous return of renal function.[102]

As noted previously, the electrolyte abnormalities associated with the tumor lysis syndrome and uric acid nephropathy are hyperkalemia, hyperphosphatemia, and hypocalcemia. These abnormalities result from the release of intracellular contents after tumor necrosis, and patients with large tumor burdens are at higher risk for tumor lysis syndrome.[90] Hyperkalemia actually occurs in less than 5% of patients after chemotherapy, but if it develops, it can occur within 24 hours of the initiation of chemotherapy and can be severe enough to necessitate emergent dialysis. In fact, sudden death has been reported as a consequence of tumor lysis–induced hyperkalemia, occurring within 48 hours of chemotherapy.[103] Hyperkalemia also is more likely to occur in patients with preexisting renal insufficiency.[102]

Hyperphosphatemia, on the other hand, is very common in the tumor lysis syndrome and occurs in virtually all patients in whom AKI develops and in 30% of patients with normal renal function.[90] The development of hyperphosphatemia also is correlated with the tumor burden. In patients with renal failure, the phosphorus concentrations average 12 mg per deciliter with a range of 7 to 22 mg per deciliter.[90,102] Hypocalcemia also is common, and the development of hypocalcemia correlates with hyperphosphatemia.[90] In the presence of hyperphosphatemia, the etiology of hypocalcemia may be the precipitation of calcium phosphate salts, as discussed in the section on Myoglobinuric Acute Kidney Injury, discussed previously. When this occurs in the kidney, it may contribute to the AKI seen in tumor lysis syndrome.[104] Hyperphosphatemia also may contribute to the development of hypocalcemia by depressing the production of $1,25(OH)_2D_3$. Hyperphosphatemia has been shown to worsen experimental AKI but the mechanism is not clear because calcium phosphate deposition could not be demonstrated in animal models.[105] Calcium deposits have been found at an autopsy in the calyces and tubules of a patient who had AKI in association with tumor lysis syndrome, hyperphosphatemia (20 mg per deciliter), and hypocalcemia.[106]

Despite these characteristic manifestations of tumor lysis syndrome, it is difficult to predict in which patients acute uric acid nephropathy will develop. Even when appropriate prophylactic measures are taken with hydration, alkalinization, and allopurinol, the following still may develop: hyperuricemia in 9%, hyperphosphatemia in 25% to 50%, hypocalcemia in 10% to 60%, and hyperkalemia in 8%. However, the incidence of clinically significant tumor lysis syndrome after chemotherapy is only 5%.[90] The likelihood for the development of acute uric acid nephropathy is increased in the presence of renal insufficiency, in patients with oliguria before therapy, and in patients with lymphoma with high serum LDH levels.[102,107]

Differential Diagnosis

The diagnosis of acute uric acid nephropathy should be suspected when AKI, in concert with tumor lysis syndrome, develops within the first 1 to 3 days after the initiation of chemotherapy for lymphoma or leukemia. The diagnosis sometimes is made difficult by the variety of drugs, radiographic studies requiring contrast exposure, and the associated clinical problems common during the early presentation of malignancies. Renal complications associated with

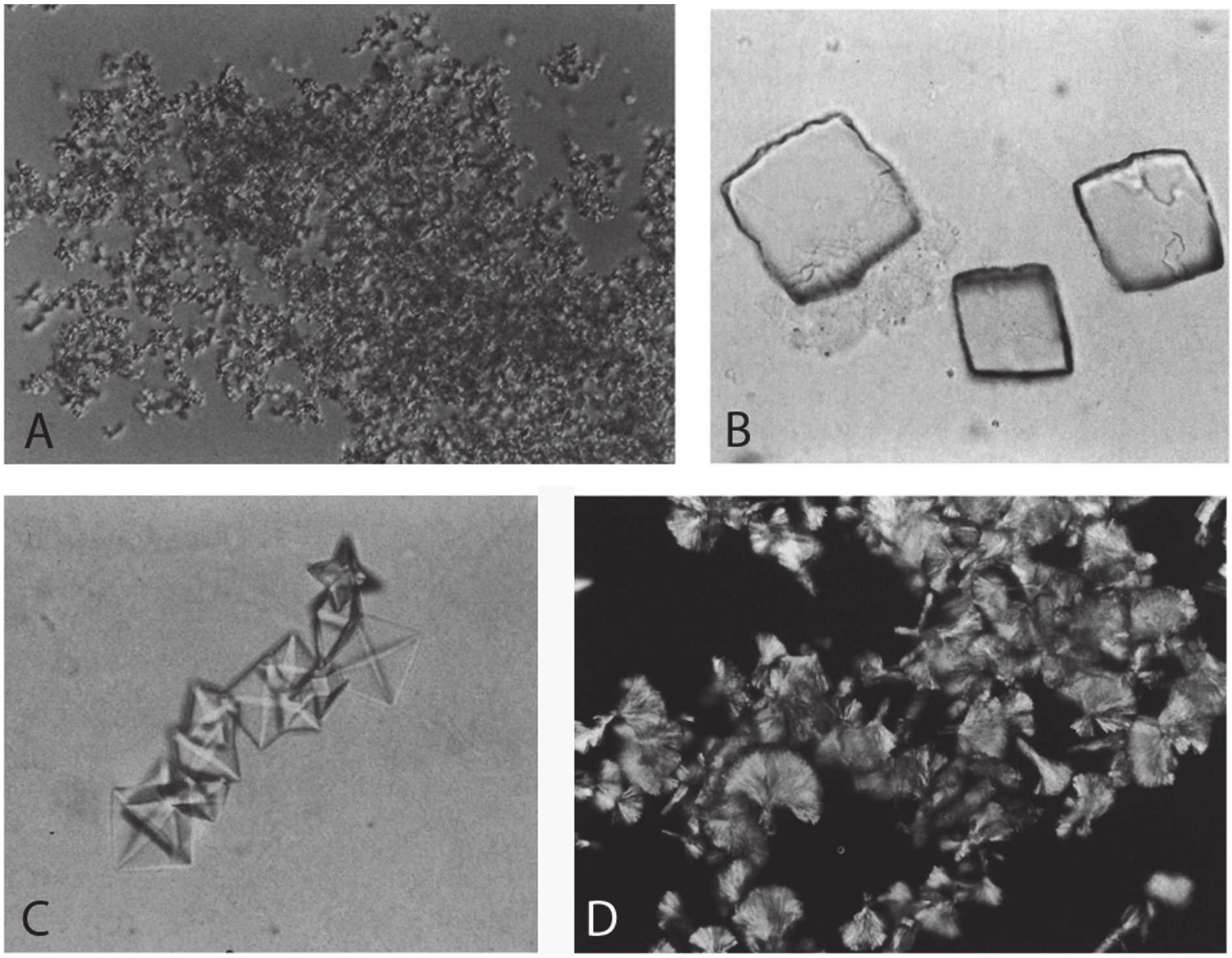

FIGURE 36.4 **A:** Amorphous urate crystals in urine. (Interference contrast microscopy, magnification ×200.) **B:** Uric acid crystals, cuboidal shape. (Magnification ×160.) **C:** Envelope-shaped calcium oxalate dihydrate form of crystal. (Note: Needle-shaped monohydrate calcium crystals are not pictured.) **D:** Sulfadiazine crystals in urine. (Polarized microscopy, magnification ×40.) These birefringent crystals often assume a "fan" or "sheaf of wheat" shape. (Courtesy of Professor M. H. Haber, Department of Pathology, Rush Medical College, Chicago, IL.)

malignancies include direct lymphomatous or leukemic renal infiltration, obstruction due to stones, and obstruction due to malignancy (summarized in Table 36.6). However, despite the common occurrence of direct lymphomatous or leukemic renal infiltration, it rarely causes AKI.[102]

Spontaneous hyperuricemia occurs more commonly with lymphomas, and some patients present with renal insufficiency before treatment, possibly because of uric acid nephropathy.[90] Ultimately, the diagnosis of acute uric acid nephropathy is made in the presence of tumor lysis syndrome with the urinary uric acid-to-creatinine concentration ratio greater than 1 and with the exclusion of other causes of AKI.

Prophylactic Measures and Treatment

Before the dialysis era, the mortality rate from AKI associated with uric acid nephropathy was 50%, but with modern treatment, including proper prophylaxis and dialysis, uric acid nephropathy is rare, but when it does occur the prognosis for the AKI is excellent.[90] The treatment approach to uric acid nephropathy is divided into two stages. The first is to prevent or minimize the metabolic consequences of the tumor lysis syndrome, and the second is to treat these consequences when they do occur (Table 36.7). The approach to both prophylaxis and the treatment of tumor lysis syndrome includes the inhibition of xanthine oxidase, forced diuresis, and urinary alkalinization. When these measures fail to prevent the consequences of tumor lysis, and AKI from uric acid nephropathy develops, dialysis must be initiated to treat uremia and severe electrolyte problems, and to control hyperuricemia. However, rasburicase, a recombinant form of urate oxidase, is an additional option for patients with AKI from uric acid nephropathy and in patients with a high risk to develop tumor lysis syndrome (see the following).[108]

36.6 Acute Renal Complications Associated with Malignancies

Prerenal
Extracellular fluid depletion (poor intake, vomiting, diarrhea, hypercalcemia)
Hepatorenal syndrome (veno-occlusive disease, hepatic resection)
Drugs (calcineurin inhibitors, nonsteroidals)
Intrinsic
Glomerular Membranous nephropathy Amyloidosis (multiple myeloma) Pamidronate-associated collapsing Glomerulopathy (incidence unknown) Light-chain deposition disease
Tubulointerstitial Acute tubular necrosis (toxic/ischemic) Lymphomatous infiltration of the kidney Light-chain deposition disease Drugs (cisplatin, ifosfamide) Intravenous contrast Cast nephropathy (multiple myeloma)
Vascular Thrombotic-thrombocytopenic purpura/ hemolytic uremic syndrome (post-HCT, gemcitabine, mitomycin C) Tumor infiltration (renal cell carcinoma with renal vein thrombosis)
Postrenal
Intratubular obstruction
Uric acid nephropathy
Methotrexate
Cast nephropathy (multiple myeloma)
Extrarenal obstruction
Bladder outlet, ureteral (primary disease, retroperitoneal lymphadenopathy, retroperitoneal fibrosis)

HCT, hematopoietic cell transplant.
Reprinted from Humphreys BD, Soiffer RJ, Magee CC. Renal failure associated with cancer and its treatment: an update. J Am Soc Nephrol. 2005 Jan;16(1):151–161, with permission.

36.7 The Approach to Uric Acid Nephropathy and Tumor Lysis Syndrome

Approach to prophylaxis for tumor lysis syndrome and uric acid nephropathy
Patients presenting prior to chemotherapy with hyperuricemia and evidence of a large tumor burden: allopurinol, 300–600 mg 4–5 L/24 hr of normal saline. Add diuretics if the patient is well hydrated and not maintaining an adequate urine output. If there is no response to diuretics, match fluid input with urine output. Urinary pH should be maintained above 7.0 by titrating intravenous bicarbonate therapy. Start with 100 mEq/L of sodium bicarbonate in D5W per hour. Bicarbonate therapy should be discontinued after serum uric acid is normalized. If clinically feasible, postpone chemotherapy until uric acid is normalized along with any other electrolyte abnormalities.
Patients presenting prior to chemotherapy and with a normal uric acid level but still at risk for tumor lysis syndrome: Allopurinol, 300–600 mg 4–5 L/24 hr of normal saline as described If clinically feasible, postpone chemotherapy until 2 days after start of allopurinol
Treatment of uric acid nephropathy and tumor lysis syndrome
Hemodialysis initiated per the routine indications including hyperkalemia, acidosis, hyperphosphatemia, volume overload, or uremia. Hemodialysis for control of hyperuricemia unresponsive to the previous measures. Adjust allopurinol doses for renal failure:

Creatinine clearance	Allopurinol dose
0	100 mg q3d
10	100 mg q2d
50	200 mg qd

After hemodialysis, supplement with 50% of allopurinol dose.
D5W, 5% dextrose with water; q3d, every 3 days; q2d, every 2 days; qd, every day.

Inhibition of Xanthine Oxidase. Allopurinol is a substrate for and a competitive inhibitor of the enzyme xanthine oxidase (Fig. 36.2). It blocks the conversion of hypoxanthine and xanthine to uric acid resulting in a reduction in both serum uric acid concentration and a urinary excretion of urates. In the presence of allopurinol, hypoxanthine and xanthine accumulate instead of uric acid, and the urinary excretion of these precursors also increases.[109] Hypoxanthine is highly soluble and even with increased renal excretion does not cause clinical problems. Xanthine, on the other hand, is less soluble than uric acid. Precipitated xanthine can be found in the urine of patients receiving allopurinol, but these precipitates do not correlate with the development of renal failure.[109] However, well-documented cases of xanthine nephropathy and xanthine calculi associated with allopurinol use have been reported.[109,110]

The half-life of allopurinol is less than 2 hours owing to prompt renal elimination and rapid conversion to its chief metabolite, oxypurinol. Oxypurinol is an active metabolite and reduces serum uric acid concentration and urinary uric acid excretion half as much as allopurinol.[111] Unlike allopurinol, oxypurinol is eliminated solely by the kidney, with a half-life of approximately 24 hours.[111] Renal clearance of oxypurinol is decreased with reduced renal function and with creatinine clearance such that with a creatinine clearance of less than 10 mL per minute, the half-life of oxypurinol is approximately 1 week.

In patients with normal renal function and hyperuricemia associated with malignancy, allopurinol decreases serum uric acid within 48 hours with a peak effect at 5 days.[112] The clinical effects of allopurinol probably are mediated by oxypurinol because the half-life of allopurinol is short. Despite the use of allopurinol, hyperuricemia and acute uric acid nephropathy sometimes cannot be avoided, and reasons for this failure include a large tumor burden, aggressive chemotherapy, and the inability to delay chemotherapy until allopurinol has decreased the serum uric acid concentration.

For optimal prophylaxis, allopurinol should be administered at least 3 days before chemotherapy. The level of existing renal function also must be considered when dosing the drug. Allopurinol can lead to a life-threatening toxicity syndrome that is characterized by a diffuse, desquamative skin rash; fever; hepatic dysfunction; eosinophilia; and worsening renal function of unknown etiology, which, however, is consistent with a diffuse vasculitis. Eighty percent of patients reported with this toxicity had renal insufficiency.[113] Improper dosing of allopurinol also can lead to xanthine nephropathy.[113]

Optimal allopurinol dosing is reflected by a therapeutic serum oxypurinol concentration that ranges from 30 to 100 μmol per liter.[114] Patients with end-stage renal disease achieve therapeutic levels of oxypurinol after one dose of allopurinol (300 to 600 mg) and maintain this level until the next dialysis, at which time the serum level is reduced by 40%.[113] Therefore, the maintenance dose must be reduced in patients with renal insufficiency to avoid an accumulation of oxypurinol. The oral route is equivalent to intravenous dosing of allopurinol; therefore, intravenous dosing should be considered only in patients unable to take anything by mouth. A rectal administration of allopurinol is not effective and should not be used.[114] Allopurinol started at 300 to 600 mg is safe and achieves therapeutic levels of oxypurinol, but the peak clinical effect on uric acid production is not seen for 3 days.

Rasburicase. As previously mentioned, most mammals degrade purines to the soluble end product allantoin, using the enzyme uricase (Fig. 36.2), which humans lack. Rasburicase, the recombinant form of urate oxidase, has several advantages over allopurinol. Rasburicase has a rapid onset of action and has been shown to return uric acid to normal levels with hours.[115,116] Unlike allopurinol, which inhibits the production of uric acid, rasburicase quickly reduces the existing uric acid levels and does not rely on the renal clearance of existing uric acid or alkalization of the urine. In one compassionate use trial, rasburicase (0.20 mg per kilogram) was administered intravenously once a day for 1 to 7 days. The mean uric acid level in 29 hyperuricemic children decreased from 15.1 to 0.4 mg per deciliter, and in 27 hyperuricemic adults, the mean level decreased from 14.2 to 0.5 mg per deciliter.[117] Rasburicase is an expensive drug and although clinical trials have compared rasburicase to allopurinol, the outcomes have been a decrement in uric acid levels rather than important metabolic outcomes or AKI.[115,116] Furthermore, there are recent reports of methemoglobinemia and hemolytic anemia that may be related to the use of rasburicase.

Forced Diuresis. Animal data have suggested that high renal tubular fluid flow induced by a solute or water diuresis is important in the prevention of acute urate nephropathy. In fact, rats treated with high-dose furosemide and Brattleboro rats with central diabetes insipidus and water diuresis both had complete protection from uric acid nephropathy, whereas rats treated solely with urine alkalinization had only partial protection.[118] Diuresis probably imparts protection by lowering the urate concentration in the collecting duct where uric acid precipitation occurs, or by effects on tubular urate handling. Whether these results can be applied to humans is not known because species differences in urate handling exist.

Despite efforts to maintain high urine flow with hydration and diuretics, a lower urine flow rate preceding chemotherapy is more common in patients who have renal failure than in those who do not.[102] Although this observation probably reflects the existence of mild spontaneous uric acid nephropathy before chemotherapy, it is reasonable to assume that increased urine flow would add protection from uric acid nephropathy. Patients should be hydrated with 4 to 5 L of normal saline every 24 hours. If the patient is well hydrated and not maintaining the expected urine output, diuretics should then be initiated. If urine output remains

low, fluid intake should be adjusted to match output in the effort to avoid fluid overload.

Urinary Alkalinization. Although evidence is lacking to confirm its role in preventing uric acid nephropathy, urinary alkalization remains a prominent component in prophylactic regimens. The theoretical benefit of urinary alkalinization is to increase the solubility of uric acid. However, in animal studies, the most important intrarenal dynamic in the prevention of acute uric acid nephropathy was high urine tubular flow.[118] In this study, the use of acetazolamide achieved only partial protection, which was likely due to the drug's diuretic effect and not its effect on urine pH.[118] Along with the inherent risk of causing a severe metabolic alkalosis when attempting to alkalinize the urine with sodium bicarbonate administration, other potential disadvantages include increasing the risk of symptomatic hypocalcemia and calcium phosphate precipitation, which can cause AKI by itself in this setting.[119] Urinary alkalinization also does not have an effect on xanthine precipitation because the pK_a of xanthine is 7.4, as opposed to 5.6 for uric acid.

Bicarbonate therapy should be included in the prophylactic regimen only when attempting to correct hyperuricemia. If hyperuricemia is present before chemotherapy, bicarbonate should be added to intravenous fluids with the aim of keeping the urine pH above 7.0. Once hyperuricemia has been corrected, bicarbonate therapy should be discontinued.

Hemodialysis. Dialysis assists in the management of acute uric acid nephropathy in two ways. First, dialytic therapy is initiated for the typical indications common in AKI such as hyperkalemia, severe hyperphosphatemia, azotemia, and fluid overload, although these indications may be more severe and may occur more rapidly than in other forms of AKI. Cases of fatal hyperkalemia have occurred within hours after the initiation of chemotherapy.[119] Second, dialysis is an effective way to reduce the serum uric acid level. This is an important role for dialysis because patients usually do not recover from acute uric acid nephropathy until the serum uric acid level is reduced.[120] Once this occurs, usually after only one to four dialysis treatments, recovery of renal function is signaled by a brisk diuresis.

Depending on the dialyzer and blood flow used, hemodialysis has a uric acid clearance rate of 90 to 150 mL per minute, whereas peritoneal dialysis clearance is only 10 to 20 mL per minute.[120,121] When starting a patient on hemodialysis, caution should be taken not to use a high-calcium bath if severe hyperphosphatemia is present because of the risk of increasing the calcium–phosphorus product. Selected patients may benefit from continuous renal replacement therapy such as CVVHD.

Ethylene Glycol Toxicity

Acute ethylene glycol intoxication is a medical emergency that, if not treated aggressively, leads to serious neurologic, cardiopulmonary, and renal dysfunction, and may result in death. Ethylene glycol, an odorless and clear liquid, is the major ingredient in antifreeze, and is most commonly consumed either intentionally by alcoholics seeking an ethanol substitute or accidentally by children. An ingestion of 100 mL is considered the minimal lethal dose of ethylene glycol.[122,123] Diethylene glycol is a condensation product of ethylene glycol production, and ingestion causes the same toxicities as ethylene glycol.[124] Diethylene glycol was used as the diluent in the first sulfa antibiotic, sulfanilamide, and consequently led to mass poisonings in 1937. One hundred five patients died from the therapeutic use of Elixir Sulfanilamide, and one important consequence of this tragedy was the 1938 Federal Food Drug and Cosmetic Act requiring proof of product safety before release of a drug.[125] Unfortunately, this kind of governmental supervision of pharmaceutical companies does not exist in other countries such as Nigeria and Haiti, where 47 and 85 children, respectively, died when diethylene glycol was used as a solvent in a preparation of cough syrup.[126,127]

Metabolism of Ethylene Glycol

The metabolism of ethylene glycol is complex and incompletely understood. As is the case with other alcohols such as ethyl and methyl alcohol, nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase is responsible for the first oxidative step converting ethylene glycol to glycolaldehyde (Fig. 36.5). After this first step the pathways have not been well elucidated in humans, but are thought to include the following: glycolaldehyde oxidized to glycolic acid by aldehyde oxidase, glycolic acid to glyoxylate by glycolic acid oxidase or LDH, and then numerous subsequent pathways for glyoxylate metabolism, including one to oxalate by LDH and glycolic acid oxidase (Fig. 36.5).[122] Glycolate is converted to glyoxylate very slowly and is probably the rate-limiting step in the metabolism of ethylene glycol, whereas glycolaldehyde and glyoxylate have very short half-lives.[128]

Ethylene glycol metabolites are thought to mediate the toxicity seen with ethylene glycol ingestion, and ethylene glycol itself is not toxic. In fact, the inhibition of ethylene glycol metabolism with ethyl alcohol or pyrazole prevents toxicity.[129,130] The observation that the mortality rate in rats is reduced by performing a partial hepatectomy before the administration of ethylene glycol and glycolate illustrates the importance of ethylene glycol metabolites on toxicity. The partially hepatectomized rats metabolized ethylene glycol more slowly to its toxic byproducts, which allowed more time for renal excretion of the nontoxic and unchanged ethylene glycol.[131] Glycolate and oxalate are thought to be important mediators of ethylene glycol toxicity.

The pathophysiologic process of ethylene glycol toxicity is multifactorial and is thought to include the accumulation of toxic ethylene glycol metabolites, calcium oxalate crystal deposition in tissues, and the effects of severe acidosis. After the administration of a lethal dose of ethylene glycol in rats, profound renal oxalosis is produced, and the same occurs with administration of glycolic acid and glyoxylic acid. Renal

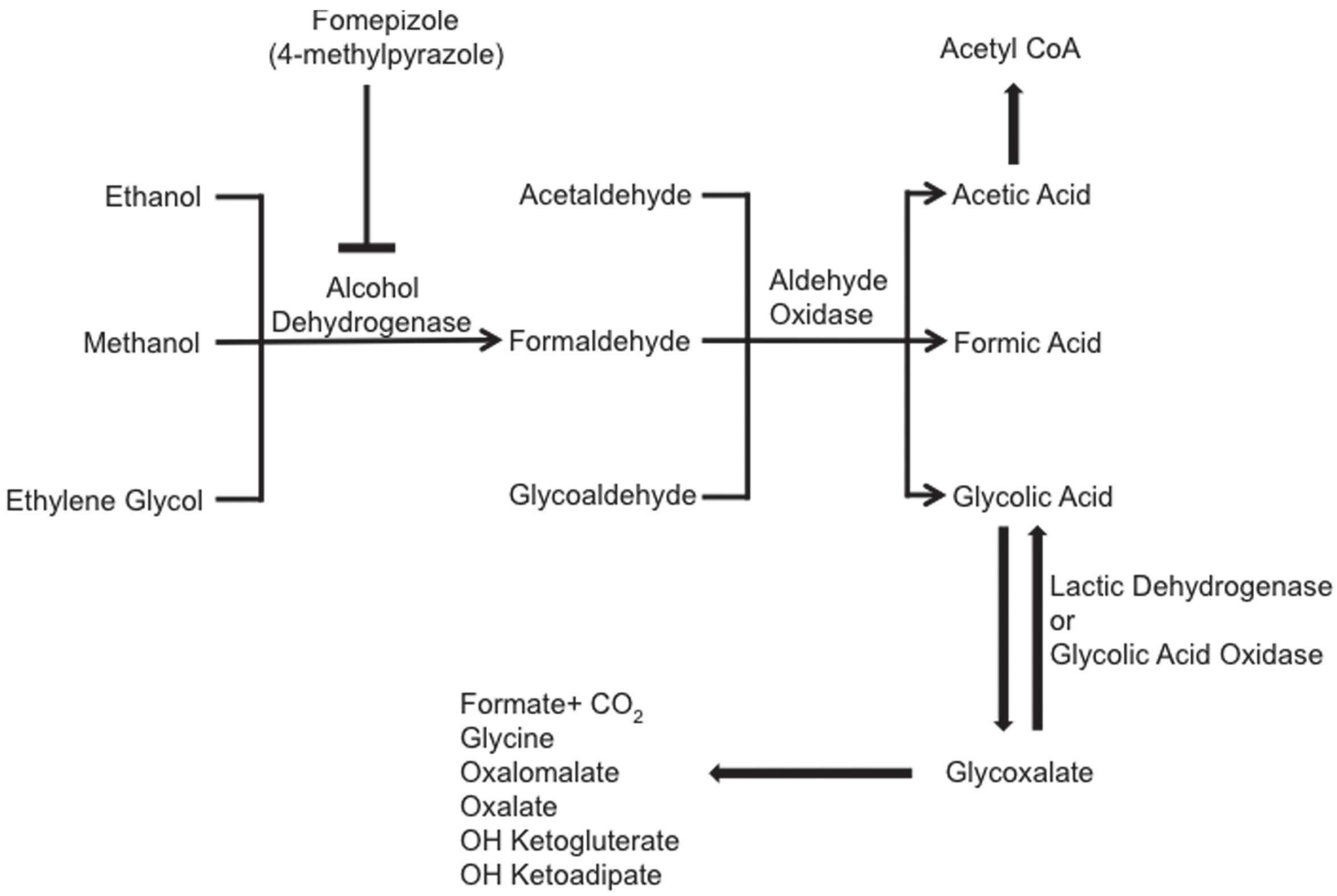

FIGURE 36.5 The pathway of ethylene glycol degradation.

pathology in these rats demonstrated calcium oxalate crystals in the proximal and distal convoluted tubules, with smaller amounts in the collecting tubule and none in the glomeruli or renal interstitium.[132] The degree of crystal formation correlated with diffuse convoluted tubular dilatation, but occasional epithelial necrosis seemed to bear a relation to the degree of crystal formation. The administration of glycolaldehyde did not produce significant crystal formation or the same degree of microscopic changes, but did lead to pronounced tubular epithelial swelling. In addition to the renal findings, oxalate crystals were found in brain tissue.[132] In dogs given nonlethal doses of ethylene glycol, renal biopsy specimens revealed interstitial edema, tubular dilatation, hydropic degeneration, and tubular cell necrosis even in areas free of crystals. Electron microscopic findings were most prominent in proximal tubule cells and included vacuolization, cellular rupture, cytoplasmic buds, and increased density of mitochondria.[133] This pattern of proximal tubule damage is similar to other models of ischemic and nephrotoxic forms of AKI.[133]

These findings are similar to human autopsy series that also have found calcium oxalate crystals in renal tubules. In these studies, renal epithelial cells appear either normal or extensively necrotic depending on the interval between ingestion and death and appear minimal if any damage of the glomeruli has been reported.[122,123] Despite severe clinical neurologic disturbances, the brain characteristically has only mild perivascular and meningeal deposition of calcium oxalate crystals, but edema, capillary engorgement, hemorrhage, and infarctions also have been described.[122,123,134] Although crystal deposition usually is not reported, myocardial tissue findings are consistent with myocarditis.[134] Autopsy and renal biopsy studies do not support the hypothesis that calcium oxalate crystallization is the primary cause of ethylene glycol toxicity; despite widespread renal tubule damage, calcium oxalate crystal deposition is patchy; and despite the presence of crystals there is no tubule obstruction or dilatation.

High–anion-gap metabolic acidosis is a major feature of ethylene glycol intoxication, and is also thought to contribute directly to the clinical toxicity. In rats poisoned with ethylene glycol, survival rates are five times greater after treatment with sodium bicarbonate alone or with ethanol alone compared with no treatment. Giving ethanol and sodium bicarbonate together improved the survival rate to six times that seen in rats with no treatment.[135] The major determinant of the metabolic acidosis is glycolic acid; in dogs and monkeys, the administration of ethylene glycol produces severe metabolic acidosis and the depressed bicarbonate is matched by the increase in glycolic acid production.[122] Oxalic acid is very toxic to kidneys and is lethal in doses much lower than toxic doses of ethylene glycol, but in rats and monkeys, only 0% to 2.5% of the original dose of ethylene glycol is excreted as oxalic acid.[136,137] In humans with ethylene glycol poisoning, the plasma glycolate concentration correlated with the increased anion gap, and the serum concentrations of oxalate and glyoxylate were negligible in these patients.[122,128] Although studies have suggested that organic acids such as lactic acid contribute to the severe metabolic acidosis of ethylene glycol intoxication, glycolic acid seems to be the main cause of the acidosis with lactic and β-hydroxybutyric acids being elevated in special circumstances such as that associated with hypotension or alcoholic ketoacidosis.[138,139]

It has been postulated that the production of glycolaldehyde, glyoxal, glycolate, and glyoxylate from ethylene glycol metabolism is important in the pathophysiologic process of toxicity. Aldehyde production is greatest 6 to 12 hours after ethylene glycol ingestion, and this is when cerebral symptoms are most severe.[122,123] However, as mentioned, glycolate is the only metabolite that accumulates; its direct toxicity has not been well studied, but it is known to be toxic in animals.[137] For example, glycolic acid given to rats is lethal and also causes renal tubular oxalosis.[132] The role that glycolic acid plays in human renal, cerebral, and cardiac toxicity remains to be proven, but it probably is one of the multifactorial causes along with acidosis and calcium oxalate crystals.

Clinical and Laboratory Manifestations

The initial reports of ethylene glycol poisoning in the 1940s and 1950s noted that the clinical manifestations of acute ethylene glycol poisoning could be divided into three stages.[140] During the first stage, occurring 30 minutes to 12 hours after ethylene glycol ingestion, the central nervous system manifestations predominate. During the second stage, occurring over the next 12 hours, cardiopulmonary dysfunction develops and includes tachypnea, pulmonary edema, and cardiac failure. In patients who survive past the first 24 hours, the third stage is characterized by prolonged renal failure. Before the advent of aggressive treatment with hemodialysis and intravenous ethanol, these stages were very typical of the clinical course of most patients, but with modern treatment and depending on the amount of ethylene glycol ingested, the sequence and occurrence of these clinical features and stages vary considerably.[122,123]

In addition to apparent inebriation without an alcoholic odor, central nervous system manifestations include nystagmus, depressed reflexes, seizures, and coma.[122,123] The delayed appearance of multiple cranial nerve deficits also has been reported, and the deficits have not always been reversible.[141] Ocular effects are a main feature of methanol ingestion but ophthalmoplegia, papilledema, loss of visual acuity, and eventual optic atrophy also have been reported with ethylene glycol ingestion.[142] Abdominal signs and symptoms including nausea, vomiting, and pain are very common.[122,123] For unexplained reasons, mild hypertension, tachycardia, and a low-grade fever sometimes are present.[122,123]

High–anion-gap metabolic acidosis with a high osmolar gap is the most striking initial laboratory finding and, when combined with clues in the history of the patient, is the main diagnostic feature. The severity of the clinical presentation depends on the quantity of ingested ethylene glycol and the elapsed time since its ingestion, but typically, patients present with a pH of less than 7.2, bicarbonate less than 10 mEq per liter, anion gap greater than 20, mean osmolal gap of 35, measured osmolality greater than 300 mOsm, and hyperkalemia.[122,123] Hypocalcemia is a frequent finding and can be severe and symptomatic leading to tetany or cardiac arrhythmia.[122,123] The onset of hypocalcemia is usually within the first 12 hours, and serum calcium usually remains low despite treatment. Hypocalcemia probably is caused by a combination of chelation of calcium by oxalate and an abnormal parathyroid hormone response.[122,123]

Lumbar puncture frequently is performed because of mental status changes, and the cerebrospinal fluid sometimes reveals pleocytosis with a sterile culture.[122,123,134] A normal hematocrit and platelet count, but a moderate leukocytosis of 10,000 to 40,000/mm^3 with a predominance of polymorphonuclear cells is seen commonly in the initial complete blood count.[122,123,134]

The urinalysis typically includes a low specific gravity, mild proteinuria, microscopic hematuria, and pyuria.[122,123] Crystalluria is not invariably present but usually is seen on presentation. The envelope-shaped calcium oxalate dihydrate form of crystal (octahedral dihydrate) (Fig. 36.4C) traditionally has been thought of as the most commonly seen crystal in ethylene glycol intoxication, but in fact needle-shaped monohydrate calcium oxalate crystals predominate in ethylene glycol intoxication.[122,123] Monohydrate calcium oxalate crystals are thermodynamically stable, and with time, the dihydrate form transforms to the monohydrate form.[143] In vitro, the dihydrate form is seen only at high concentrations of both calcium and oxalate.[143] The pattern of oxalate crystals in individual patients transforms from the envelope-shaped crystals to the needle-shaped crystals in a matter of hours.[138]

The cardiopulmonary consequences of ethylene glycol intoxication now are rarely seen with prompt, aggressive treatment. After the ingestion of ethylene glycol sufficient to cause metabolic acidosis, oliguric renal failure develops in most patients.[144,145] If aggressive treatment, including dialysis and fomepizole or ethanol, is provided soon after the ethylene glycol is ingested, renal failure can be avoided. However, most patients do not seek medical attention until symptoms develop, which usually is many hours after ingestion. Thus, renal failure is common and may develop as soon as 24 hours after ingestion.[122,146] The course of the renal failure is typical of oliguric acute tubular necrosis. The oliguria lasts 4 to 5 days and is followed by a diuretic phase. BUN and serum creatinine usually peak at 7 to 10 days, and most patients require only 1 to 2 weeks of dialytic support.[147,148] However, some patients require dialysis for many months, and despite the return of sufficient kidney function to stop dialysis, kidney function does not always return to baseline values.[138]

Diagnosis

In the absence of ketoacidosis, and in the presence of the characteristic signs and symptoms, it should be assumed that all patients presenting with metabolic acidosis combined with increased anion and osmolal gaps have either methanol or ethylene glycol poisoning.[148] The prognosis of both these poisonings is improved with early diagnosis and treatment, and therefore, if the diagnosis cannot be confirmed with serum levels of methanol or ethylene glycol treatment with

bicarbonate and ethanol infusion and hemodialysis should be initiated.[148] Determining specific levels of each alcohol is the most specific test. Because these tests are not available in all hospitals,[149] adding fluorescein to the urine and then observing for urine fluorescence with an ultraviolet Wood lamp is helpful in making the diagnosis.

Once the diagnosis of ethylene glycol poisoning has been confirmed and the blood concentration of any concomitantly ingested ethanol has been determined, the serum ethylene glycol level can be estimated using the osmolal gap.[150,151] Ethylene glycol levels above 20 mg per deciliter can be lethal if not treated aggressively.[144]

Clinical Course and Treatment

Initial Emergency Department Treatment. Gastric lavage should be initiated to reduce further drug absorption if the patient is seen in the first few hours after ethylene glycol ingestion.[148,152] Both methanol and ethylene glycol intoxication were previously treated with an ethanol infusion (to prevent the production of toxic metabolites) followed by hemodialysis to remove the actual substance from the body.[148,152] However, fomepizole (4-methylpyrazole; Antizol) is now the drug of choice.[153,154] Hemodialysis to provide a source of bicarbonate and to clear ethylene glycol and its metabolites is the therapy of choice for the treatment of the acidosis. However, hemodialysis usually is delayed and during this waiting period, patients require large doses (300 to 500 mEq) of sodium bicarbonate, and the metabolic acidosis is not corrected until hemodialysis is initiated.[155]

Correction of the acidosis may increase the likelihood of symptomatic hypocalcemia such as seizures, tetany, and cardiac dysfunction. Intravenous calcium supplementation should be given cautiously because of the potential risk of further calcium oxalate precipitation. Calcium should be given if clinical signs or symptoms of hypocalcemia develop, but not prophylactically.[148,152] Thiamine and pyridoxine are cofactors required in the nontoxic metabolic pathways of ethylene glycol (away from oxalate), and early replacement of these cofactors is advocated to prevent potential depletion.[148,152]

The administration of ethanol and hemodialysis has traditionally made up the definitive treatments for ethylene glycol intoxication. Compared with ethylene glycol, ethanol has a higher affinity for alcohol dehydrogenase and therefore inhibits the metabolism of ethylene glycol to the toxic metabolites, permitting the ethylene glycol to be renally excreted or dialyzed. With a blood ethanol level of 100 mg per deciliter liver alcohol dehydrogenase is saturated, and the half-life of ethylene glycol increases from 3 to 17 hours.[122,148,152] Since the first report of ethanol treatment in humans, ethanol has been used in conjunction with dialysis in the treatment of ethylene glycol poisoning, and ethanol is not recommended as a sole treatment.[129] Although there have been reports of successful treatment with ethanol without dialysis, these were isolated cases in which ingestion only of small amounts of ethylene glycol occurred.[156]

For the maximal inhibition of ethylene glycol metabolism, the plasma ethanol concentration should be maintained between 100 and 200 mg per deciliter. This is achieved with a loading dose of 0.6 g per kilogram, followed by a maintenance dose of 66 mg per kilogram in nondrinkers, and 154 mg per kilogram in regular alcohol consumers. During dialysis, 7.2 g per hour should be added to the maintenance dose.[157] Oral ethanol also can be used, but the dose should be increased by 50% if given soon after the administration of charcoal.[157] Intravenous ethanol comes in 5% and 10% solutions diluted in dextrose and water, whereas a 20% or 50% solution usually is used for oral or nasogastric administration. The specific gravity of ethanol is used in calculating the correct dose.[157] Until the correct dose to achieve a level between 100 and 200 mg per deciliter has been ascertained, hourly ethanol concentrations should be checked. The dosing guidelines for ethanol in ethylene glycol toxicity is summarized in Table 36.8.

36.8 Dosing Guidelines for Ethanol Treatment

Ethanol Solution and Route of Administration	Specific Gravity of Ethanol (g/dL)	Loading Dose (100 mg/dL of ethanol × 0.6 L/kg) (mL/kg)	Maintenance Dose in Nondrinkers 66 mg/kg/hr (mL/kg/hr)	Maintenance Dose in Drinkers 154 mg/kg/hr (mL/kg/hr)	During Dialysis Add the Following to the Maintenance Dose (mL/hr)
5% IV	3.9	15.4	1.7	3.9	185
10% IV	7.8	7.7	0.84	2.0	90
20% PO	15.8	3.8	0.42	1.0	45
50% PO	39.5	1.5	0.17	0.4	18

Oral ethanol dose should be increased by 50% after charcoal therapy. IV, intravenous; PO, orally.

4-Methylpyrazole (Fomepizole). Fomepizole is a potent inhibitor of alcohol dehydrogenase, and animal studies have shown that fomepizole prevents ethylene glycol–related mortality and toxicities, and increases the urinary excretion of ethylene glycol by preventing its metabolism.[130] In humans, fomepizole has been shown to normalize acidosis within hours, to prevent decreases in renal function if used early, and to decrease serum levels of ethylene glycol toxic metabolites.[153,154,158] In humans without AKI, treatment results in an increase in the ethylene glycol half-life from 3 to 14 hours, an increase in urinary excretion of ethylene glycol, and the prevention of clinical toxicity.[153,154]

Fomepizole offers advantages over ethanol treatment including predictable pharmacokinetics, avoiding the need to achieve and maintain the desired blood ethanol level, and avoiding an ethanol-induced central nervous system depression. Fomepizole is available as a parenteral solution. The loading dose is 15 mg per kilogram intravenously, followed by 4 more doses of 10 mg per kilogram every 12 hours, after which it is continued at a rate of 15 mg per kilogram every 12 hours until the ethylene glycol concentration is undetectable or the patient is asymptomatic with a resolution of the high–anion-gap metabolic acidosis. Like ethanol, the dose of fomepizole is adjusted during dialysis therapy. At the start of dialysis, the next scheduled dose is given if it has been longer than 6 hours since the last dose, but if it has been less than 6 hours, the next scheduled dose is held. Fomepizole is then given every 4 hours during dialysis. At the completion of dialysis, no additional dose is given if it has been less than 1 hour since the last dose, one-half of the next scheduled dose is given if it has been 1 to 3 hours since the last dose, and the next scheduled dose is given if it has been longer than 3 hours since the last dose. The maintenance dose off dialysis is continued 12 hours after the last dose.[153,154]

Fomepizole has been used to treat ethylene glycol poisoning successfully without hemodialysis or ethanol, but these patients had normal renal function, and fomepizole treatment was initiated soon after ethylene glycol ingestion.[159,160] In mild cases of ethylene glycol poisoning, as evidenced by normal renal function and no high–anion-gap acidosis, ethanol or fomepizole is used by some as sole therapy without dialysis. However, in these cases, forced diuresis with intravenous fluids or furosemide should be used to avoid dehydration, minimize renal calcium oxalate crystal formation, and maintain the renal clearance of ethylene glycol.[159] More recent data suggest that an abnormal presenting serum creatinine concentration ($\geq$1.5 mg per deciliter) predicts significantly prolonged ethylene glycol elimination during fomepizole therapy, and in the presence of metabolic acidosis, patients should undergo hemodialysis.[161]

Hemodialysis. Hemodialysis is indicated in all cases of confirmed or strongly suspected ethylene glycol poisoning presenting with renal failure, metabolic acidosis, and/or deteriorating clinical status. Ethylene glycol and glycolate have low molecular weights, no protein binding, and a volume of distribution of 0.8 and 0.55 L per kilogram, respectively, making them easily dialyzable.[147,148,152] Large surface-area dialyzers ($>$2 m^2) can achieve a clearance of ethylene glycol of greater than 200 mL per minute, and with smaller surface-area dialyzers (1.1 to 1.6 m^2) clearance of ethylene glycol and glycolate typically ranges from 150 to 190 mL per minute and 140 to 170 mL per minute, respectively.[147,148,152] The renal clearance of ethylene glycol can be as high as 30 mL per minute in patients with preserved renal function, but the importance of hemodialysis is illustrated by the fact that most patients present with renal insufficiency, and in these patients the renal clearance of ethylene glycol and glycolate is negligible.[162] The length of the hemodialysis session should be determined by the quantity of ethylene glycol ingested, but this rarely is known. Although blood ethylene glycol levels are helpful, they do not necessarily reflect the total quantity ingested because the blood ethylene glycol level is influenced by time since ingestion and amount metabolized. Dialysis should be continued for 8 hours if ethylene glycol levels are not available, and when levels are available, the dialysis prescription should be calculated using the total body water, the blood ethylene glycol level, and the manufacturer-specified dialyzer urea clearance (in milliliters per minute) at the initial observed blood flow rate.[163] Bicarbonate-based dialysate is probably optimal compared with acetate dialysate, which is associated with greater hemodynamic instability, more central nervous system symptoms, and more oscillations in plasma bicarbonate.[164] Although peritoneal dialysis clears ethylene glycol and oxalate, it should not be used over hemodialysis because of the high efficacy of hemodialysis.[165]

Sulfonamide Antibiotics, Indinavir, and Acyclovir

Crystal-induced AKI also can be caused by drugs used for therapeutic purposes. If the solubility limit of a given drug is exceeded in the renal tubules, the drug can then crystallize and possibly cause obstructive nephropathy. Certain sulfonamide antibiotics and acyclovir are the most common drugs that can cause crystalline AKI, but other drugs such as methotrexate, triamterene, acetazolamide, several herbal medicines, and high-dose vitamin C potentially can crystallize and cause stones or obstructive nephropathy.[166–169] Before the AIDS era, crystalline AKI had become fairly rare, but with the frequent use of high-dose sulfadiazine, sulfamethoxazole, indinavir, tenofovir, and acyclovir in this population, it is again an important cause of AKI.[170,171]

Sulfonamides. The sulfonamides were introduced into medical practice in 1936, and early animal experiments recognized that sulfonamides of low solubility were able to crystallize in the urinary tract and the renal parenchyma, causing obstructive nephropathy.[172,173] Reports of patients with hematuria, crystalluria, renal colic, and renal failure were common until the 1950s, when sulfonamides with greater solubility became available.[172] In patients with AIDS, high-dose sulfadiazine is again commonly being used in

conjunction with pyrimethamine for the treatment of toxoplasmosis. After an oral dose, sulfadiazine is rapidly absorbed and then partially acetylated in the liver. The half-life of sulfadiazine is 8 to 17 hours in patients with normal renal function and is 22 to 34 hours in patients with severe renal insufficiency.[171] Renal crystal formation in the nephron is promoted as the filtrate is concentrated and acidified. The solubility of sulfadiazine is almost 10-fold higher at a pH of 7.5 than at a pH of 6.5.

Patients with sulfonamide-induced AKI classically present with renal colic, hematuria, and oliguria or anuria.[174] Although renal failure develops in most patients in the first week after the start of the sulfadiazine patients also can present months after the start of the medication. Delayed presentation of AKI usually occurs with the concurrent development of volume depletion, often due to diarrhea, and these patients can be managed with hydration without stopping the sulfadiazine.[175] The urinalysis usually shows hematuria, mild pyuria, and "shock of wheat" crystals (Fig. 36.4D). Renal ultrasonography may reveal multiple echogenic foci in the renal parenchyma, but occasionally may show frank hydronephrosis with ureteral stones.[175,176]

AKI should be managed with intravenous fluids containing sodium bicarbonate with the aim of maintaining urine pH over 7.15 and urine output over 1 L per day. Urologic intervention sometimes is required in patients who remain anuric. Bilateral retrograde ureteral catheterization with warm 5% sodium bicarbonate solution, ureteral stents, and stone extraction with a stone basket all have been used in cases of ureteral obstruction with stones.[177] Although temporary hemodialysis sometimes is necessary, the recovery of renal function to baseline is the rule within 7 days.[176]

Patients starting sulfadiazine therapy should receive prophylaxis against renal toxicity. To minimize crystal formation, patients should be encouraged to maintain fluid intake over 2 to 3 L per day and should be started on sodium bicarbonate (6 to 12 g per day) to maintain a urine pH higher than 7.15.[174] Patients with renal insufficiency, diarrhea, or volume depletion should be monitored closely with urinalyses looking for hematuria and crystalluria, and sulfadiazine levels should be considered in patients with renal insufficiency.[174]

Indinavir. Indinavir is one of the most common protease inhibitors used in patients with AIDS as part of a highly active antiretroviral therapy. Indinavir causes nephrolithiasis in 3% to 4% of patients, and symptomatic urinary tract disease, including nephrolithiasis with renal colic, flank pain without evidence of stones, and dysuria or urgency in 8% of patients taking the drug.[178,179] Most patients presenting with symptomatic urinary tract disease have crystalluria, and many have radiographic evidence of either stones or renal parenchyma filling defects. However, only a minority of patients have mild-to-moderate renal insufficiency. Hydration can prevent symptomatic urinary tract disease, but a permanent discontinuation of indinavir is necessary in some because of recurrence of symptoms. Asymptomatic indinavir crystalluria is found in 20% of patients receiving the drug in the normal dosage of 800 mg orally three times a day, and the drug should not be discontinued for asymptomatic crystalluria. The presence of crystalluria and pyuria may signal the presence of interstitial nephritis, which may not reverse with conservative treatment with hydration alone.[180]

Acyclovir. High-dose acyclovir also is associated with AKI and crystalluria. Early preclinical toxicology studies in animals clearly demonstrated that high-dose acyclovir given to rats resulted in the precipitation of drug crystals in the distal nephron and also caused reversible obstructive nephropathy.[181,182] Although it has been assumed that intratubular acyclovir crystallization is also responsible for the renal failure observed in humans, the pathophysiologic process is not entirely clear. Most reported kidney biopsy or autopsy specimens have not demonstrated intrarenal crystals, but typically show normal glomeruli, no obstruction, occasional ruptured tubules, and minimal focal areas of interstitial hemorrhage, congestion, and inflammatory infiltrates.[183,184] In one case report of acyclovir nephrotoxicity, the renal biopsy was consistent with acute tubular necrosis without any evidence of intratubular crystals.[185] Crystal dissolution during tissue fixation or the time interval between discontinuation of acyclovir and obtaining the renal biopsy could account for the inconsistent demonstration of crystals in renal tissue.[185]

Renal impairment after intravenous acyclovir was commonly observed when bolus injections were used instead of slow infusions; one series reported that increased BUN or serum creatinine levels developed in 58 of 354 (16%) of patients 24 to 48 hours after the administration of acyclovir.[182,186] Unlike all other subsequent reports, one infant in this series with renal failure did show birefringent crystals in the renal tubules at the postmortem examination.[182] In contrast to bolus injections, renal failure after slow intravenous infusions or oral acyclovir is less common but does occur, especially in patients with renal insufficiency or volume depletion.[184]

AKI caused by acyclovir typically develops 24 to 72 hours after the first dose of intravenous acyclovir. Unlike with the sulfonamide antibiotics, most patients do not have renal colic, stones, or ultrasonographic findings of obstruction. Many patients also have neurotoxicity, including headache, irritability, tremulousness, ataxia, nystagmus, lethargy, dysarthria, confusion, and coma.[184] The urinalysis usually reveals both mild hematuria and pyuria, and an examination of the urine with a polarizing microscope may show birefringent, needle-shaped crystals within leukocytes.[184,187]

Risk factors for the development of acyclovir-induced AKI include volume depletion, bolus dosing, chronic renal failure, and an acyclovir serum level of greater than 25 μg per milliliter.[182,188] The renal function in patients with AKI usually normalizes within 4 to 9 days after drug discontinuation.[184,188] Conservative management, with hydration and the discontinuation of acyclovir, is sufficient in most

patients, but in patients with combined severe neurotoxicity and nephrotoxicity, hemodialysis can be used to reduce serum acyclovir levels. This results in the prompt reversal of acyclovir-associated neurologic symptoms.[187] In cases of mild renal failure, acyclovir nephrotoxicity can be managed by hydration and dose reduction of acyclovir.[189]

The half-life of acyclovir is 3 hours, and renal excretion is the major route of elimination. For example, over 90% of a given dose of acyclovir can be recovered unchanged in the urine of subjects with normal renal function 12 hours after dosing.[190] There is a linear relationship between creatinine clearance and the renal clearance of acyclovir. The renal clearance of acyclovir is three times that of a given creatinine clearance, indicating significant tubular secretion.[190] In subjects with preexisting renal insufficiency, the half-life of acyclovir can be as high as 20 hours, and dosing in renal insufficiency should be adjusted according to the level of renal function.[190,191] Hemodialysis effectively removes acyclovir, reducing the half-life to 5 hours, and can effectively remove 40% of acyclovir in body stores.[190,192]

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CHAPTER

37 Acute Kidney Injury Secondary to Tropical Hemorrhagic Viral Infections and Snakebites

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TROPICAL HEMORRHAGIC VIRUS INFECTIONS

Viral hemorrhagic fevers (VHF) are a group of highly infectious diseases with similar clinical presentations, characterized by an intense inflammatory status, endothelial injury, increased vascular permeability, and bleeding. They are caused by RNA viruses from the families Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae, and can be transmitted directly or by tick or mosquito bites. Among the VHF, dengue and yellow fever are the most medically relevant for the tropical areas and can cause acute kidney injury.

Dengue

Dengue is a mosquito-borne infectious disease caused by small (50 nm) single-stranded RNA arboviral viruses, from the Flaviviridae family. There are four closely related, yet antigenically distinct, serotypes of dengue virus: DVN1, DNV2, DNV3, and DNV4. Infection by one serotype does not confer lasting immunity to the others.[1–5]

The main vector for dengue infection is the female of the Aedes aegypti mosquito. They stay in or around houses where they emerge as adults and usually acquire the virus while feeding on the blood of infected individuals. The male mosquito does not transmit the disease because they feed on plant juices. Other species of mosquitoes may also transmit dengue, such as Aedes albopictus, Aedes polynesiensis, and Aedes scutellaris.[1–5]

In the last 50 years, the world has seen an explosive outbreak of dengue, which is now considered the most important human viral mosquito-borne infection and an extremely significant global health problem. The average number of cases annually reported to the World Health Organization (WHO) jumped from 908 during 1955 to 1959 to 925,896 during 2000 to 2007. In the same time frame, the number of countries reporting dengue has been augmented from less than 10 to more than 60, and in the last 10 years the disease has spread from the rural to the urban setting.[1–5]

Currently, more than 50 million dengue cases are estimated to occur annually. Dengue is considered endemic in more than 100 countries in all WHO regions except Europe and 2.5 billion individuals, representing two-fifths of the entire world population, are at risk of dengue. Dengue affects mainly tropical developing countries, but warmer areas of developed countries, such as the southern parts of the United States, are also affected.[1–7]

Multiple factors have led to this increasing incidence. Growth in international business and tourism travelling; immigration flow from countries where dengue is endemic; the occurrence of climate changes, such as global warming, and increases in the intensity and duration of the rainy season, which facilitates the Aedes aegypti dispersion; the growth of uncontrolled and unplanned urbanization; and the difficulty in effectively eradicating the disease vector will likely maintain and even worsen the dengue global health problem in the future.[5]

Dengue is a flulike illness with a multifaceted clinical picture. It can be asymptomatic, or it can manifest as an undifferentiated fever without symptoms, similar to several other acute febrile diseases. It can also appear as classic dengue fever (DF), a painful but self-limited disease course, or as a life-threatening form, namely dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[1–5]

DF is characterized by the high and abrupt onset of fever lasting 2 to 7 days, prostration, severe headaches, retro-ocular pain, diffuse body aches, myalgia, arthralgia, mild hemorrhagic manifestations, a positive tourniquet test, facial flushing, and a diffuse cutaneous erythematous maculopapular rash. Anorexia, nausea, vomiting, biphasic fever, and liver enlargement may occur. A laboratory examination typically reveals leukopenia, and varying degrees of low platelet count. Increased liver enzymes, especially serum aspartate transaminase (AST), are frequently seen.[1–5,8,9]

DHF and DSS are the most severe varieties of dengue disease presentations. The early manifestations of DHF and DSS are similar to DF. In fact, there is controversy if DHF and DSS are single nosologic entities or the ends of a continuum of the same illness. Children, infants, and adult patients with secondary heterotypic infections are at a higher risk of developing DHF/DSS.[1–5]

DHF is defined as a clinical picture with a simultaneous occurrence of high fever, thrombocytopenia ($\leq$100,000/mm^3), severe hemorrhagic phenomena, and evidence of increased vascular permeability. This includes hemoconcentration (increased hematocrit), hypoalbuminemia, pleural effusion, and ascites. Depending on the amount and the rate of fluid leaving the intravascular space, the patient may develop hemodynamic instability, tachycardia, signs of poor capillary perfusion, and finally, hypotension and shock, thus characterizing DSS. If this picture is not quickly and efficiently reverted, the patient frequently experiences multiple organ failure and massive bleeding.[1–5] Whereas mortality rate in DF is less than 1%, it rises to approximately 12% in DHF and to up to 40% in DSS.[10]

The rapidly changing pattern of dengue epidemiology exposed the idea that the classification of dengue in DF, DHF, and DSS was frequently inadequate for use in the clinical setting.[11] Furthermore, an increased number of life-threatening cases of dengue that did not strictly adhere to DHF criteria have been observed.[11] Therefore, although the classification of DF, DHF, and DSS continues to be widely used, the WHO has suggested a new one, dividing the dengue spectrum into nonsevere dengue (without or with warning signs), corresponding to DF and severe dengue, that will include and expand the concept of DHF and DSS.[5] Severe dengue was defined by the WHO as the presence of one or more of the following:

1. Plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress.
2. Severe bleeding.
3. Severe organ impairment, even in the absence of plasma leakage or shock (liver, kidneys, central nervous system, heart).

This new classification is strongly based on patient behavior during the three pathophysiologic phases of the disease. The first one is the febrile phase, when viremia occurs, and it is similar between nonsevere and severe dengue forms. It comprises the clinical and laboratory picture already described for DF. The second is the critical phase, when after 3 to 7 days of high fever, the temperature decreases or normalizes. At this point variable degrees of endothelial dysfunction causing increased capillary permeability and plasma leakage will occur. This phase lasts for 1 to 2 days and it is critical in differentiating nonsevere and severe dengue. Some patients have mild and limited increases in capillary permeability and will start to improve and recover. Others have significant and maintained plasma leakage and will progress to severe dengue. These patients usually have warning signals, such as abdominal pain or tenderness, persistent vomiting, body fluid accumulation, mucosal bleeding, lethargy, restlessness, liver enlargement, and their hematocrit increases simultaneously with a rapid platelet count decrease. The third phase, or the recovery phase, begins when the critical phase ends and it is characterized by the halting of plasma leakage and the reabsorption of fluids from the extravascular compartment. The patient's clinical recovery is manifest and laboratory parameters go back to normal. The presence of a petechial rash, with multiple small round islands of unaffected skin ("isles of white"), and generalized pruritus are distinctive findings of this phase.[5]

The diagnosis of dengue should be suspected in individuals with a suggestive clinical picture who live or have visited an endemic area for this illness. Considering the alarming increase in the number of patients infected with dengue viruses, the occurrence of several outbreaks of the disease, the spread of the vector, and the high number of international traveling days, even medical staff working in dengue-free areas must be aware of this diagnostic possibility in febrile patients.[12,13]

Laboratory diagnostic methods for confirming a dengue virus infection include immunoglobulin M (IgM) and IgG antibodies, dengue antigen, virus or viral nucleic acid detection, or a combination of these procedures. After the start of the disease, the virus can be found in corporal fluids and tissues for 4 to 5 days. During the early stages of the disease, it can be isolated and its nucleic acid or antigen can be detected. At the end of the acute phase of dengue infection, when there is no more fever, serology is the method of choice for diagnosis.[14]

The differential diagnosis for dengue, principally in the acute febrile phase, includes other febrile viral diseases (yellow fever, Hantavirus, influenza, Chikungunya virus, O'nyong-nyong virus, acute viral hepatitis, measles, rubella, and enteroviruses), bacterial infections (leptospirosis, typhoid fever, meningococcemia, and bacterial sepsis), rickettsial diseases, malaria, autoimmune diseases, and severe acute hematologic diseases.[1–5,14]

There is no specific treatment for dengue. Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided. Early and adequate support, maintaining adequate blood pressure, and intravascular volume repletion are absolutely essential in severe dengue.[1–5,14]

Dengue-Induced Renal Disease

There are no prospective studies specifically designed to assess the effects of dengue infection on renal function and structure, and most of the available information is derived from retrospective data, small case series, and case reports.

Serum creatinine (SCr) elevations, acute kidney injury (AKI), acute tubular necrosis (ATN), hemolytic uremic syndrome (HUS), proteinuria, nephrotic syndrome, and glomerulonephritis have been described in dengue patients.[15–45]

The frequency of AKI reported in dengue range widely, from approximately 1% to 3% to 27% to 35% (Table 37.1).[23,26,32,34,35,37,40,42,44] Most of the series studied DHF/DSS, but AKI has also been reported among DF patients. The use of different definitions of AKI, the diverse ethnic background of infected individuals, and the clinical heterogeneity of the assessed population make it hard to compare the diverse studies. DHF/DSS, obesity (in children), older age, and simultaneous bacterial infection have been pointed to as possible risk factors for AKI development, which consistently carried higher mortality for dengue patients.[35,37]

37.1 Series of Dengue Patients and Frequency of Acute Kidney Injury

Author, year (ref)	Country	Number of Patients	Type of Dengue	AKI (%)
Mendez/Gonzales, 2003 (23)	Colombia	617 children	DHF	1.6
Lee et al., 2005 (26)	Taiwan	88 adults	DHF/DSS	10.2
Wang, 2007 (32)	Taiwan	606 adults	N/A	1.2
Khan, 2008 (34)	Saudi Arabia	91 adults/children	93% DF; 7% DHF	2.2
Kuo, 2008 (35)	Taiwan	273 adults	79.4% DF; 20.6% DHF	27.1
Lee, 2009 (37)	Taiwan	304 adults	DHF	3.3
Laoprasopwattana, 2010 (40)	Thailand	2,893 children	DHF/DSS	0.9
Parkash, 2010 (42)	Pakistan	699 (>14 y)	87% DF; 13% DHF/DSS	2.7
Basu, 2011 (44)	India	28 adults	N/A	35.7

AKI, acute kidney injury; DHF/DSS, dengue hemorrhagic fever/dengue shock syndrome; DF, dengue fever; N/A, not available.

Renal histology data are scarce in AKI patients. One case report found acute tubular necrosis in the renal biopsy of a patient who had DF-associated AKI requiring dialysis.[38] An autopsy of a fatal dengue case disclosed renal cortical hemorrhage of glomerular capillaries and proximal tubules, and a medullar mononuclear infiltrate around the collecting ducts, with hemorrhagic foci, interstitial edema, and vascular congestion.[46] Renal biopsies were performed 11 to 44 days after AKI development in three children who survived an episode of DSS. Two had interstitial nephritis and one only had slight mesangial matrix expansion. Immunofluorescence studies have found traces of C3 with a granular pattern at some vessels in one patient.[40]

Renal replacement therapy has been performed in about 7% to 44% of the patients with dengue-associated AKI, and both peritoneal and extracorporeal blood purification techniques have been used.[40,44,45]

The pathogenesis of renal injury in dengue is not clearly established. Different mechanisms have been proposed, such as systemic hemodynamic instability, shock, rhabdomyolysis, and hemolysis. However, dengue-induced severe rhabdomyolysis and myositis with dark urine and high creatine phosphokinase (CK) levels have been reported without the development of AKI.[25,47–51] Cases of DF-associated AKI without rhabdomyolysis or hemodynamic instability have also been described,[33,38] suggesting a possible direct or cytokine-mediated effect of the dengue virus on kidney function. Supporting this hypothesis, viral particles have been found in the renal tissue from humans and from animals infected with dengue.[46,52–54]

Other renal complications that have been associated with dengue include proteinuria and glomerulonephritis. The prevalence of proteinuria was described to be over 70% in children with DHF[15] and in hospitalized patients during a dengue outbreak in Australia.[19] Moreover, proteinuria in the nephrotic range was described in dengue patients.[19,43,55] Renal histology results confirmed the association between dengue and glomerular injury. Renal biopsies performed in children with DHF and proteinuria, hematuria, or both demonstrated hypertrophy and hyperplasia of mesangial and endothelial cells, monocytelike cells in some glomerular capillary lumens, and focal thickening of the glomerular basement membrane. Glomerular and arteriolar IgG, IgM, or both, and C3 immune complexes were found in biopsies obtained 2 weeks after the onset of symptoms.[16] More recently, transient IgA nephropathy was reported in a biopsy from a 15-year-old patient with DF-associated severe AKI.[39] Experimental studies carried out in mice showed that dengue virus inoculation causes early glomerular structural changes (diffuse glomerular proliferation, glomerular volume enlargement, increased endocapillary and mesangial cellularity), and glomerular IgM deposition.[52,56]

In conclusion, dengue is associated with several different forms of renal injury. The development of AKI in dengue patients is related to unfavorable outcomes. Probably, the renal involvement in dengue is underestimated. Prospective studies assessing kidney function in dengue are deeply necessary.

Yellow Fever

Yellow fever (YF) is a mosquito-borne infectious disease caused by the yellow fever virus, a 40-nm diameter arbovirus, which is considered the prototype for the Flavivirus genus and Flaviviridae family. The name yellow fever originated from the striking jaundice observed in the severe cases of this disease.[10,57–61]

YF originated in Africa and was spread to the American continent and to Europe through commercial routes and the slave trade. Devastating and deadly epidemics occurred throughout the 17th, 18th, and 19th century in North, South, and Central America, and European cities. The development of large-scale public health campaigns aiming at control of the mosquito vector and the development of efficacious vaccines virtually eradicated the disease in North America and Europe, and drastically decreased its incidence in Central and South America and Africa. However, because the natural epidemiology of YF in Africa and the Americas encompass a cycling of the virus between forest mosquitoes and wild nonhuman primate hosts, it is not possible to completely eradicate the disease.[59,60]

The current vectors for YF are blood-eating mosquitoes from the Culicidae family, belonging to the Haemagogus genus in the Americas and to the Aedes genus in Africa. There exist three possible cycles of YF transmission: jungle (sylvatic), intermediate, and urban transmission. In the jungle cycle, the virus is transmitted between nonhuman primates and mosquito species inhabiting the forest canopy. Occasionally, infected sylvatic mosquitos contaminate individuals working or visiting the forest. This cycle occurs in Africa and South and Central America rainforests.[10,57–61] One important difference between the American and African cycles is that although in the American continent the infected monkeys frequently die, in Africa they usually survive without signs of infection.[58] An intermediate cycle is characterized by the YF virus (YFV) transmission to both nonhuman primates and human individuals. It occurs in the humid African savanna bordering the equatorial forests, when Aedes sp. mosquitoes indiscriminately feed on nonhuman primates and humans. The intermediate YF cycle produces small-scale outbreaks in rural villages, which are currently the most usual form of YF epidemics in Africa.[10,57–61] The urban cycle involves the transmission of YFV from human to human by Aedes aegypti domestic mosquitoes in urban areas. It is a major cause of concern because explosive, large-scale outbreaks can occur. It is at this time present in Africa, particularly in Nigeria.[10,57–61] It has not been described in tropical South America since 1942, in Brazil.[58]

YF is nowadays endemic in tropical regions of Africa and South America and in Panama. The existing data on the YF burden are probably inexact due to the underreporting of the disease and the limitation of diagnostic capabilities in many regions where YF is endemic.[59] In South America, the rate of transmission of YF is lower than in Africa, most likely due to the launching of mass vaccination campaigns as a reaction to outbreaks of the disease.[59]

YF currently affects over 200,000 individuals annually in Africa and South and Central America, with approximately 30,000 fatalities. Forty-four countries, 32 in Africa and 12 in South and Central America, are within the YF endemic zone, with almost 900 million people at risk for infection. The recent resurgence of virus circulation, urban reinfestation by the A. aegypti, and the expansion of the YF endemic zones in Africa and South America make for a dramatic potential scenario for the reemergence of explosive epidemics of urban YF.[10,58–62]

YF is a multisystem viral sepsis, which might be asymptomatic or have a clinical spectrum ranging from a nonspecific febrile disease to a severe and fatal hemorrhagic illness associated with shock, liver, kidney, heart, and nervous system injury. YF characteristically evolves through three phases. The first, known as the infection phase, when viremia takes place, starts 2 to 6 days after the virus inoculation and lasts for several days. The symptoms comprise an abrupt onset of high fever, chills, anorexia, nausea, vomiting, irritability, dizziness, mild hemorrhagic phenomena, malaise, tiredness, headache, generalized myalgia, lower back pain, and Faget sign, which is increasing fever with a paradoxical bradycardia. Laboratory abnormalities include low white blood cell count and increased serum transaminases. The second phase, remission, is characterized by a rapid (24 to 48 hours) reduction of fever, the abatement of other symptoms, and the viral clearance from the blood. Approximately 80% of the patients will have a benign course of the disease, recovering at this phase without the development of jaundice. The third and last phase, the intoxication phase, is distinctive as the most severe, hemorrhagic form of the disease and occurs in about 20% of the patients. The high fever returns and the patient presents with epigastralgia, vomiting, and jaundice. Multiorgan failure affecting the liver, the kidneys, and the central nervous system develops. The disease evolves with exuberant hemorrhagic diathesis, characterized by petechiae, ecchymoses, epistaxis, and hematemesis (the characteristic YF "black vomit") due to liver failure, platelet dysfunction, and disseminated intravascular coagulation. Laboratory examination will show intense leukopenia, very high transaminases and bilirubin levels, blood coagulation disorders, increased serum creatinine, and albuminuria. At this phase, the viruses are usually missing in the blood and antibodies will appear. Later shock, confusion, seizure, and coma will develop, presaging death. The lethality rate of YF ranges from 20% to 50%. Infancy, older age, and the development of jaundice and the intoxication phase are associated with an increased severity and lethality of YFV infection.[10,14,57,58,60,61]

YF can be distinguished from the other viral hemorrhagic viruses by the characteristic severity of liver damage and the development of jaundice. The disease can be diagnosed by serology (detection of IgM), virus isolation, immunohistochemistry, and reverse transcription polymerase chain reaction (RT-PCR).[10,14,57,59–61]

Kidney Injury in Yellow Fever

The kidneys are frequently cited as target organs in severe cases of YF,[59,60] but there is very little consistent information about the types, evolution, prevalence, and mechanisms of YF-associated kidney injury in the literature.

Oliguria has been described to occur after 5 to 7 days in severe YF, and even earlier in African patients without jaundice or liver abnormalities.[57,63] Gross pathology of fatal

human YF usually revealed grossly enlarged, congested, and edematous kidneys. Renal histology disclosed acute tubular necrosis.[60]

The mechanisms causing the kidney injury in YF have been poorly studied. Studies performed in rhesus monkeys suggested that prerenal mechanisms predominate until the late phase of the disease, when frank acute tubular necrosis develops.[64–66] In severe YF cases, the presence of microcirculatory failure and tissue ischemia, due to shock and disseminated intravascular coagulation associated with extremely high bilirubin levels, are possible mechanisms for AKI development. The finding of viral antigens in the renal epithelium of three fatal cases of YF and the virus isolation from renal tissue from patients with YF vaccine-induced viscerotropic disease[67,68] suggest a possible direct effect of the YF virus on the kidneys. Consistent with this hypothesis, YF viral antigens were detected in glomeruli 2 to 3 days after experimental inoculation in monkeys,[66] and renal structural changes culminating in renal cell necrosis were observed 24 hours after infection in a murine YF model.[69]

SNAKEBITES AND ACUTE KIDNEY INJURY

The estimated number of existing snake species is 3,000, of which around 19% are considered venomous, belonging to the families Elapidae, Viperidae, Colubridae, Hydrophiidae, and Atractaspididae.[70–72]

Snake venom is highly modified saliva that is injected by specialized fangs to immobilize and digest prey, and for self-defense against predators. Snake venoms contain a multitude of components; 90% to 95% of its net weight is composed of proteins, which are responsible for the venom's toxic and lethal effects. The nonproteic fraction, which is responsible for nonlethal biological effects, contains carbohydrates, lipids, metals, biogenic amines, free amino acids, and nucleotides. The venom composition can differ among the same species of snakes, depending on the geographic area and food accessibility, and even in the same animal, depending on its age.[70,73]

The vast majority of venomous snakebites affect previously healthy, young male farmers and agricultural workers in the rural areas of poor health resourced tropical countries. Accordingly, venomous snakebite is in fact considered an occupational hazard in the tropical areas of the developing world, leading to significant mortality and disability in the young rural active labor force of these countries. Children are also frequent victims of snakebite accidents.[70,71,73–77] The most often affected anatomic sites are feet, legs, ankles, and hands.[78] Consequently, many survivors will be afflicted by permanent disability caused by the necrotic effects of the venom on the tissue.

The exact global burden of venomous snakebite incidences and its related morbidity and mortality is elusive. The limited information available comes mainly from data obtained from the hospital records or from published epidemiologic studies.[79,80] This methodology clearly underestimates the real problem because most victims do not have access to medical facilities and, consequently, the accidents and the complications are not recorded.[70,71,76,81,82] The more recent publication, which combined the literature and was analyzed with the WHO mortality data, reported an approximate annual global burden of 1,200,000 to 5,500,000 snakebites with 421,000 to 2,650,000 envenomations and 20,000 to 94,000 deaths (Table 37.2).[81] The highest burden exists in South and Southeast Asia, in sub-Saharan Africa, and in Central and Latin America.[70,71,80–82] South Asia, and particularly India, have the highest incidence and mortality rates from snakebites worldwide.[70,83,84] Recently, a clear negative association between snakebite mortality and government spending on health was demonstrated, confirming the existence of a strong association between snakebite-induced mortality and poverty. The same authors pointed out that venomous snakebites have higher mortality rates in the tropics than do some of the world's recognized neglected tropical diseases (NTDs), such as schistosomiasis and leishmaniasis.[76]

37.2 Global Estimates of Annual Snakebite Morbidity and Mortality

	Swaroop & Grab, 1954[79]	Chippaux, 1998[80]	Kasturiratne, 2008[81]
Assessed population	$1{,}122 \times 10^6$	$5{,}840 \times 10^6$	169 countries
Snakebites	500,000	5,400,000	1,200,000–5,500,000
Envenomation	NR	2,682,500	421,000–2,650,000
Deaths	30,000–40,000	123,345	20,000–94,000

NR, not reported.

At this time, the only treatment with confirmed efficacy against snakebite envenoming is the early administration of the specific antivenom in a correct dosage and by an adequate route, which is preferentially intravenous.[82]

Snake venom may cause several clinically relevant kidney disorders, such as AKI,[73,74,85–111] proteinuria and/or hematuria,[112–114] nephrotic syndrome,[115] and hemolytic-uremic syndrome (Table 37.3).[116–119] Histopathologic descriptions of the effects of snake venom on renal structures are relatively limited, but the occurrence of acute tubular necrosis,[120–125] acute cortical necrosis,[85,124,125,126–133] acute interstitial nephritis,[134–136] papillary necrosis,[137] necrotizing vasculitis,[138] and glomerular injury[123,124,139–148] have been described (Table 37.3).

AKI is considered one of the main toxic effects induced by snake venom in humans, and has been pointed out as the main cause of mortality in individuals who survived the first impact of the venom.[73,74,87,96,102]

The reported frequency of AKI after a venomous snakebite varies widely from approximately 1% to 40%. The majority of cases have been reported in Asia, Latin America, and Africa. Many factors can affect this percentage, such as the snake species, the size and age of the snake, the amount of venom inoculated, the geographic area, the severity of the accident, the time for antivenom treatment, among others. Moreover, many different definitions of AKI and methods for evaluating renal function were used in the published studies, making it difficult to compare results.[72–74,89–92,95,98–103,107,108,111,149–153] Finally, very few studies were prospective and were specifically designed to evaluate the snake venom-induced kidney injury.[102,107]

37.3 Clinical and Histopathologic Presentations of Snakebite-Induced Kidney Injury

Clinical	Histopathologic
Acute kidney injury	Acute tubular necrosis
Proteinuria	Cortical necrosis
Hematuria	Acute interstitial nephritis
Hemolytic-uremic syndrome	Papillary necrosis
Nephrotic syndrome	Necrotizing vasculitis
	Glomerular injury ■ Mesangial proliferation ■ Diffuse proliferative glomerulonephritis ■ Membranoproliferative glomerulonephritis

A delay in receiving the appropriate antivenom treatment, residing in a rural area, the creatine phosphokinase (CK) levels at admission, myalgia, neurotoxic facies, younger age (children), and advanced age have been associated with an increased risk of developing AKI after a snakebite.[74,90,94,100,102,105,108,154]

The etiology of the kidney injury after a venomous snakebite has been attributed to a venom-induced development of hemodynamic instability or shock, renal vasoconstriction, deposition of fibrin thrombi in microcirculation due to coagulation system activation, systemic rhabdomyolysis, hemolysis, cytotoxicity, oxidative stress, platelet activating factor, renal infiltration by venom-activated macrophages, direct venom nephrotoxicity, and hypersensitivity to the venomous or antivenomous proteins.[72,73,86,88,90,93,102,111,120,140,141,143,149,153,155–174]

Snake-bite induced AKIs have been described after accidents with practically all snakes with medical relevance, especially from the snakes of the Elapidae, Viperidae, and Colubridae families.[72,73,103,107,108,110,111,124,133,148–154] However, a careful review of the medical literature reveals that three snakes are responsible for the large majority of venomous snakebite-induced AKI. They are Russell's vipers (Daboia russelii) in Asia, the South American rattlesnake (Crotalus durissus) in South America, and members of the genera Bothrops in Central and in South America (Table 37.4).[72–74,85–105,107–112,121,123–127,129–132,148–155,161,175]

Russell's Viper (Daboia Russelii)

The Russell's viper (Daboia russelii) is a nocturnally active venomous snake with an average length of 1.2 m from the Viperidae family and the Viperinae subfamily. It is usually found in grassy and bushy areas, forested plantations, and farmland, such as paddy fields used for growing rice. Because this snake feeds mainly on rodents, which are frequently found around humans, it is attracted to the rural settlements and to highly populated areas. When threatened, Russell's vipers become very aggressive and can attack in a very fast and unpredictable way. Humans are bitten on the footpaths and roads at dusk and in the early night hours, or during the day in the paddy fields.[101] There are two peaks of accidents during the year, which correspond to the times of agricultural harvesting and cultivation.[101]

Russell's vipers are widely and equally distributed throughout South Asia, where they account for most of the snakebite incidents and deaths. In fact, it is the principal cause of lethal snakebites in India, Pakistan, Sri Lanka, Bangladesh, Burma, Thailand, and in parts of Indonesia.[72,83,176]

The principal toxic components of the Russell's viper's venom are the phospholipase A_2 (70% of the protein content), the coagulation factor V and X activators, a protease inhibitor, and the hemorrhagins.[176,177] The clinical and laboratory picture of the envenoming includes swelling at the bite site (and more rarely, local necrosis), coagulopathy (which may manifest as abnormal laboratory parameters, bleeding diathesis, systemic thrombosis), thrombocytopenia, hemolysis,

37.4 A Summary of Some Characteristics of the Principal Snakes Causing Acute Kidney Injury Worldwide

Name	**Russell's Viper** (Daboia russelii)	Bothrops **Genus**	**South American Rattlesnake** (Crotalus durissus)
Scientific classification	Family Viperidae Subfamily Viperinae	Family Viperidae Subfamily Crotalinae	Family Viperidae Subfamily Crotalinae
Geographic distribution	South Asia	Southern Mexico, some Caribbean islands, Central and South America	South America, few Caribbean islands
Average size	1.2 m	50 cm to 2 m (depending on species)	1 m
Habitat	Grassy, bush areas Forested plantations Farmland (paddy fields)	Humid environments Tropical forests Grassland Plantations	Semi-arid grassy areas, such as savannas and the Caatinga
Behavior	Aggressive; fast and unpredictable attack	Very aggressive	Less aggressive
Clinical picture	Bite site swelling Nephrotoxicity Coagulopathy (bleeding) Hypotension, shock Hemolysis Neurotoxicity Rhabdomyolysis Thrombocytopenia[a]	Bite site edema, blisters, ecchymoses, tissue necrosis Nephrotoxicity Coagulopathy (bleeding) Hypotension, shock[b]	Mild or absent bite site injury Nephrotoxicity Neurotoxicity Rhabdomyolysis Coagulopathy (rarely bleeding)
AKI etiopathogenesis	Direct nephrotoxicity Glomeruli microthrombi Hemoglobinuria Hypotension Renal vasoconstriction Myoglobinuria	Direct nephrotoxicity Glomeruli microthrombi Hemoglobinuria Hypotension Renal vasoconstriction Platelet activating factor Oxidative stress Cytotoxicity	Direct nephrotoxicity Renal vasoconstriction Myoglobinuria Oxidative stress Activated macrophage
Histopathologic pattern	ATN, CN, AIN, PN, glomerular injury, vasculitis	ATN, CN, glomerular injury	ATN
AKI prevalence	8%–86.3%	1.6%–38.5%	10%–29%

[a]It may differ among different geographic areas.
[b]It may differ between young and older snakes.
AKI, acute kidney injury; ATN, acute tubular necrosis; CN, cortical necrosis; AIN, acute interstitial nephritis; PN, papillary necrosis.

neurotoxicity, nephrotoxicity, rhabdomyolysis, and hemodynamic instability.[72,93,101,105,110,112,155,161,176–180] These clinical manifestations differ among the different geographical areas. For instance, in Sri Lanka, the venom is very neurotoxic, which is not true for the venom in India and Burma. Likewise, it causes rhabdomyolysis in Sri Lanka and South India, but not in Burma and Thailand.[101,176,177,179]

AKI is a universal, usual, and severe complication after a Russell's viper snakebite.[173] The reported frequency of nephrotoxicity and AKI ranges respectively from 18% to 41.7%,[101,112] and from 8% to 86.3% in a small series of 22 patients from Sri Lanka.[101,110,112,177–180] It occurs early after the bite (within hours), the AKI is frequently oliguric and may require renal replacement therapy, and the most frequently used modality in the reported series is peritoneal dialysis.[93,101,105,112,178,179] Increased urinary N-acetyl-β-D-glucosaminidase (NAG), seen 2 hours after the bite, was highly predictive of AKI development.[158] The described histopathologic patterns in Russell's viper–induced AKI comprise ATN,[123,124] cortical necrosis,[124,129,130] glomerular injury,[123,124,145] acute interstitial nephritis,[134,135] and vasculitis.[138] The early administration of the antivenom was associated with a decreased frequency of AKI.[105,108] The mechanisms possibly related to the development of AKI are venom direct nephrotoxicity, venom-induced renal vasoconstriction, hypotension, hemoglobinuria, myoglobinuria, and microthrombi deposition in the glomeruli.[72,93,101,111,112,137,150,153,155–159,161,162,173,176–182]

Bothrops **Genus Snakes (Lanceheads)**

Bothrops snakes belong to the Viperidae family and the Crotalinae subfamily. The Bothrops genus encompasses more than 30 species, with the most medically relevant being B. asper, B. atrox, B. erythromelas, B. neuwiedi, B. moojeni, B. jararaca, B. jararacussu, and B. alternates.[73,149,183]

Bothrops snakes mainly have crepuscular and nocturnal habits, when they prey on small mammals. Its size varies widely, from 50 cm to 2 m, depending on the observed species. They favor humid environments, such as the tropical forests, but can also live in the grassland regions or on plantations. They can be found in the rural areas and in the rural/urban fringes of large cities, in places with high rodent concentrations, such as in silos and wood deposits. Bothrops snakes have an extremely aggressive defensive behavior when they feel threatened. A higher frequency of accidents occurs in the rainy season and at times of the year when soil preparation, planting, or harvesting occurs.[73,149,183,184]

The Bothrops species are widely distributed in the American continent, from Southern Mexico, Central America (all countries), some Caribbean islands, through South America (all countries except Chile). Members of this genus account for the greater part of venomous snakebites in Central and South America (approximately 90% of bites), particularly the B. asper, B. atrox, and B. jararaca species.[73,149,183]

The venom composition, effects, and the envenoming clinical picture are similar among the different Bothrops species. The venom is proteolytic, anticoagulant, hemorrhagic, and nephrotoxic.[73,149,183,184] The clinical picture after a Bothrops snakebite is characterized by the rapid onset of pain and edema at the bite site. Ecchymoses, blisters, and local bleeding may occur. The local injury can evolve to severe tissue necrosis, resulting in relevant disability. The most important systemic manifestations are the development of hemorrhagic phenomena due to blood incoagulability, hypotension, shock, and AKI.[73,92,95,97–99,125,149,183,184,185] The venom composition and the clinical picture might differ in the same species, according to the snake's age and the geographic area. For instance, young B. jararaca venom is more procoagulant and less necrotic than the venom from the adults of the same species.[73,149,183,184]

AKI is the most severe complication of bothropic envenomation and it is the principal cause of death in patients who survived the initial effects of the venom.[96] The reported frequency (all the studies are retrospective) of Bothrops venom–induced AKI ranges from 1.6% to 38.5%.[149] The risk factors possibly related to the development of AKI include older patient age,[92,185] the snake species and size, the quantity of venom injected, and the time to receive specific antivenom in an adequate way.[65,149,170] In fact, a study of B. asper snakebites in Colombia found that a time interval of 2 hours between the bite and the administration of antivenom was associated with AKI development.[65] Confirming this clinical observation, the Bothrops venom–induced renal proximal tubule injury was blocked only when the antivenom and the venom were administered simultaneously.[170]

AKI may develop within hours to a few days after a Bothrops snakebite. It is frequently oliguric and severe, with 33% to 75% of the cases requiring the use of renal replacement theraphy (RRT). The mortality rate of Bothrops venom–induced AKI has been reported to range from 13% to 19%.[85,87,99,149] This mortality is very significant when we realize that most of the patients are previously healthy, young, working individuals.

The renal structural injuries caused by Bothrops venom are acute tubular necrosis,[120,125,143,186,187] cortical necrosis,[85,127,131] and glomerular changes.[140,141,143] The mechanisms possibly related to the Bothrops venom–induced AKI are renal ischemia due to microcirculation and/or glomeruli fibrin deposition, hypotension, renal vasoconstriction, platelet activating factor release, oxidative stress, cytotoxicity, venom direct tubular toxicity, and hemoglobinuria.[73,120,149,163,166–168,170,172,188,189]

South American Rattlesnake (Crotalus durissus)

The South American rattlesnake belongs to the Viperidae family, the Crotalinae subfamily, and the Crotalus genus. C. durissus terrificus and C. durissus collilineatus are the C. durissus subspecies with the higher medical relevance.[71,149] The South American rattlesnake average size is around 1 m, but it may reach 1.8 m in length. Its most remarkable characteristic is the existence of a rattle at the tail end, used to avert and to turn away predators. C. durissus snakes are not as aggressive

as Russell's viper and Bothrops snakes; they try to avoid humans and only attack if threatened. In Brazil they are responsible for approximately 10% of venomous snakebites.[92,149,190] Like the other venomous snakes studied in this chapter, accidents are more frequent in times of the year corresponding to agricultural harvesting and cultivation.[91] They habitually live in semiarid grassy areas, such as savannas and the Caatinga region in Brazil; have vespertine and crepuscular habits; and feed on small animals, such as rodents and birds. C. durissus can be found in all South America countries (except Ecuador and Chile) and in a few Caribbean islands.[92,149,190]

The venom of C. durissus contains several toxic peptides, such as crotoxin, crotamine, giroxin, convulxin, and a thrombinlike enzyme.[71,149] Crotoxin, which constitutes more than 50% of the venom protein content, is the major determinant for the neurotoxic, myotoxic, and nephrotoxic actions of the venom.[71,149,165,169,191]

The clinical picture seen after a C. durissus accident is typically characterized by mild or absent injury at the bite site and severe systemic manifestations.[71,89–91,100,102,149] Neurotoxicity is usually manifested by palpebral ptosis, visual disturbances, and facial muscle paralysis, but respiratory muscle impairment and acute respiratory failure have also been described.[71,89–91,96,100,102,149] The venom myotoxicity causes diffuse rhabdomyolysis that results in generalized muscle tenderness, myoglobinemia and myoglobinuria, dark urine, and elevated CK levels.[71,85–91,96,100,102,149] The thrombinlike enzyme disturbs the coagulation system, causing blood incoagulability and afibrinogenemia in approximately half of patients, but bleeding diathesis is a rare event.[71,85,89–91,96,100,102,149,192]

AKI is the most important complication in individuals who survived the first impact of the venom and is considered the main cause of death among these patients.[87,96] It is noteworthy that even considering that Crotalus durissus snakebites are 10 times less prevalent than Bothrops snakebites, the absolute number of AKI cases reported is similar for both snakes.[120,175] The frequency of AKI after Crotalus durissus snakebites ranges from 10% in retrospective studies[90,91,100,149] to 29% in the only prospective study available.[102] In this prospective study, the authors also noted that even patients that did not meet the criteria for AKI (defined as a creatinine clearance $<$60 mL/min/1.73m^2 in the first 72 hours after a snakebite) had a discharge glomerular filtration rate (GFR) significantly higher than the nadir values during hospitalization, indicating that some degree of renal dysfunction occurred in all of the patients. Finally, the same study identified that a time interval longer than 2 hours between the Crotalus durissus bite and the adequate antivenom administration, CK admissions levels over 2,000 UI per liter, and an age $<$12 years were independent risk factors for AKI development.[102]

AKI develops within the first 24 to 48 hours after the accident and may be severe enough to require RRT.[91,102,149] A high fractional excretion of sodium and mild proteinuria were documented in patients developing C. durissus–induced AKI.[102] The histologic lesion found in these patients was acute tubular necrosis.[86,149] The mortality rates reported for AKI after C. durissus envenomation range from 8% to 17%.[26,90,102,149]

Experimental studies and clinical reports indicate that the etiology of C. durissus venom–induced AKI is probably related to rhabdomyolysis, renal vasoconstriction, direct venom nephrotoxicity, activated macrophages, and oxidative stress.[71,86,88,149,165,169,171,174,191–193]

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CHAPTER

38 Acute Kidney Injury following Hematopoietic Cell Transplantation and Severe Burns

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Acute kidney injury (AKI) occurs, with varying prevalence, across virtually all hospital settings. The most common etiologies of hospital-based AKI—acute tubular necrosis (ATN), contrast induced nephropathy, and prerenal azotemia—are familiar to every practicing nephrologist. However, AKI can also be uniquely associated with specific conditions. Two such situations are following hematopoietic cell transplantation (HCT) and in the setting of severe burns. Care for HCT and burn patients is highly specialized, typically restricted to tertiary care centers, and involves a limited number of patients. Thus, many nephrologists may have limited experience evaluating and managing AKI in these settings. However, although not commonly encountered, HCT and burns are associated with a remarkable incidence of AKI that portends subsequent worse outcomes. Therefore, this chapter explores the epidemiology, pathogenesis, and outcomes of AKI in these unique settings.

BACKGROUND

HCT is an increasingly common treatment that offers the possibility of cure for a variety of once fatal malignant and nonmalignant disorders. Initially conceived in the late 1950s as a treatment for hematologic malignancies, the spectrum of illnesses treated with HCT has burgeoned to include solid tumors,[1] red blood cell dyscrasias,[2] inborn errors of metabolism,[3] and autoimmune disorders.[4] The coming years hold the potential of an even greater role for HCT including the tantalizing possibility of inducing tolerance in solid organ transplantation and treating chronic ailments such as Parkinson disease and diabetes mellitus.[5] Current common indications for transplantation are listed in Table 38.1. Overall, approximately 50,000 to 60,000 HCTs are performed annually worldwide with roughly 40% of these occurring in the United States (www.cibmtr.org). The 5-year survival rate has improved steadily and now stands at over 50%. Additional improvements have been achieved over the past two decades in engraftment time, graft-versus-host disease (GVHD), relapse or malignant progression, and nonrelapse mortality.[12] Much of this improvement is attributable to more accurate human lymphocytic antigen (HLA) matching, more effective and tolerable infection prophylaxis, and a reduction in the intensity of chemoradiation conditioning regimens. Despite these striking advances, the procedure remains fraught with potential complications, one of the most severe of which is AKI.

The development of AKI can be detrimental, beyond its own sequelae, because it interferes with the usual adequate dosing of immunosuppressants and thus predisposes for GVHD, rejection, and interruption of treatment. AKI can also contribute to other organ dysfunction, such as lung and liver, due to volume overload, coagulation abnormalities, and cytokine mediated pulmonary dysfunction. There are at present three types of transplantation: myeloablative autologous (autologous), myeloablative allogeneic (allogeneic), and nonmyeloablative allogeneic, or reduced intensity conditioning (RIC). In the United States, 57% of transplants are autologous with the remainder being conventional and reduced intensity allogeneic.[6,7] The incidence, risk factors for development, etiology, severity, and outcomes of AKI differ markedly among the three transplant modalities.

Sources of Cells

In the early days of HCT bone marrow was the source of cells for most transplants, reflected in the older term "bone marrow transplant." However, the potential sources of cells for HCT have expanded over time. The use of recombinant human granulocyte colony stimulating factor, which can increase the entry of stem cells into the peripheral blood by up to 1,000-fold, has allowed cytapheresis harvested peripheral cells to become the current dominant source for HCT. Autologous transplants involve the harvesting of one's own bone marrow or peripheral blood cells or, most recently, the use of banked umbilical cord cells. Allogeneic transplants utilize family members or HLA-matched unrelated donors and can again derive

38.1 Common Indications for Hematopoietic Cell Transplantation

Autologous	Allogeneic
Multiple myeloma	Acute myelogenous leukemia
Non-Hodgkin lymphoma[a]	Acute lymphocytic leukemia
Hodgkin lymphoma	Myelodysplastic syndrome/ myelodysplastic disorder
Other cancers[c]	Non-Hodgkin lymphoma[a]
Neuroblastoma	Nonmalignant disease[b]
Breast cancer	–AL amyloidosis –Paroxysmal nocturnal hemoglobinuria –Thalassanemia –Sickle cell disease –Systemic lupus erythematous Chronic myelogenous leukemia Aplastic anemia Multiple myeloma

[a]Non-Hodgkin lymphomas include lymphoblastic, Burkitt, diffuse large B cell, follicular, mantle cell, T cell.
[b]Nonmalignant disease includes Fanconi anemia, Blackfan-Diamond anemia, rheumatoid arthritis, Crohn disease, multiple sclerosis, systemic sclerosis, juvenile rheumatoid arthritis, Evan syndrome, chronic inflammatory demyelinating polyneuropathy, Niemann-Pick disease, Kostmann syndrome, osteopetrosis, Hurler syndrome, adrenoleukodystrophy (ALD)/metachromic dystrophy (MLD), Di-George syndrome, chronic granulomatous disease, common variable disease, severe combined immunodeficiency, Wiskott-Aldrich syndrome, POEMS syndrome, familial erythrophagocytic lymphohistiocytosis.
[c]Other cancers include chronic lymphocytic leukemia, Ewing sarcoma, rhabdomyosarcoma, medulloblastoma, Wilms tumor, osteogenic sarcoma, hepatic blastoma, juvenile chronic myeloid leukemia, desmoplastic small round cell tumor, germ cell, ovarian, renal cell, small-cell lung, soft cell sarcoma.
Data is from references 6 to 11.

cells from bone marrow, peripheral blood, or umbilical cord blood. By 2007, 80% of allogeneic and nearly 100% of autologous transplants involved the use of peripheral blood cells.[6,7] AKI has been found to be more common in recipients of marrow transplant as opposed to peripheral stem cells in some studies[11] but not in others.[13] Conversely, chronic GVHD (cGVHD) may be more prevalent in recipients of peripheral stem cell transplantation due to the greater dose of delivered T cells.[13,15–17] No association has been established between the number of infused stem cells per body weight and AKI.[18]

Myeloablative versus Nonmyeloablative

Conventional myeloablative allogeneic HCT involves high-dose chemotherapy and radiation to eradicate the underlying disease and immunosuppression to prevent rejection of the transplant graft. The allograft then serves to reconstitute the marrow and correct the treatment associated pancytopenia. Survival is contingent on recovery from the cytoablative therapy, successful engraftment, prevention and treatment of infections and GVHD, and eradication of the underlying disease. The potent myeloablative procedure is associated with significant rates of acute conditioning associated complications due to its high-dose chemotherapy and total-body irradiation (TBI), restricting its use to younger, healthier HCT recipients. However, many of the hematologic and malignant conditions wherein HCT holds the greatest potential for cure present primarily in patients older than the standard myeloablative cutoff of 55 to 60 years old or with baseline organ dysfunction or previous high-dose chemotherapy sufficient to render them ineligible for the procedure.

For years these patients were necessarily excluded from treatment. In 1997, a nonmyeloablative procedure was proposed wherein a reduced intensity conditioning regimen would allow its use in older patients and those with significant burdens of comorbidities.[19] Unlike in myeloablative transplant, the low dose chemoradiation in RIC is not intended to kill all residual cancer cells but to provide sufficient immunosuppression so as to allow engraftment of the transplanted stem cells, facilitating their subsequent eradication of the cancer via a donor mediated immune response known as the graft-versus-tumor effect.[19–23] RIC has demonstrated low toxicity and similar efficacy compared to conventional myeloablation in several malignancies without an increased rate of disease recurrence.[19,20] RIC constituted approximately 20% of allogeneic transplants in 2006 and it is increasingly employed in low-grade lymphoma, chronic leukemia, acute leukemia in remission, renal cell carcinoma, and multiple myeloma.[24] Utilization of RIC is likely to continue to increase given the aging population, expanded indications, and technologic advances for transplants across histocompatibility barriers. Distinguishing features of the three types of hematopoietic cell transplants are charted in Table 38.2.

Conditioning Treatments and Prophylaxis

Although all modalities of HCT expose recipients to potentially nephrotoxic medications in the course of conditioning and infection prophylaxis, the specific risks vary by type of transplant. Myeloablative allogeneic regimens typically are cyclophosphamide based along with either TBI or busulfan whereas autologous recipients receive a combination of cyclophosphamide or busulfan along with other agents. For RIC, a very low dose of TBI or fludarabine is substituted for cyclophosphamide or cyclophosphamide dosing is individualized. Allogeneic recipients receive prophylaxis against acute graft-versus-host disease (aGVHD) with

38.2 Distinguishing Features of the Three Modalities of Hematopoietic Cell Transplant

	Myeloablative Autologous	Myeloablative Allogeneic	Nonmyeloablative Allogeneic
Number in U.S. (annual)	21,000	4,300	2,700
Age % recipients age >60	Mostly younger 34%	Younger <10%	Older 40%
Conditioning regimen Radiation Cytotoxic therapy	 +/− High dose	 12 Gy High dose	 2 Gy Low dose
Donor cells	PB (98%) Other (2%)	PB (68%) BM (24%) CB (8%)	PB (96%) Other (4%)
GVT effect	None	Mild/moderate	Main effect
SOS (incidence)	4%–7%	2%–54%	Rare
TM (incidence)	0%–27%	0%–76%	Extremely rare
Pancytopenia	Shorter (~2 weeks)	Longer (~3 weeks)	Shorter (~2 weeks)
Acute GVHD (II-V) Timing Incidence Prophylaxis	 N/A N/A N/A	 Early (weeks) 7%–91% MTX/CsA ±Pred	 Later 13%–77% CsA or Tac ±Pred
Chronic extensive GVHD (incidence)	N/A	13%–71%	11%–73%
Overall mortality 100 days 1 year	 ~5%–20% ~25%–30%	 ~20%–25% ~40%–45%	 ~5%–15% ~35%–40%

HCT, hematopoietic cell transplantation; U.S., United States; Gy, Gray; PB, peripheral blood; BM, bone marrow; CB, cord blood; GVT, graft-versus-tumor; SOS, sinusoidal obstruction syndrome; TM, thrombotic microangiopathy; GVHD, graft-versus-host disease; N/A; not applicable; MTX, methotrexate; CsA, cyclosporine; Tac, tacrolimus; Pred, prednisone.
Adapted and modified from reference 10. Data from references 6, 7, 10, and 25 to 29.

immunosuppressive drugs, typically cyclosporine or tacrolimus plus methotrexate, although mycophenolate mofetil and sirolimus can also be used.[30] Prophylactic drugs may be withdrawn earlier in RIC to facilitate graft-versus-tumor effect. Prophylaxis for infection includes acyclovir for patients seropositive for herpes simplex virus (HSV), trimethoprim/sulfamethoxazole to prevent Pneumocystis jiroveci infection, oral fluconazole for fungal prevention, and preemptive ganciclovir or foscarnet for cytomegalovirus (CMV) infection in viremic recipients.[31,32] Amphotericin, voriconazole, or micafungin are used for patients at risk for aspergillus infection.[33] Comparing outcomes in allogeneic recipients in the periods between 1993 and 1997 and 2003 and 2007, there has been a significant decrease in bacterial, fungal, and CMV infections.[12] In addition to improvements in the use of prophylactic antimicrobials, the increased use of peripheral blood donor cells has resulted in significantly faster neutrophil engraftment and earlier reconstitution of immunity.

Epidemiology of Acute Kidney Injury

Estimates of the incidence of AKI post-HCT vary widely, ranging from 14% to 100%.[14,34] The likelihood of kidney injury is impacted by transplant type (allogeneic or autologous), donor type (related or unrelated), degree of HLA matching (full match or mismatched), conditioning regimen (myeloablative or nonmyeloablative), and specific

38.3 Commonly Utilized Definitions of Acute Kidney Injury in Hematopoietic Cell Transplant Studies

RIFLE	
Risk	Increase in Scr ≥ 1.5 × baseline or decrease in GFR ≥25%
Injury	Increase in Scr ≥ 2 × baseline or decrease in GFR ≥50%
Failure	Increase in Scr ≥ 3 × baseline or decrease in GFR ≥75% or an absolute Scr ≥4.0 mg/dL with an acute rise of at least 0.5 mg/dL
Loss	Persistent AKI >4 weeks
ESRD	ESRD >3 months
AKIN	
Stage 1	Increase in Scr ≥0.3 mg/dL or increase to 150%–199% (1.5–1.9 fold) from baseline
Stage 2	Increase in Scr 200–299% (>2.0–2.9 fold) from baseline
Stage 3	Increase in Scr ≥300% (≥3-fold) from baseline or Scr ≥4.0 mg/dL with an acute rise of at least 0.5 mg/dL
Parikh-Schrier	
Grade 0	Decrease in GFR <25% of baseline
Grade 1	Increase in Scr <2-fold from baseline with a decrease in GFR >25% but <50% of baseline
Grade 2	Increase in Scr ≥2-fold from baseline but not requiring dialysis
Grade 3	Increase in Scr ≥2-fold from baseline and need for dialysis

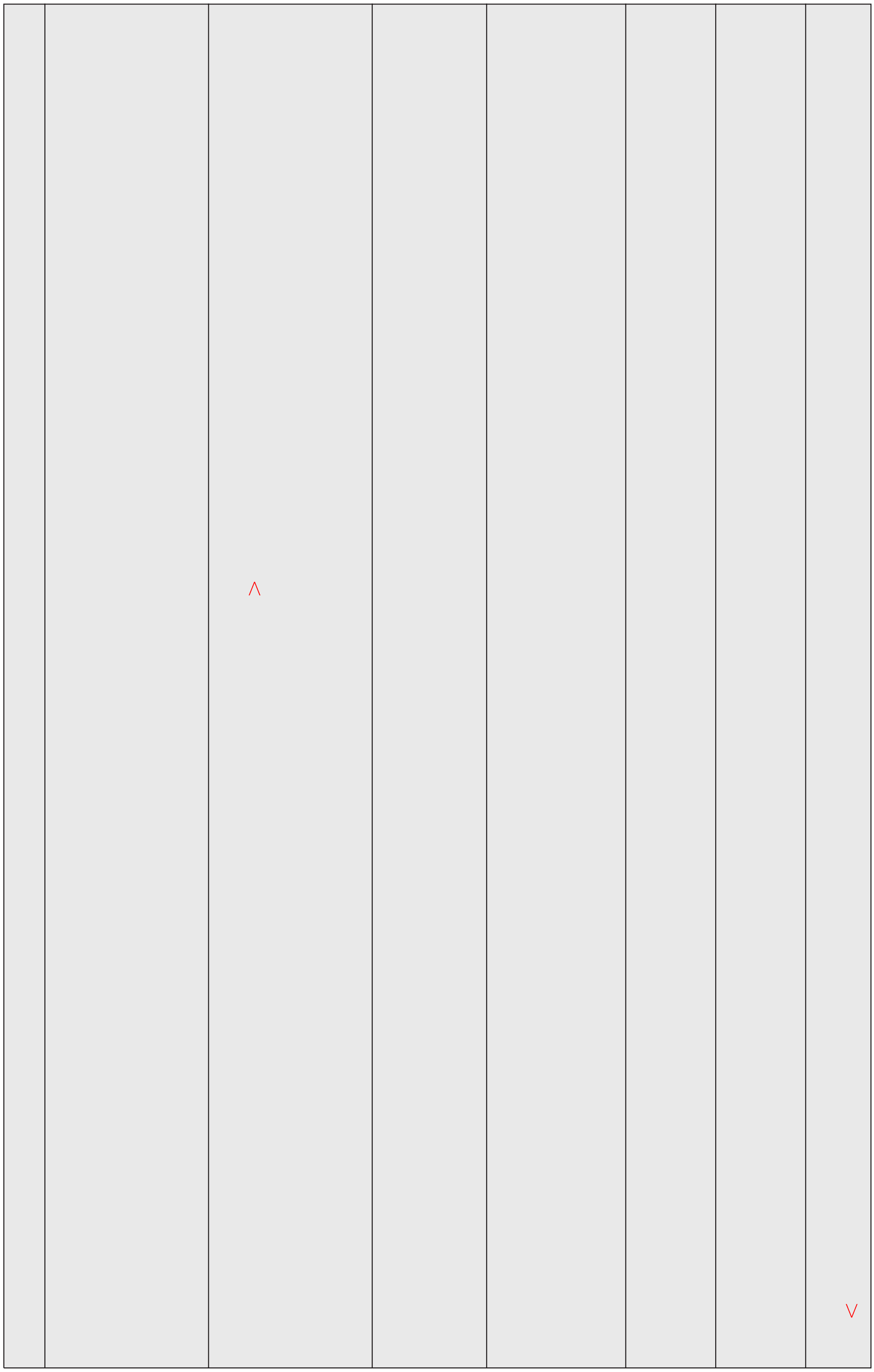
AKI, acute kidney injury; RIFLE, risk, injury, failure, loss, end-stage renal disease; Scr, serum creatinine; GFR, glomerular filtration rate; mg/dL; milligrams/deciliter; ESRD, end-stage renal disease; AKIN, Acute Kidney Injury Network.

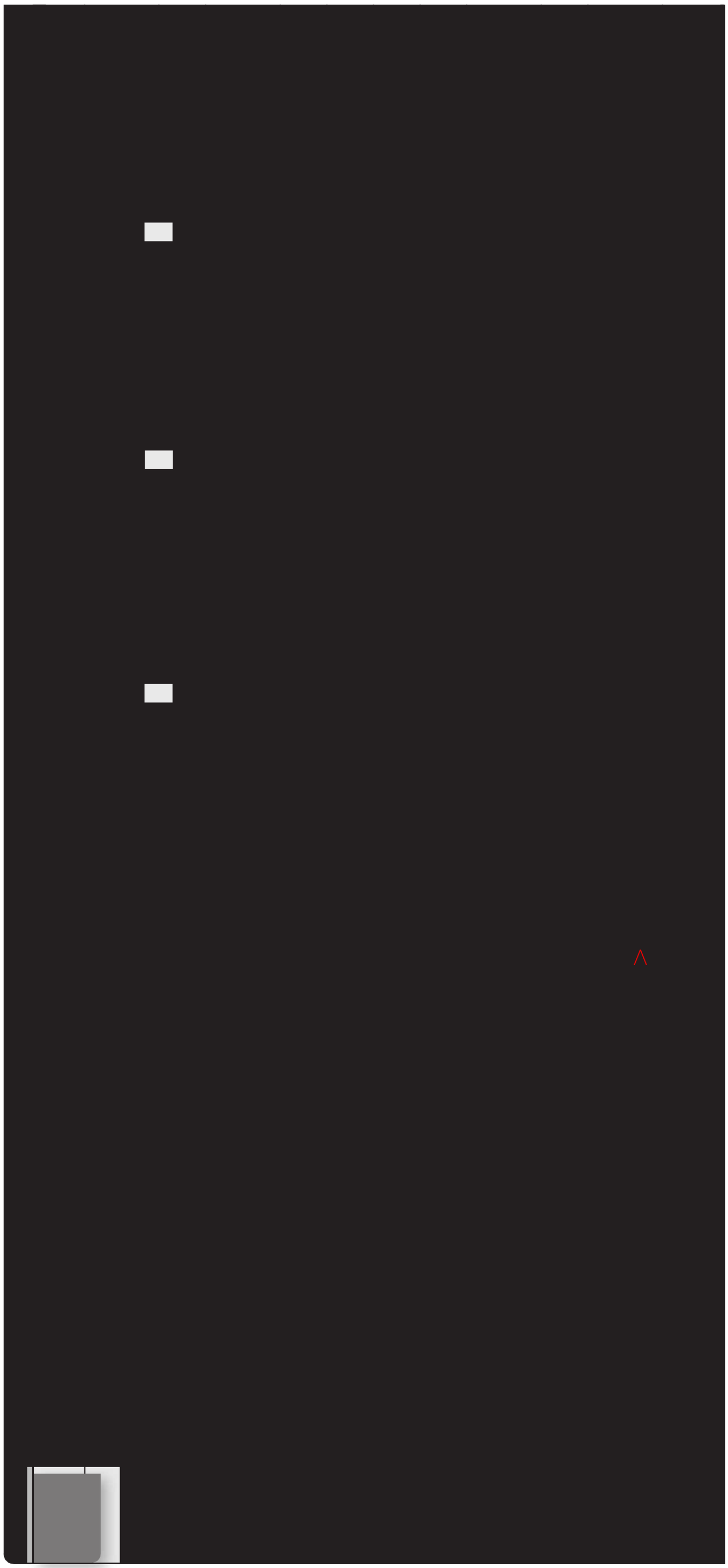
conditioning regimens, immunosuppressants, prophylactic medications, and length of follow-up. In addition, the lack of a standardized definition for AKI has been vexing to those seeking to understand the epidemiology of the disease. Two recently proposed sets of diagnostic criteria, RIFLE (risk, injury, failure, loss of kidney function, end-stage kidney disease)[35] and AKIN (Acute Kidney Injury Network),[36] have facilitated diagnostic standardization and allowed for significant advances in understanding the epidemiology and outcomes of AKI at large.[37] The definitions employed by three common classification systems for AKI following HCT are shown in Table 38.3. Although many studies of post-HCT AKI have used a variation of the classification system utilized by Parikh et al.,[38] the sensitivity of the RIFLE and AKIN criteria has recently been evaluated for diagnosis and prediction of long-term, all-cause mortality associated with post-HCT AKI.[39] AKIN identified the smallest number of patients as having AKI across all three modalities due to a reduced sensitivity for identifying the lowest category of AKI. For severe AKI, denoted as RIFLE ≥ injury, AKIN ≥ stage 2, or Parikh ≥ grade 2, all three systems performed identically. As seen in multiple other settings,[40,41] RIFLE and AKIN stages, along with those of the Parikh classification, were associated in a stepwise manner with mortality. The HCT-Comorbidity (HCT-CI) index is composed of cardiac, pulmonary, hepatic, gastrointestinal, and renal function tests that are sensitive for the detection of subclinical organ impairment and has been shown to predict nonrelapse mortality.[42,43] Both intermediate, hazard ratio (HR) 2.42, and high risk, HR 4.69, HCT-CI scores were independently associated with the development of RIFLE "I" and "F" in a combined cohort of myeloablative and RIC allogeneic recipients.[43]

INCIDENCE AND TIMING OF ACUTE KIDNEY INJURY

Myeloablative Allogeneic

Following the initial seminal study by Zager et al.,[44] AKI has been recognized as a common and devastating complication of myeloablative allogeneic HCT. In that initial cohort of 272 patients, 53% developed AKI at a median of 14 days. Numerous retrospective and prospective studies have since evaluated the incidence of AKI following myeloablative allogeneic HCT (Tables 38.4 and 38.5). Utilizing multiple definitions, AKI has been noted in 21% to 100% of patients with a weighted mean of 60%. Severe AKI occurs in a weighted mean of 40% of recipients. Although AKI following myeloablative allogeneic HCT has historically been thought to occur primarily in the first 2 to 3 weeks posttransplant, reflective of conditioning toxicity,[44,52] the median onset across all studies ranges from 15 to 60 days with a weighted mean of 30 days. The requirement for dialysis has ranged from 0% to 36%,[8,18,28,44,49,51–55,60,62,64,67,68] with some the lowest incidences[18,64] occurring more recently, perhaps reflecting refinements in conditioning and prophylactic regimens.





Befitting the discrepant patient populations, conditioning regimens, and prophylactic medications seen in these studies, myriad risk factors have been found for AKI. Conflicting results have been reported for multiple factors. Additionally, many studies have not utilized adjusted analysis, calling the true influence of postulated risk factors with potential colinearity into question. Those that have been found significant after adjustment in multiple studies include sinusoidal obstruction syndrome (SOS),[28,48,50,51,52,53,63,67,68] cyclosporine use/toxicity,[45,53,64] hyperbilirubinemia/jaundice,[46,48] amphotericin,[28,50] aGVHD,[48,51] and chronic kidney disease (CKD).[46,55] A full listing of risk factors for AKI in myeloablative allogeneic as well as autologous and RIC HCT are provided in Table 38.6.

Nonmyeloablative Allogeneic

Given the older age, poorer performance status, and increased comorbidities in those patients selected for RIC, a higher incidence of AKI than is seen with myeloablative transplant might be expected. Instead, when compared directly, RIC has consistently been associated with equal[14,43] or reduced rates of AKI.[39,63] Overall, the reported incidence is similar to myeloablative, ranging from 17% to 94% with a weighted mean of 65%. However, severe AKI is less common, occurring in a weighted mean of 30% of recipients. Reflecting this, the need for dialysis is lower and has been noted in 0% to 5% of patients.[14,25,26,29,38,39,43,58,59,63] Comparing the two modalities concurrently, myeloablative HCT was associated with an odds ratio (OR) of 4.8 for AKI.[14] Because the level of posttransplant immunosuppression and the rate of GVHD are similar between the two regimens, it appears to be the milder preconditioning regimen that reduces the incidence and severity of AKI. A gentler chemoradiation procedure alleviates both direct nephrotoxicity as well as damage indirectly caused by myeloablative associated SOS, infection, and thrombotic microangiopathy (TMA).[38] The median onset of AKI in RIC studies ranges from 17 to 60 days with a weighted mean of 46 days. This is 2 weeks later than in myeloablative, again reflecting the lesser role of conditioning toxicity in the etiology of RIC associated AKI. The onset of AKI is fairly evenly distributed over the first 3 months but subsequently tapers off significantly.[25,38] As with myeloablative allogeneic HCT, there has been little agreement between studies on independently significant risk factors for AKI following RIC HCT, with only aGVHD[25,58] noted in multiple studies.

An additional contribution to the discordant incidence and severity of AKI in MA and RIC may lie in the recipient's capacity to repair conditioning mediated tubular injury. Hematopoietic stem cells have shown the capacity to differentiate into renal tubular cells and home to the site of injury after ischemic renal insults.[69,70] It is possible such cells may contribute to the repair and regeneration of damaged tubular cells and thus mitigate AKI. Renoprotective activity of stem cell lineages has been documented in ischemically injured kidneys,[71,72] as well as with cisplatin-induced toxicity.[73,74] It is possible obliteration of endogenous stem cells in myeloablative regimens contributes to the higher observed rates of AKI.

Myeloablative Autologous

Although autologous transplants outnumber allogeneic worldwide,[6,7] comparatively few studies have investigated renal outcomes following this procedure.[29,35,40,44,58,59,61–64] The incidence of AKI after myeloablative autologous HCT is significantly lower than in either myeloablative allogeneic or RIC, ranging from 7% to 56%, with a weighted mean of 30%. Severe AKI occurs in a weighted mean of 19% of recipients. Dialysis is required following 0% to 9% of transplants.[29,35,44,58,59,61,63] Few studies have evaluated the timing of AKI onset following autologous transplantation. The lower incidence of AKI occurs despite an older patient population and the corresponding prevalence of more comorbidities.[8,9,39,67] This paradoxical outcome is due to several factors. First, autologous recipients receive comparatively mild chemoradiation conditioning regimens, thus affording protection from many conditioning associated complications. Second, autologous recipients by definition cannot suffer from acute or chronic GVHD, both of which expose the kidney to direct and indirect damage. Correspondingly, there is no need to treat autologous recipients with calcineurin inhibitors to prevent or treat GVHD and these patients are thus spared the risk of calcineurin inhibitor nephropathy (CIN). Third, associated with the milder conditioning and lack of GVHD, SOS and TMA are much less common.[13,38,75] Finally, there is more rapid engraftment without foreign cells, shortening neutropenia, lessening sepsis, and minimizing exposure to nephrotoxic antimicrobials.[64] No risk factors have been found across studies to independently associate with AKI following autologous HCT.

General Risk Factors

Chronic Kidney Disease

CKD is a well-established risk factor for AKI in the setting of potential kidney insults with nephrotoxins such as radiocontrast media[76] and aminoglycosides.[77] Surprisingly, the inverse has been found in multiple studies of HCT. Zager assessed the relationship in 3,325 patients between pretransplant eGFR and renal function at 1 year following HCT.[78] Outcomes included both "renal functional impairment," defined as a 25% reduction in eGFR from baseline, as well as absolute change. A striking inverse correlation was seen between baseline function and 25% decrease, $r = 0.92$, and absolute decrease, $r = 0.97$. The authors speculate it may be a form of acquired cytoresistance as seen in several animal models.[79–84] Although not universally noted,[55] a similar finding of elevated pretransplant GFR as a risk factor for AKI has been found in several RIC[38,58] and myeloablative allogeneic studies.[50] Although the concept of ischemic preconditioning to prevent AKI is intriguing,[85,86] some part of the effect in this setting is likely due to the utilized AKI definitions in that the

38.6 Risk Factors Independently Associated with Acute Kidney Injury Following Hematopoietic Cell Transplant

Risk Factors	MA	Autologous	RIC
SOS/liver toxicity	6 (8)[a]	1 (1)	– (0)
Cyclosporine use/toxicity	3 (7)	– (0)	1 (0)
Ventilator use	2 (2)	1 (1)	– (0)
aGVHD	2 (8)	– (0)	2 (2)
Amphotericin	2 (6)	– (1)	– (0)
Hyperbilirubinemia	2 (5)	– (0)	– (0)
Diminished baseline renal function	2 (7)	– (1)	– (0)
Sepsis/septic shock	1 (5)	1 (1)	– (0)
Hypertension	1 (3)	– (0)	– (0)
ICU admission	1 (1)	– (0)	– (0)
Unrelated donor/incomplete HLA-match	1 (9)	– (0)	1 (4)
Foscarnet	1 (2)	– (0)	– (0)
Age	1 (7)	– (2)	1 (3)
Methotrexate	– (3)	– (0)	1 (1)
Diabetes mellitus	– (0)	– (0)	1 (1)
(GVHD, sepsis, SOS) composite	– (0)	– (0)	1 (1)
>3 cycles of prior chemotherapy	– (0)	– (0)	1 (1)
Absence of vascular disease	– (0)	– (9)	1 (1)
High baseline GFR	– (0)	– (0)	1 (1)
Bacteremia	– (0)	1 (0)	– (0)
Melphalan	– (0)	1 (0)	– (0)
Proteinuria	– (0)	1 (0)	– (0)

[a]Number of studies with statistically significant increased risk of AKI (number of studies where risk factor has been evaluated for independent association).
AKI, acute kidney injury; HCT, hematopoietic cell transplantation; MA, myeloablative allogeneic; RIC, reduced intensity conditioning allogeneic; SOS, sinusoidal obstruction syndrome, aGVHD, acute graft-versus-host disease; ICU, intensive care unit; GFR, glomerular filtration rate.

absolute change required for doubling of serum creatinine is less in patients with lower baselines, with the same following for GFR, and thus those patients with preserved baseline function require smaller changes to meet AKI criteria.

Genetics

SNPs in genes associated with the urea cycle (CPSI) and hemochromatosis (HFE) have been shown to influence susceptibility to SOS.[87–90] Additionally, donor–recipient genotype combinations in the killer immunoglobulin-like receptors (KIRs) present on NK and some T cells are important determinants of aGVHD.[91] Finally, the presence of the DD allele in the ACE gene may slow the decline in creatinine clearance in the year following HCT.[92]

Additional Markers of Kidney Injury

Despite the tremendous incidence of AKI documented in these studies, they are likely to be significantly underestimating the true occurrence of kidney injury following HCT. As is widely recognized in nephrology, serum creatinine is a poor and insensitive marker for kidney function. Due to renal functional reserve and tubular creatinine secretion, serum creatinine can remain in the normal range even when the glomerular filtration rate (GFR) has fallen to 50% of baseline. In addition to actual changes in GFR, creatinine levels are also influenced by body weight, race, age, gender, muscle mass, protein intake, and drugs. In HCT patients in particular, given the severity of their disease, it is common to see significant weight loss, loss of muscle mass, and decreased protein intake.

^{99m}Tc-DTPA was used to sequentially measure GFR in pediatric patients post-HCT.[47] GFR fell precipitously from baseline at 30 and 100 days but rebounded somewhat by 180 days. The estimated GFR utilizing creatinine was significantly higher than ^{99m}Tc-DTPA measured GFR. N-acetyl-β-D-glucosaminidase (β-NAG), a biomarker of tubular damage,[93] was significantly elevated at 30 days but returned to baseline by 180 days. Near ubiquitous early elevation in urinary α-1 microglobulin and β-NAG and a decrease in phosphate reabsorption after conditioning indicated rampant tubular damage.[28] After 2 years, although only 5% of the children had inulin measured GFR $<$90 mL/min, approximately 40% maintained elevated urinary α-1 microglobulin levels or decreased phosphate reabsorption. Based on these more sensitive markers it appears nonspecific tubular damage in the peritransplant period is ubiquitous and recovery is often incomplete, even if subclinically so.

Additional novel markers may assist in ascertaining the true incidence of injury. Cystatin C, a low molecular weight protein (13kDa) synthesized by all nucleated cells, freely crosses the glomerular filtration barrier and is almost completely reabsorbed by the cells of the proximal tubule. Cystatin C has demonstrated superior diagnostic sensitivity for the detection of AKI compared with creatinine.[94] Cystatin-C has retrospectively been assessed as a marker for post-HCT kidney dysfunction.[45] Although noting a strong inverse correlation between cystatin C and estimated GFR, the authors did not compare the ability of cystatin C and creatinine to detect AKI. A significantly higher rate of worsening of preexisting chronic kidney disease (CKD) was found during the first year post-HCT in patients with a pretransplant cystatin C level $\geq$0.90 mg per L relative to patients with levels below this cutoff.

In the context of validating the results of a urine proteomic study of novel markers predictive of clinical AKI, an AKI-specific peptide panel was assessed in 31 patients undergoing allogeneic HCT, 13 of whom developed AKI.[95] Although absolute peptide levels differed from those of patients studied in an intensive care unit (ICU) setting, the panel showed excellent discriminatory ability with an area under the receiver operating curve (AUC) of 0.90, a sensitivity of 94%, and a specificity of 82% to predict AKI as defined by a rise in serum creatinine of $\geq$50% within 48 hours.

ETIOLOGIES OF ACUTE KIDNEY INJURY

AKI seen in the context of HCT is often multifactorial and identifying a specific etiology can be challenging. Several factors unique to the transplant patient and procedure contribute to the extreme incidence and severity of AKI observed post-HCT. Various classification systems have attempted to distinguish between causes associated with early, middle, and late onset, as variably defined.[50,96–98] The most common etiologies will be discussed in chronological order, beginning at the time of induction.

Medullary Infusion Toxicity

Infusion of cryopreserved marrow or blood progenitor cells may lead to kidney injury via two mechanisms. Cell lysis products and debris associated with the freeze/thaw cycle can cause direct glomerular damage and proteinuria. Additionally, dimethyl sulfoxide, a cryopreservative primarily used in autologous cell storage, may cause hemolysis and hemoglobinuria mediated nephrotoxicity.[99–101]

Tumor Lysis Syndrome

Tumor lysis syndrome is a condition in which the rapid obliteration via radiation and chemotherapy of a high bulk of tumor cells results in a massive release of nephrotoxic debris. Renal injury is primarily mediated via hyperphosphatemia as well as urate and xanthine nephropathy. Precipitation of these products in the renal tubules leads to intratubular obstruction and AKI. Additionally, hyperuricemia has been associated with renal vasoconstriction and pro-oxidative and pro-inflammatory mediators.[102] Those at greatest risk are patients with high tumor bulk such as acute leukemias with high white cell counts and Burkitt's lymphoma.[103] Fortunately, due the fact that many patients are in remission at the time of therapy and are treated appropriately with

prophylactic intravenous fluids, urinary alkalinization, and allopurinol, the incidence of tumor lysis syndrome following HCT is rather low (approximately one in 400).[101]

Sepsis

Patients undergoing HCT are at tremendous risk for bacterial and fungal infection and resulting sepsis due to the profound neutropenia engendered by induction chemotherapy and radiation, which is then compounded by the use of immunosuppressants to prevent aGVHD. The inflammatory response to disseminated infection leads to systemic arterial vasodilatation and capillary leak, precipitating hypotension and renal hypoperfusion with resultant pre-renal azotemia and acute tubular necrosis. This hypoperfusion can be potentiated by vomiting and diarrhea associated with the intense conditioning regimens. However, even in the absence of hypotension, sepsis may provoke AKI via cytokine and chemokine mediated renal vasoconstriction and direct intrarenal inflammatory and complement associated kidney injury.[104] Additionally, the antimicrobials used in the prevention and treatment of sepsis are often nephrotoxic (see later).

Nephrotoxic Medications

Many of the chemotherapy agents used for induction have the potential to be nephrotoxic including carboplatin, vincristine, melphalan, busulfan, etoposide, carmustine, cytarabine, and cyclophosphamide.[100,105–107] The nephrotoxicity of antineoplastic medications is treated in depth elsewhere in this text. Amphotericin B is utilized for patients at risk for or with evidence of aspergillus infection and is a well-recognized nephrotoxin. Though liposomal amphotericin has generally been associated with lessened nephrotoxicity,[108] studies show both preparations confer an equal increase in risk of AKI following HCT.[28,50] The use of aminoglycosides has declined with the advent of alternative broad spectrum antibiotics but when utilized still carry a marked potential for nephrotoxicity. Additional medications found to associate with AKI following HCT include vancomycin[33,43] and methotrexate.[25] Given the potential nephrotoxicity of calcineurin inhibitors (see later), sirolimus has been investigated for GVHD prophylaxis. Although there is no data on monotherapy, sirolimus has been shown to potentiate the risk of TMA (OR 2.79)[109] and SOS (OR 2.76)[27] when used with calcineurin inhibitors without affecting mortality. The risk of SOS was even higher when sirolimus was used in conjuncture with busulfan.

Calcineurin Inhibitors

Calcineurin inhibitors (cyclosporine [CSA] and tacrolimus) are routinely used as immunosuppressants to prevent GVHD. In myeloablative allogeneic transplant patients, they are frequently combined with methotrexate or mycophenolate mofetil while they are used with steroids in non-myeloablative transplantation. Calcineurin inhibitors are well-known nephrotoxins, inducing potent renal vasoconstriction, direct endothelial injury, and provoking thrombotic microangiopathy. Toxicity has been thought to correlate well with serum drug concentration.[110] Although the expected association was seen between CSA exposure and AKI in several older studies,[19,20,111] the majority of recent studies have failed to replicate this finding.[25,38,44,49,50,58,112] There does not appear to be any difference in the incidence of AKI utilizing cyclosporine versus tacrolimus.[54] In two studies where the etiology of AKI was chart adjudicated, elevated CSA levels were associated with AKI in 67% of all patients[28] and 100% of patients with grade 2 AKI.[38] However, despite this clinical association and the biologic plausibility of causation, CSA levels were not found in either study to be independent risk factors for AKI on multivariable analysis.

This discordance between statistical analysis and chart reviewed diagnosis may be due to variability of levels and troughs within a given patient and the transient effect of CSA on short-term renal function. Additionally, targeted CSA therapeutic ranges vary significantly across studies (see Table 38.4) and, in analyzing CSA as a risk factor, it has sometimes been treated as a continuous variable based on levels and sometimes categorical regarding its use or the surpassing of the designated toxic threshold. Renal dysfunction may also lag peak concentration.[67] Finally, CSA toxicity can occur despite low or normal serum concentrations and thus may be complicated by a patient's genetic susceptibility. Woodhahl et al. investigated four single nucleotide polymorphisms (SNPs) in genes regulating cyclosporine metabolism and transport which may affect tubular intracellular drug levels in 121 patients following myeloablative transplant.[113] Although no findings reached statistical significance, there was a trend toward a higher OR for AKI with SNPs in ABCB1, encoding the transporter P-glycoprotein.

Sinusoidal Obstruction Syndrome

SOS, formerly known as hepatic veno-occlusive disease, is initiated by chemoradiation-induced injury to sinusoidal epithelial cells. Damage to these cells leads to activation of stellate cells and deposition of extracellular matrix in sinusoids and zone 3 hepatocytes with resulting thrombosis and necrosis. Subsequent portal hypertension stimulates a shift in fluid from the intravascular to extravascular space, increased activation of the renal-angiotensin-aldosterone system, sodium and water retention, ascites formation, and ultimately AKI in a presentation similar to hepatorenal syndrome.[75] However, patients with SOS and AKI have also been found to have higher β-NAG, α-1 microglobulin, and albumin excretion than those without AKI, implying there is a component of tubular and perhaps glomerular injury as well.[100] Signs and symptoms include hepatomegaly, right upper quadrant pain, ascites, weight gain, and increased serum aminotransferase and bilirubin levels.[114] SOS is only rarely seen with RIC[14] and is less common in autologous than allogeneic transplants.[62] In myeloablative regimens, the development of SOS is strongly associated with post-HCT AKI,[14,44,52,68] with an incidence of 0% to 60%,[8,30,114] and

increases with AKI severity. Ileri et al. documented SOS in 6% of pediatric recipients without AKI, 8% with grade 1, 22% with grade 2, and 100% with grade 3.[28]

Risk factors for the development of SOS include age, preexisting hepatic disease, hepatitis C, TBI, CMV seropositivity, and multiple medications including cyclophosphamide, busulfan, vancomycin, amphotericin B, acyclovir, and methotrexate.[64,114,115] Although the exact contribution of individual medications can be difficult to untangle in overlapping regimens, cyclophosphamide in particular is thought to directly contribute to liver injury.[116] Using personalized dosing based on measurement of carboxyethylphosphoramide mustard, a metabolite that functions as a reporter molecule for hepatotoxins from cyclophosphamide, and utilizing Bayesian parameter estimates, patients that received reduced cyclophosphamide had lower bilirubin levels, and suffered less AKI, 62% vs 77% ($P = .03$), than those receiving conventional dosing.[117]

HCT Associated Thrombotic Microangiopathy

Thrombotic microangiopathies (TMA) are characterized by systemic or intrarenal platelet aggregation, thrombocytopenia, and microvascular fragmentation of erythrocytes. Diagnostic evaluation divulges schistocytes on peripheral smear and increased serum lactate dehydrogenase (LDH). In the archetypal TMA, thrombotic thrombocytopenia purpura (TTP), abnormalities in ADAMTS-13 von Willebrand factor cleaving protease activity have been associated with inherited and acquired disease. Decreased ADAMTS-13 activity results in a preponderance of large multimers of von Willebrand factor and subsequent platelet aggregation.[118] However, ADAMTS-13 deficiency is not involved in the pathophysiology of HCT associated TMA.[119–123] Additionally, autopsy studies have demonstrated post-HCT TMA to be primarily renally dominant, similar to hemolytic uremic syndrome rather than TTP.[119,124] Correspondingly, the neurologic features of TTP are typically absent.[124]

The inciting event in post-HCT TMA is endothelial damage initiated by the conditioning regimen, particularly TBI and busulfan.[121,125] The initial injury is potentiated by infections, lipopolysaccharides, calcineurin inhibitors, sirolimus, and aGVHD.[122,126,127] GVHD may mediate endothelial damage in the kidney via circulating inflammatory cytokines related to systemic disease or may inflict direct injury to endothelial cells in the kidney due to localized activated donor cells.[128] This kidney specific GVHD may also be associated with reduced VEGF function.[128–130] Owing to this damage, a loss of endothelial resistance to thrombus formation, subsequent leukocyte adhesion, and an increased vascular shear stress ensue and augment the injury. In addition to AKI, patients often present with anemia, thrombocytopenia, hypertension (HTN), edema, proteinuria, and hematuria. Disease onset typically is within 100 days of HCT. The clinical spectrum of kidney disease in HCT patients with TMA ranges from an indolent course resulting in CKD to a fulminant presentation with AKI and death.[128] Different forms of overlapping thrombotic microangiopathic syndromes have been described. Patients with TMA after HCT have thus been diagnosed with HCT nephropathy, radiation nephropathy, hemolytic uremia syndrome, TTP, or transplant associated microangiopathy.[127] Making a confident diagnosis is challenging because anemia, thrombocytopenia, and elevations in LDH and creatinine are common post-HCT due to delayed engraftment, fungal and viral infections, medications, and GVHD.[121,131] Biopsy is often contraindicated due to patient instability and thrombocytopenia. Confounding the confusion, no fewer than 28 different sets of diagnostic criteria have been proposed in 35 articles detailing post-HCT TMA.[124] Two distinct consensus definitions have also been set forth.[120,131]

Not surprisingly, the estimated incidence of TMA in the setting of HCT varies widely from 0.5% to 76%[34,122,128] with the alleged mortality ranging from 0% to 100%. Changsirikulcai et al. retrospectively analyzed autopsy samples from 322 patients who had died a median of 43 days posttransplant; 41% had suffered AKI within 2 weeks preceding their deaths.[128] On biopsy, 20% met criteria for TMA whereas an additional 15% had a singular glomerular or vascular thrombus. There was no statistically significant difference in serologic abnormalities (hematocrit, platelets, LDH, elevated creatinine) between patients with these findings and those with negative biopsies. Histologic evidence of TMA in the kidney did not correlate with the laboratory evidence of AKI in the 2 weeks prior to death. On chart review, a clinical diagnosis or suspicion of TMA correlated with histologic evidence of TMA in 35.5% of the positive samples versus 4.5% of the negative ($P < .001$). Other studies have shown mixed results in the correlation between TMA as diagnosed by clinical criteria and histologic findings on autopsy.[99,119,124] An extensive litany of risk factors for post-HCT TMA have been described including advanced age, female sex, female recipient–male donor, unrelated donor, HLA-mismatch, T-cell depletion, high intensity conditioning regimens, high dose busulfan, fludarabine, TBI, sirolimus, calcineurin inhibitors, sirolimus with calcineurin inhibitors, aGVHD, methylprednisolone therapy, adenovirus, fungal or viral infection, and SOS.[38,109,128,132–137]

Treatment options for post-HCT TMA, outside of withdrawing any identifiable inciting agents, are scarce. Indeed a review article found that any attempt at treatment led to increased mortality as opposed to no treatment at all, although this finding may suffer confounding by indication.[121] Although plasma exchange, a staple of treatment in ADAMTS-13 associated TMA, has been reported to be effective in rare instances,[138,139] the majority of studies have seen little or no benefit.[124,131,140–143] Rituximab, an anti-CD20 monoclonal antibody, showed promise in a small series treating post-HCT TMA which had failed plasma exchange.[144] In patients who have developed GVHD and TMA, withdrawal of calcineurin inhibitors and treatment with daclizumab, a humanized anti-CD25 antibody, have shown promise in the treatment of TMA but poor response in terms of complete remission of

GVHD.[126] Defibrotide, a polydeoxyribonucleotide salt, protects against endothelial damage by inhibiting tumor necrosis factor (TNF)-α mediated endothelial cell apoptosis and exhibits profibrinolytic, antithrombotic, and anti-inflammatory activity.[122] It has been used in treating SOS associated with HCT and with some success in small studies in TMA.[145]

Graft-versus-Host Disease

GVHD has classically been thought not to involve the kidney. aGVHD develops in 50% to 60%[146] of sibling-matched allografts and cGVHD involves 30% to 60%.[147] Any association between GVHD and AKI in these patients has been attributed to volume depletion due to gastrointestinal tract involvement with diarrhea and an increased need of nephrotoxic drugs for GVHD treatment. Recent evidence, however, suggests that GVHD may affect the kidney directly. aGVHD has been found an independent risk factor for post-HCT AKI in multiple studies.[25,48,58,63] Such toxicity may be due to cytokine mediated inflammatory injury, glomerular deposits leading to nephrotic syndrome (see later), and tubulitis secondary to activated cytotoxic T cells. Documentation of severe renal infiltration by cytotoxic T cells during GVHD has been documented in mice,[148] but confirmation of the role of GVHD in human AKI awaits further study.

Biopsy Data for Etiologies

Biopsies are rarely performed in patients with AKI following HCT due to thrombocytopenia, hemodynamic instability, and infection. Several series have been performed, often retrospectively, in patients with AKI or worsening CKD months to years out from transplant.[149–153] Among frequently overlapping pathology, findings consistent with TMA, polyoma virus nephropathy, ATN, interstitial nephritis, tubulointerstitial scarring, and podocytopathy are commonly found. El-Seisi et al. performed an autopsy study of 26 patients who had died a median 3 months after transplant; 15 had AKI.[99] Global segmental sclerosis, tubular epithelial atypia, tubular calcification, mild tubulitis, interstitial fibrosis, and thrombotic microangiopathy were all common. Despite the expected renal insults common in hospital-based death, very little classical ATN was noted. No association was seen between clinical SOS and histology consistent with TMA. Interestingly, only 1/12 patients with TMA on biopsy had clinical evidence of the disease on retrospective chart review. In assessing the generalizability of these findings to all AKI post-HCT, the study suffers from selection bias in that patients would have been much less likely to be autopsied if they died of disease relapse or at home.

PROGNOSIS

Mortality

Nonrelapse mortality after HCT occurs primarily due to complications of the HCT procedure, with AKI being one of the most prominent. The pathway linking HCT associated

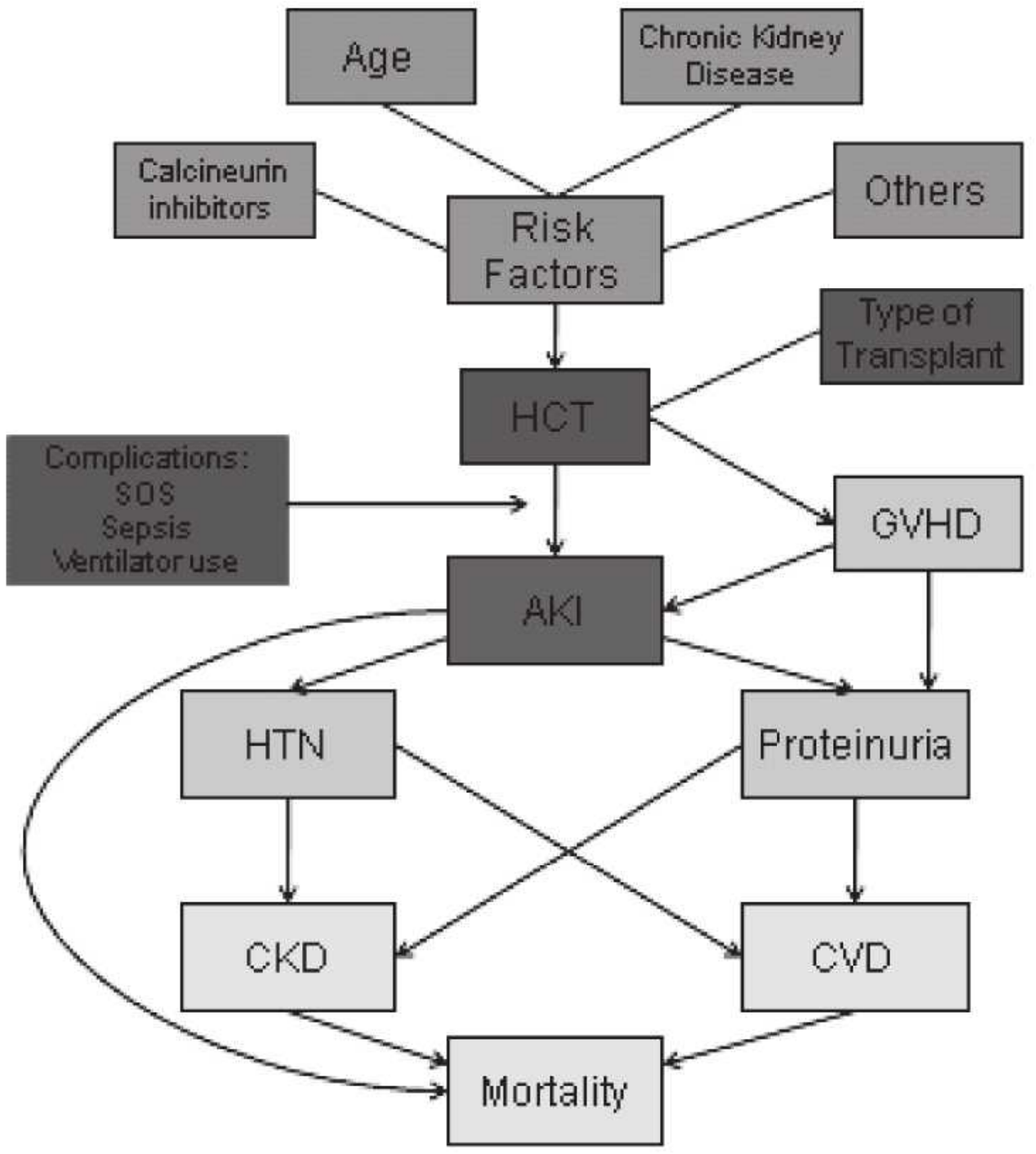


FIGURE 38.1 Mechanisms and outcomes of hematopoietic cell transplant associated acute kidney injury. AKI, acute kidney injury; CKD, chronic kidney disease; CVD, cardiovascular disease; GVHD, graft-versus host disease; HCT, hematopoietic cell transplant; HTN, hypertension; SOS, sinusoidal obstruction syndrome.

AKI with mortality and other long-term outcomes is shown in Figure 38.1. AKI can cause mortality directly via consequences of compromised renal function including metabolic acidosis, cardiac dysrhythmias, HTN, electrolyte imbalances, gastrointestinal dysfunction, and hemorrhage.[154] AKI can also contribute to dysfunction in other organ systems such as lung and liver via volume overload and confers an increased risk of sepsis due to altered leukocyte function.[154–156] Additionally, cytokine or immune-mediated injury may be culpable for the dysfunction in distant organs seen with AKI.[157] Finally, AKI interferes with adequate dosing of immunosuppressants and may predispose to GVHD and rejection while complicating their treatment.[158]

Across modalities, AKI following HCT has consistently been linked with an increased risk of death on both short- and long-term follow-up. Overall mortality following AKI has been found to range from 4% to 61%.[34,64] In one of the earliest studies involving myeloablative allogeneic transplant, Zager et al. found an index hospitalization mortality of 17% in those without AKI, 37% in non–dialysis-requiring AKI, and a whopping 84% in dialysis-requiring AKI.[44] AKI has since been found to confer a significant independent risk for mortality in myeloablative allogeneic,[14,33,49,50,52,67] myeloablative autologous,[67,114] and RIC[25,28,38] transplants. The strength of this association has consistently been found to be dose dependent and contingent on the severity of the

AKI as well as the length of follow-up.[14,25,43,52,58,59,66,67,112,159] Increased mortality has been found at 100 days,[28,52,67] 6 months,[46,49] 1 year,[25,38] and 5 years.[26] Patients with AKI have significantly decreased 1-, 2-, and 3-year survival when stratified by AKIN stage.[66] Utilizing the Parikh scale, Yu et al. found 29% mortality without AKI, 57% with Grade 1. and 79% with Grade 2-3.[63] In a prospective study evaluating the RIFLE, AKIN, and Parikh diagnostic criteria in 249 patients receiving a mixture of myeloablative allogeneic, RIC, and myeloablative autologous transplants, the mortality rates at 1,000 days for patients with AKI were approximately 54% in myeloablative allogeneic, 48% to 50% in RIC, and 11% to 20% in autologous recipients.[39] The variance in mortality rates may be due to the previously noted different etiologies of AKI in these three modalities. A very significant stepwise increase in mortality hazard ratio was seen in all three classification systems. On multivariable analysis, RIFLE "I" and "F" were associated with overall nonrelapse mortality, HR 2.1 ($P = .01$) and 6.15 ($P < .01$). Finally, a meta-analysis of over 1,200 patients receiving a myeloablative allogeneic transplant revealed a doubling of the relative risk of death 6 months after AKI.[86] The adjusted odds of 6-month mortality were elevated nearly sevenfold. The outlook for patients requiring dialysis is especially grim, with mortality ranging from 75% to 100%.[10,25,38,43,44,49,51,54,58,60,62,68,112]

Significantly, AKI is an independent predictor of overall and nonrelapse 5-year mortality even in RIFLE stage "R," demonstrating that small changes in creatinine may be associated with long-term mortality.[26] Such a connection between mild AKI and mortality has also been demonstrated in cardiac surgery, acute myocardial infarction (MI), and the critically ill.[160–163]

AKI has been postulated to drive mortality by means of instigating HTN, CKD, and increased cardiovascular disease. Specific to HCT, AKI interferes with dosing of immunosuppressive medications, including calcineurin inhibitors, and may thus contribute to the development of cGVHD and resultant mortality.[155] The extent to which the association between AKI and long-term mortality is causal remains unknown.[164] Although it plausibly could be a direct mediator through the above mechanisms, AKI may associate with mortality merely as a surrogate for unappreciated or inadequately adjusted comorbidities. Additionally, many of the causes of AKI, including SOS, infection, and GVHD, independently lead to mortality.[97] In one study where AKI had a marked dose response association with mortality, correcting for aGVHD, SOS, TMA, and ICU admission abrogated the association.[49] However, another study showed AKI continuing to confer a robust OR of 6.8 for mortality even on a model adjusted for comorbidities and complications.[112]

Chronic Kidney Disease

The increased incidence of CKD following AKI has been well established.[162–165] The first report of CKD following HCT was in 1978.[166] Since then, estimates of the incidence of CKD in survivors of AKI have ranged from 3.6% to 89%.[153] The marked discrepancy in these estimates is due to variation in the definition of CKD, changes in conditioning regimens and posttransplant prophylactic and therapeutic medications, as well as evolving patient populations. Additionally, autologous, conventional allogeneic, and RIC transplants all confer different risks for AKI and their proportional representation among cohorts will influence the observed CKD rate. AKI following HCT has been identified as an independent risk factor in the development of CKD in multiple studies.[45,50,53,153,167,168] Indeed, in some pediatric populations, AKI has been identified as the only consistently significant risk factor for post-HCT CKD.[53,55] A systematic review by Ellis et al. identified 28 studies containing 5,337 patients who survived at least 100 days following HCT and who were evaluated for CKD.[169] With significant heterogeneity in definitions, 16.6% of all patients were reported to have developed CKD with 0.8% progressing to end-stage renal disease (ESRD). AKI following HCT was assessed as a risk factor for the development of CKD in four studies involving 2,038 patients. The cumulative OR for CKD after AKI was found to be 2.57. Worldwide, 5,100 post-HCT patients develop CKD annually whereas 300 progress to ESRD.[170] Survival in patients with HCT associated ESRD may be worse than in the general ESRD population.[171]

On systematic review, the incidence of CKD following allogeneic and autologous transplants is similar.[169] In addition to AKI, multiple risk factors for post-HCT CKD have been identified, although inconsistently, including age, prior transplant, aGVHD, cGVHD, calcineurin use >60 days, baseline GFR, microalbuminuria, multiple myeloma, and female gender.[31,32,153,167,169] Although TBI has often been found to be weakly, if at all, associated with CKD,[32,167,172,173] other studies have found a strong and indeed preeminent association.[169,174,175]

Post-HCT CKD can be secondary to glomerular (see later) or interstitial and vascular disease. Although many cases of interstitial CKD are readily attributable to TMA (often associated with TBI), from 17.5% in myeloablative to 66% in nonmyeloablative recipients have idiopathic disease.[32] This CKD is multifactorial but it seems likely that AKI plays a prominent role in its development. In a retrospective study of 123 patients receiving allogeneic transplants, CKD developed in 40% within 24 months.[167] The occurrence of AKI post-HCT conferred an OR of 4.54. Additional risk factors included age and baseline GFR <90 mL per minute. Patients who would and would not go on to develop CKD had a near identical drop in GFR at 3 months (28 vs. 26). However, by 24 months, GFR had dropped further to −36 mL per minute from baseline in CKD patients but rebounded to −17 mL per minute in those not destined for CKD. Only 3% of recipients developed CKD subsequent to 24 months. It may be that much of this "early" CKD is secondary to progression of AKI in patients with lower baseline GFR and less renal functional

reserve.[168] Indeed, nonrecovery of GFR by 100 days post-HCT has been documented even in mild AKI.[62]

Proteinuria

On systematic review, the rate of proteinuria following HCT is 7.8%[169] but was rarely reported. Thought to be a marker of endothelial dysfunction and inflammation, proteinuria post-HCT may reflect systemic endothelial injury. As such, proteinuria is intricately associated with SOS and GVHD. Hingorani et al. prospectively followed 142 patients receiving myeloablative allogeneic, RIC and autologous transplants.[31] Microalbuminuria was present in 37% at baseline and 64% at day 100, with an overall prevalence of 94% in the first 100 days post-HCT. Overt proteinuria was seen in 4% at baseline and in 15% by day 100. Mean proteinuria peaked at 35 days and declined at 100 days and again by 1 year. The development of SOS was associated with a markedly higher level of albuminuria in the first week post-HCT. Median albuminuria was significantly higher in allogeneic recipients than autologous at 35 and 100 days. Interestingly, albuminuria was not associated with the development of AKI but was, as a time-varying term, a risk factor for the development of aGVHD. On multivariable analysis, overt proteinuria at 100 days conferred an HR 2.4 for overall mortality and 6.8 for nonrelapse mortality.

The presence of albuminuria prior to the clinical presentation of aGVHD suggests it as an early marker of systemic and renal inflammation and endothelial damage. It also implies the kidney as a target of aGVHD. Such targeting is also insinuated by the association between SOS, strongly associated with aGVHD, and proteinuria. Both membranous nephropathy and minimal-change disease post-HCT are thought to be renal manifestations of GVHD. In minimal change, the lack of infiltrates on biopsy and the increased production of tumor necrosis factor-α and interferon-γ suggest cytokine mediated glomerular injury in response to extrarenal alloantigens.[176] In contrast, in membranous nephropathy, subepithelial immune complex deposition is present along the basement membrane, consisting of antigen-antibody complexes that resemble GVHD. GVHD may thus cause proteinuria via both inflammation and direct glomerular and tubular injury. The near universal incidence of microalbuminuria see by Hingorani and others[57] may thus suggest ubiquitous subclinical immune mediated kidney injury in HCT despite the observation that only 30% of patients in this study met clinical diagnostic criteria for AKI.

Frank nephrotic syndrome is rare after HCT, with an incidence of 0.4% to 0.8%.[96,177] The most common etiology, membranous nephropathy (MN), was first reported in 1989,[178] and has been documented in nearly 60 cases.[151] Although the antigenic target has yet to be elucidated, MN following HCT is intimately associated with cGVHD. Much like chronic allograft nephropathy, cGVHD is increasingly recognized to feature a role for B cells and antibodies in its pathophysiology, altering the classically understood T-cell centric paradigm.[45] Additional glomerulopathies reported after HCT include MCD, focal segmental glomerular sclerosis, membranoproliferative glomerulonephritis, diffuse proliferative glomerulonephritis, antineutrophil cytoplasmic antibody (ANCA)-associated GN, and IgA nephropathy.[151,179–186] Case reports suggest nephrotic syndrome associated with post-HCT MN may be responsive to treatment with angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs).[187]

Hypertension

HTN is an integral component of the classically described transplant nephropathy. Few prospective studies have evaluated the incidence of new-onset HTN following HCT. Hoffmeister et al. followed 689 pediatric patients receiving a mixture of myeloablative allogeneic, RIC, and autologous transplants.[188] Seventeen percent of recipients developed new-onset HTN during a median follow-up of 16 years. In those who experienced AKI within 100 days of transplant, HTN developed in 22% versus 14% in those without AKI. On multivariable analysis, the hazard ratio for HTN given post-HCT AKI was 2.53 ($P < .0001$). Additional risk factors for the development of HTN were high dose TBI, autologous transplant, and time-dependent obesity, diabetes, and growth hormone usage. Overall rates of HTN on follow-up were found to be two to three times higher than the age appropriate population prevalence. In 73 autologous recipients, 72% developed new-onset or worsening of prevalent HTN at 1 year post-HCT. HTN rates were higher in those patients that had developed CKD. Although HTN was also associated with calcineurin inhibitor use, elevated blood pressure persisted in 64% of hypertensive patients 6 months after stopping calcineurin inhibitors.[153] A systematic review showed HTN developing in 108/873 (12.4%) patients across modalities but was unable to assess risk factors or the time frame of HTN onset in HCT recipients.[169]

Conclusion

AKI is remarkably prevalent following all modalities of HCT and is independently associated with mortality. The etiology is multifactorial and varies by conditioning regimen. Although the incidence of AKI has fallen over the past two decades,[12] it remains one of the most common and devastating complications of HCT. Given the data on biomarkers for tubular damage, it is likely that the incidence of subclinical injury following HCT far outstrips even the remarkable rates noted in studies to date. With the survival rate following HCT improving, the importance of the long-term effects associated with peritransplant complications such as AKI will become increasingly preeminent. The relationship between even mild AKI and both CKD and mortality is now well established.[189] It is possible that strategies to minimize AKI post-HCT, while improving short-term outcomes, may also mitigate the subsequent incidence of these devastating long-term sequelae.

ACUTE KIDNEY INJURY FOLLOWING SEVERE BURNS

In the United States, approximately 45,000 people are hospitalized annually for burn injuries.[190,191] The prognosis for these patients depends substantially on the extent of their burns. Although burn patients are increasingly receiving specialized care in dedicated burn units, their hospitalizations continue to be associated with significant complications resulting in a mean length of stay of 9 days and mean hospital costs of $60,000. One of the most devastating of these complications is AKI. Indeed, it was not until 1965 that a patient was reported to survive the combined insults of a severe burn and AKI.[192] As seen in general critical care, AKI following burns is often the heralding sign of impending multiorgan failure. The impact of severe AKI requiring renal replacement therapy (RRT) on postburn outcomes has long been recognized but only recently has the significant sequelae of milder disease been appreciated. Over the past 20 years, survival rates following severe burns have improved modestly associated with evolving critical care techniques and burn specific therapeutic protocols,[193,194] but the diagnosis of AKI complicating a severe burn continues to portend a grim prognosis. For the purposes of this review, severe burns will be understood to involve at least 10% of total body surface area (TBSA), with more stringent requirements present as noted.

Epidemiology and Risk Factors

Estimates of the incidence of AKI following severe burns vary widely. A portion of this variance can be attributed to differences amongst the literature in what constitutes a "severe" burn and to heterogeneous patient populations, types of burns, and ranges of TBSA percentage burns included in different studies. However, the primary source of the discordant findings in the epidemiology of AKI following burns has been the lack of a standardized definition for AKI. A recent systematic review identified 57 pediatric and adult studies providing data on the prevalence and mortality of AKI in patients with severe burn injuries, 9 of which were prospective.[195] An astounding 23 different definitions of AKI were used, illustrating the difficulty in comparing results across the literature. Definitions were founded on fixed serum creatinine cutoffs, relative increases in creatinine, urine output, blood urea nitrogen (BUN) levels, and need for RRT.

In this review, a total of 34,868 patients were evaluated across studies. In those studies permitting assessment of AKI prevalence, 1,872/34,771 (5.4%) patients developed AKI. The prevalence of AKI varied considerably and ranged from 0.2% to 64%. Unsurprisingly, AKI rates are highly contingent on diagnostic criteria, ranging from 34.9% in those studies utilizing a fixed serum creatinine cutoff to 3.5% in those identifying AKI based on urine output. When employing the sensitive RIFLE criteria, AKI was seen in 608/2,111 (30.9%) of patients versus 334/9,443 (17%) where AKI was more stringently defined by a requirement for RRT. It is likely that even those studies utilizing a definition involving relative changes in serum creatinine, such as the RIFLE system, may actually underestimate the true prevalence of AKI. Major burn patients are often initially resuscitated at an outside hospital prior to transferring to a specialized burn center and patients' initial presenting creatinine may thus already be elevated from their true baseline. Overall, the median prevalence was noted to be 14.5%, which is lower than that seen in ICU populations at large.[196,197] This discrepancy may be due to burn patients often being younger and with fewer comorbidities than are present in the general ICU population.

Across studies, RRT was performed in 30% of AKI patients or 3% of the total population with burn injuries.[195] This is slightly less than seen in studies of general ICU populations. This lower utilization of RRT may again reflect younger, healthier patients who are better able to withstand AKI without progressing to a requirement for RRT. However, the mortality of burn patients on RRT (see below) is worse than even the dreadful rates of 50% to 70% witnessed in general ICUs,[196–199] indicating the extreme severity of these patients' critical illness. It is thus possible that lower prevalence of RRT use in burn patients may alternatively be related to withholding of treatment for perceived therapeutic futility. Finally, difficulties in obtaining access or concerns of possible catheter associated infection may induce a reluctance to initiate RRT in patients with severe burns.

Utilizing a Pearson correlation test, Brusselaers et al. found the prevalence of AKI has increased over time ($r = 0.31$; $P = .02$) but the prevalence of AKI requiring RRT has declined ($r = 0.36$; $P = .05$).[195] This discrepancy can be explained by changes in the sensitivity and specificity of AKI definitions concurrent with improvements in critical care techniques and protocols for the treatment of burn victims.

The primary risk factor for the development of AKI following a severe burn is the size of the burn itself, as expressed in TBSA percentage.[200–204] Greater burn size predisposes to greater fluid and albumin loss as well as increasing the likelihood of infection. The incidence of AKI begins to increase with burns of >10% TBSA.[200] AKI is associated with burn size in a dose-dependent manner, with each 10% increase conferring an increased risk of death up until approximately 60%, when mortality levels off, albeit at a level of close to 50%.[200] A burn size of >65% of TBSA is associated with RR for AKI of 9.9 compared to <65% on multivariable analysis.[203]

Additional risk factors noted in the literature include sepsis,[190,202,204,205] inhalation injury (IHI),[190,200,204] age,[201,205,206,207] overall severity of critical illness or multiorgan dysfunction (acute physiology and chronic health evaluation II [APACHE II], pediatric risk of mortality [PRISMA]),[204,206–208] catheter infections,[190] adequacy of preadmission fluid resuscitation,[205] percentage of full thickness burns (FTB),[201] and admission thrombocytopenia and elevated bilirubin.[201] Interestingly, whereas Steinvall et al. noted age, TBSA percentage, and extent of FTB to be associated with

the development of AKI, these factors did not correspond to severity by RIFLE stages.[201]

ETIOLOGIES

Early Acute Kidney Injury

Renal Hypoperfusion

AKI associated with burns is bimodal, occurring either within the first 7 days after injury or during the second to third week. Causes of AKI in these early and later periods are listed in Table 38.7. Burns lead to an intense inflammatory response and release of vasoactive agents, increasing capillary permeability to fluid and albumin.[209] Albumin exhibits a significant leak within 6 to 18 hours postburn.[210] As compared to other critically ill patients, burn victims experience inflammation of a greater intensity and significantly longer duration.[211] The resulting loss of oncotic pressure is compounded by a decrease in albumin synthesis after severe burns.[212] As a result, fluid is shifted from the intravascular to extravascular space. Additionally, compromise of the barrier integrity of the skin engenders massive insensible fluid losses. The resulting intravascular hypovolemia and subsequent renal hypoperfusion are the primary cause of early AKI. Severe burns with extensive tissue destruction also decrease cardiac inotropism via endotoxin mediated apoptosis[213] while simultaneously elevating local and systemic stress-related hormones such as catecholamines, angiotensin II, aldosterone, and vasopressin with resulting systemic and renal vasoconstriction, a condition known as burn shock.[200,202,203,214–216]

These physiologic responses to a severe burn occur within hours and thus compel rapid and aggressive fluid resuscitation to prevent renal hypoperfusion and resulting oliguria. Indeed, a delay in adequate fluid resuscitation has been associated with poor prognosis in burn associated AKI.[217,218] Fluid requirements are contingent on the patient's initial weight, burn size, and need for mechanical ventilation.[193] With recognition of the importance of timely resuscitation and the adaptation of aggressive, standardized protocols[219,220] early AKI following severe burns has become less frequent.[200] In modern studies, however, there is not always an association between resuscitation volume and the development of AKI.[205] It may be that once a critical volume threshold is reached, no further benefit may be realized as etiologies other than hypovolemia come to predominate. Indeed, administration of fluid >25% more than that predicted as appropriate by the Parkland burn formula has been associated with increased odds of pneumonia, acute respiratory distress syndrome (ARDS), multisystem organ failure (MOF), and death.[221]

38.7 Common Causes of Acute Kidney Injury Following Severe Burns

Early
Volume depletion/hypoperfusion
Inhalation injury
Rhabdomyolysis/hemolysis
Late
Sepsis
Abdominal compartment syndrome
Nephrotoxic medications
Hypercalcemia

Inhalation Injury

Early AKI is often preceded by or concurrent with systemic inflammatory response syndrome (SIRS) and respiratory disease.[201] Pulmonary injury associated with trauma or burns promotes pathogenic systemic inflammation and the development of MOF, including AKI.[222] The extravasation of intravascular fluid initiated by the burn may be potentiated by inflammation triggered by IHI. Supporting this, burn victims with IHI have been documented as having significantly higher fluid and sodium replacement requirements than those without IHI.[223] IHI associated respiratory failure results in hypoxia and acidemia, further compromising vascular tone. Renal dysfunction is more common among patients with the most severe respiratory dysfunction.[224] Prevalence of inhalation injury is higher and the number of days on mechanical ventilation is greater in pediatric burn patients with AKI than in those without.[204]

Rhabdomyolysis/Hemolysis

Rhabdomyolysis (RML) and hemolysis can result in AKI via the release of free hemoglobin and myoglobin. Both molecules are freely filtered by the glomerulus, absorbed by tubular epithelial cells, and degraded into heme and globin, respectively. Each of these pigment molecules can be directly toxic to tubular cells via generation of oxygen free radicals as well as causing cast precipitation and tubular obstruction. AKI is associated with RML in one third of patients with myoglobinuria due to this oxidative stress and myoglobin cast nephropathy.[225,226] In patients with RML, myoglobinuria is strongly associated with the development of AKI, with an area under the receiver operating curve (AUC) of 0.88 and an optimal cutoff of 3865 ng per mL.[227] In the early period following a severe burn, patients are at substantial risk for AKI secondary to RML and, to a lesser degree, hemolysis.

RML is most frequently associated with electrical burns.[228–230] However, RML can also follow deep flame burns which produce direct myocyte damage or else induce limb ischemia and secondary RML through circumferential burns resulting in eschar formation and compartment syndrome.[231] Burn victims are at additional risk for RML due to prolonged immobilization, IHI, sepsis, pharmacologic

agents, obesity, and large surgical surface areas, which promote the destruction of striated muscle cells.[206,232] Once present, the nephrotoxic effects of RML are potentiated by such frequent comorbidities of burns as acidosis, intravascular volume depletion, and oliguria.[202]

In a retrospective study of 714 burn victims, RML, diagnosed by serum creatinine phosphokinase (CPK) and myoglobin levels as well as the documented presence of pigmented urine, was noted in 8/714 (1%) of patients,[206] six of whom (75%) developed AKI. Although serum CPK levels were similar between those with and without AKI, myoglobin trended toward being higher in the AKI patients, 10,600 μg per L versus 4989 μg per L, but the results were not statistically significant. While RML can develop several weeks after a severe burn, it is a significantly more common cause of AKI in the early period. Patients with AKI due to RML are more likely to present with early injury, 12/16 (75%), than those with AKI secondary to other causes, 31/77 (40%), and RML induced AKI requires RRT more frequently 9/16 (56%) than other AKI etiologies 23/77 (30%).[233] In a study of 48 patients with AKI requiring RRT, myoglobinuria occurred significantly more frequently in those with AKI <5 days postburn than those with later injury ($P < .0001$).[200] Although not statistically significant, Steinvall et al. examined 31 patients with burn-associated AKI and found mean myoglobin levels were higher in those with early AKI as opposed to late, 1,167 μg per L versus 220 μg per L.[201]

Outcomes following RML are better after electric burn than flame due to the severity of burn required to cause RML with thermal injury.[233] Early escharotomies and fasciotomies so as to minimize tissue ischemia are key to reducing the incidence of RML.[233] Although of unproven benefit, urine alkalinization and forced diuresis using lactated Ringer's (LR) solution, sodium bicarbonate, and mannitol or furosemide is typically employed. Myoglobin has a molecular weight of 17 kD and can theoretically be eliminated by ultrafiltration but the half-life of systemic myoglobin has not been shown to differ in burn-associated AKI patients treated with continuous venovenous hemodiafiltration (CVVHDF) as compared to LR alone.[206,234]

Proteinuria

Burn shock associated with sepsis may incite lesions in the glomerulus and tubules and is often associated with high and low molecular weight proteinuria.[235] Plasma from septic burn victims with AKI induces a pro-apoptotic effect in tubular cells and podocytes that correlates with the extent of proteinuria. Specifically, alteration of polarity in tubular cells and reduced expression of megalin, nephrin, and the tight junction protein ZO-1 have been demonstrated.[236]

The development of proteinuria may be a more sensitive indicator of kidney injury than definitions predicated on serum creatinine. Kang et al. evaluated 24-hour urine protein excretion daily for 3 days and then weekly for 3 weeks in 12 patients following a severe burn injury.[237] None of the patients experienced a rise in serum creatinine or a decrease in urine output. Mean proteinuria rose from 139 mg per 24 hours on day 0 to 835 mg per 24 hours by day 3 and remained above baseline at 3 weeks with the majority of protein being nonalbumin. Additional confirmation of tubular injury was found by a sustained elevation in N-acetyl-β-D-glucosaminidase (NAG), a specific marker of renal tubular pathology.[238] Sabry evaluated 40 patients with TBSA burns >20%, nine (22.5%) of whom developed AKI.[202] Although serum creatinine in those patients with AKI was not significantly higher than in those without AKI until hospital day 7, the patients with AKI were found to have significantly higher microalbuminuria at admission. It is unclear if this is an example of microalbuminuria being an early biomarker of tubular injury or if these patients had microalbuminuria at baseline and hence were at higher risk for AKI.

Late Acute Kidney Injury

Patients who develop AKI in the later period following burns are younger with lower TBSA percentage burns and less FTB.[201] Due to the profound and prolonged inflammation engendered by burns, patients 2 to 3 weeks postinsult continue to express elevated cytokines, including TNF-α and IL-1, with resulting vasoconstriction and renal hypoperfusion. These ongoing physiologic derangements render them highly susceptible to the predominant renal insults, sepsis, and nephrotoxic medications, encountered in this period. The resulting development of ATN compromises the kidney's ability to retain sodium and concentrate urine and thus perpetuates intravascular volume depletion. With improved resuscitation protocols having minimized hypoperfusion associated early AKI, late-onset injury now predominates.[200] The factors influencing the development of late AKI are shown in Figure 38.2.

Sepsis

The primary causes of late AKI following severe burns are sepsis and associated MOF.[200,203,217,218,239] Sepsis has been noted in 64% of late-onset AKI compared to 28% in early AKI[233] although such a disparity is not always seen.[201] Holm et al. found sepsis to be present in 30/33 (91%) of burn victims whose AKI developed >5 days postinjury but in 0/15 of those with AKI within the first 5 days.[200] Despite the extensive use of topical and systemic antibiotics, the nidus of infection in postburn sepsis remains the burn itself. However, as seen in ICU populations at large, impairment of the GI mucosal barrier with subsequent bacterial translocation and endotoxemia may be an additional contributor to late onset sepsis after burns.

The onset of sepsis in a burn patient is an ominous development. In a study of 76 patients with AKI, sepsis was seen in 96% of nonsurvivors versus 44% of survivors ($P < 0.001$).[217] However, not all episodes of severe sepsis cause AKI and the chronology is not always consistent. Among survivors of AKI, Chrysoloulo et al. found kidney injury often preceded clinical evidence of sepsis, rather than following

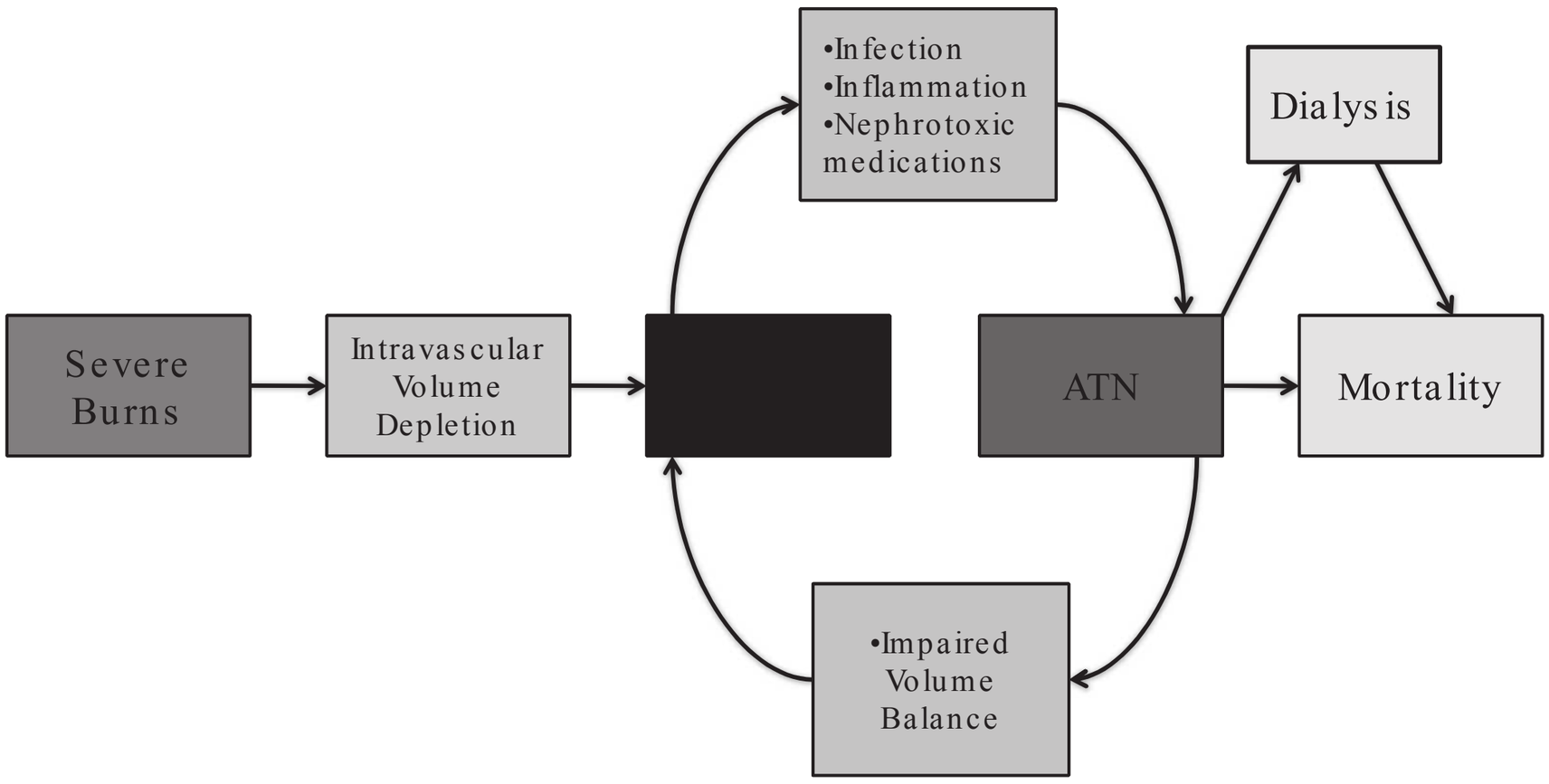


FIGURE 38.2 Progression of late kidney injury after severe burns. AKI, acute kidney injury; ATN, acute tubular necrosis.

it.[217] Remarkably, all diagnoses of sepsis occurred after onset of AKI in survivors whereas nonsurvivors were stricken both before and after inception of AKI. Taken together, these findings suggest that when AKI follows sepsis, it is part of MOF and results in the expected devastating mortality. The mere development of sepsis in the setting of prevalent AKI, however, allows for the possibility that the AKI's initial etiology was primarily hemodynamic and thus may be associated with more favorable outcomes.

Abdominal Compartment Syndrome

Aggressive volume resuscitation, although minimizing early intravascular hypovolemia, hypotension, and renal hypoperfusion, may ultimately lead to a significantly positive fluid balance. In the setting of increased capillary permeability and subsequent fluid translocation, excessive extravascular volume puts patients at risk for intra-abdominal hypertension and abdominal compartment syndrome.[240–242] First reported following a burn in 1994,[243] abdominal compartment syndrome has been diagnosed in 40% of patients meeting RIFLE "F,"[204] although the temporal relationship between intra-abdominal hypertension and AKI is not always clear. Abdominal compartment syndrome impairs cardiac function, reduces pulmonary compliance, and truncates renal perfusion.[243–245] In a study of 123 burn victims, 9/56 (16%) of those with AKI were diagnosed with compartment syndrome whereas none of the 67 without AKI developed this complication.[204] Surgical decompression has been shown to improve APACHE scores and increase urine output in post-burn compartment syndrome.[244]

Nephrotoxic Medications

Nephrotoxic medications, particularly antibiotics, are well-known risk factors for AKI. The use of potentially nephrotoxic antibiotics has been reported as nearly ubiquitous in late onset AKI after severe burns.[200,233] Additionally, in patients diagnosed with AKI by RIFLE criteria, use of known nephrotoxic medications was much more prevalent in those patients who ultimately progressed to a higher stage of AKI severity (86%) than in those who did not (27%) ($P = .02$).[208] However, the concurrent presence of sepsis in nearly all patients on antibiotics is an obvious potential confounder and confirmation of the independent role of nephrotoxic medications in the development of late AKI awaits future controlled studies. The use of nephrotoxic antibiotics has also been reported in up to 57% of early AKI cases but the drugs were only initiated 1 to 3 days prior to occurrence of AKI and thus cannot be said to be causal.[233]

Hypercalcemia

Burn injuries and their management frequently result in hypercalcemia. Kohut et al. found hypercalcemia in 22/73 (30%) of patients with TSBA >20% burns and/or ICU stay >20 days.[246] Elevation of calcium in this setting is multifactorial but primarily related to prolonged immobility, with subsequent increase in bone resorption, and high protein feeds stimulating calcium mobilization and hypercalciuria.[247] Hypercalcemia leads to renal hypoperfusion both through direct vascular action causing renal vasoconstriction and by stimulation of natriuresis and aquaresis. Treatment of hypercalcemia involves aggressive hydration, volume expansion, and early mobilization. Bisphosphonates have also proven successful in preserving bone mass following severe burns.[248]

Treatment

Volume Resuscitation

The critical impact of volume depletion on outcomes following severe burns has long been recognized.[249] Immediate and aggressive fluid resuscitation so as to maintain circulating

blood volume and ensure adequate tissue perfusion and oxygenation is a critical component of the acute treatment of burn patients. The time between burn injury and fluid resuscitation is significantly less in survivors than nonsurvivors.[217] Of multiple proposed resuscitation formulas,[219,220] the Parkland formula[250] is one of the most widely utilized. This protocol calls for the administration of LR at 4 mL/kg/TBSA percentage burned, half to be given in the first 8 hours and the remainder over the next 16 hours. Fluids are then adjusted to maintain urine output of 0.5 to 1.0 mL/kg/hr. Resuscitation guided by the Parkland formula has been shown to result in less volume administered without difference in cardiac output parameters than that guided by pulmonary artery catheter.[251] Colloid solutions have increasingly fallen out of favor as they have not been shown superior to saline in general ICU patients[252] and a decrease in GFR following colloid administration has been observed in burn victims.[253]

Early wound excision and grafting may temporize the inflammatory response and subsequent release of inflammatory mediators, thereby improving renal blood flow and mitigating AKI. However, the effect of early excision on mortality has been variable.[217,218]

Vitamin C

Vitamin C has been utilized following thermal injury due to its antioxidant properties. Activation of mast cells is instigated by burns, resulting in histamine release, increased xanthine oxidase activity, and resultant free radical production.[254] By serving as a free radical scavenger, vitamin C may reduce postinjury membrane lipid peroxidation and attenuate vascular permeability.[255] Concerns have been expressed about the potential of vitamin C to initiate an osmotic diuresis and worsen renal injury. However, addition of vitamin C to resuscitation fluids has been shown to reduce fluid requirements, minimize weight gain, and lessen wound edema.[256] Patients receiving vitamin C experienced fewer ventilator days and demonstrated improved PaO2:FiO2 ratios.[256] Kahn et al. prospectively compared conventional LR resuscitation and LR with vitamin C at 66 mg/kg/hr.[257] The vitamin C group displayed a decreased fluid requirement but increased urine output. Although no difference was noted in mortality, the study involved only 33 patients and was likely underpowered for such hard outcomes.

Fenoldopam

Fenoldopam, a highly selective dopamine-1 receptor agonist, decreases renal vascular resistance, increases glomerular filtration rate (GFR), stimulates natriuresis through inhibition of the Na/H exchanger and Na/K/ATPase mediated sodium reabsorption, and facilitates aquaresis via antagonism of antidiuretic hormone (ADH).[258] Fenoldopam administration has been associated with increased cortical and medullary renal blood flow via nonnitric oxidase mediated arterial dilitation.[259,260] In a retrospective study of 758 patients admitted to an intensive care burn unit, 76 diagnosed with AKI on AKIN criteria were treated with fenoldopam.[258] Serum creatinine improved within 48 hours across all AKIN stages and urine output increased without adjustment in fluid administration in AKIN stages 2 and 3. The study, however, was not powered to assess RRT requirement, length of stay, or mortality.

Outcomes

Progression/Resource Utilization

Palmieri et al. evaluated the prognostic ability of the RIFLE criteria in 60 patients, 32 of whom (53%) were diagnosed with AKI.[208] Following initial fulfillment of criteria, 13 patients subsequently progressed over a mean of 9 days to a higher class. Mortality was 0% in those without AKI, 8% in those who did not progress beyond their initial stage ("R" or "I"), and 46% in those who progressed to a higher class. Progression was not associated with age, comorbidities, or severity of illness scores at admission but rather factors arising subsequent to admission such as sepsis, use of nephrotoxic drugs, cumulative fluid balance, and number of surgeries.

Evaluating the impact of AKI on length of hospital stay is difficult due to the competing risk of death associated with AKI. Nonetheless, significantly longer ICU,[200,204,208] and overall hospital[201,204] stays have been noted in patients with AKI. Similarly, the number of required ventilator days has also been demonstrated to be higher with AKI[200,204,208] and to increase in a stepwise manner with AKI severity.[200]

Dialysis

Although the first report of survival in a burn victim with AKI requiring RRT was in 1965,[192] the subsequent 20 years continued to produce very poor outcomes. Burn patients typically have massive obligatory fluid input in the service of maintaining hemodynamics, providing parenteral nutrition, and administering medications. Given these patients' low oncotic pressure and increased capillary permeability, much of this fluid is extravasated into third spaces, resulting in anasarca and placing patients at risk for impaired wound healing, skin breakdown, and pulmonary edema. During the period preceding the mid-1980s, the only treatment option was standard intermittent hemodialysis, which typically was employed daily in an attempt at aggressive ultrafiltration. With sepsis often compounding patients' hypotension and intravascular volume depletion, intermittent intense fluid removal was often impossible and outcomes were very poor. In the mid-1980s, continuous arteriovenous, followed by continuous venovenous, methods of RRT were introduced and are now preeminent in ICUs. Continuous RRT offers the advantage of gentle yet large volume ultrafiltration, allowing for aggressive nutritional support. The physiology of burn patients makes such techniques an especially appealing option in this setting.

Multiple venovenous therapeutic modalities (hemodialysis, hemofiltration, hemodiafiltration) have been successfully employed in patients with AKI and severe burns.[261] In a review of 16 studies, the incidence of renal replacement therapy after severe burns ranged from 0.7% to 14.6%.[201] A 10-year experience at a large burn unit showed 1% of all admissions, and 2.7% of patients with TBSA >10% burns, required RRT.[239] A systemic review found RRT was performed in 30% of AKI patients or 3% of the total population with burn injuries.[195] RRT is typically initiated approximately 2 weeks after hospital admission, consistent with the period of late onset sepsis/MOF associated AKI.[201,239,261,262] Reported mean duration of treatment has ranged from 10 to 24 days.[201,239,261,262]

Early studies reported a significantly higher rate of bleeding complications in burn patients receiving CRRT than in general ICU CRRT patients, 56% versus 15% (P = .002).[261] This increased risk may have resulted from the interaction of anticoagulation and the frequent thrombocytopenia and consumptive coagulopathy seen following burns. The patients in this study received a mixture of arteriovenous and venovenous dialysis. When the same investigators assessed a later cohort using only venovenous techniques, no bleeding complications were noted.[262] Encouragingly, survival improved from 18% in the first time period (1987–1994) to 50% in the second (1995–1998) within the same institution.

Inpatient Mortality

Prior to the widespread availability of RRT, the survival rate in patients with severe burns and AKI was abysmal. Of 119 patients in the literature published between 1953 and 1979, only 8/119 (7%) survived.[216] In the ensuing years, outcomes have improved considerably but the diagnosis of AKI following a severe burn still portends a grim prognosis. Analyzing data culled from nearly 30 years of experience at a single center, Jeschke et al. identified 60 children who had experienced AKI following a severe burn. Dividing the patients into those presenting before and after 1984, they found that the mortality rate had decreased from 100% to 56%.[218]

On systematic review, mortality rates vary by AKI definition, ranging from a median of 35% in studies utilizing RIFLE criteria to 88% in three older studies where AKI status was determined by elevations in BUN levels.[195] The overall mean mortality in severely burned patients with AKI was 55%, with a median across studies of 77%. The median mortality in burn patients not afflicted with AKI was 13%. In the 30 studies with data available on control populations, AKI was clearly associated with an increased risk for mortality with an RR of 4.85. An increased risk of mortality has been documented with both early and late AKI. Retrospectively examining 62 patients meeting RIFLE criteria within 24 hours of admission, Mosier et al. found hospital mortality significantly elevated compared to burn patients without early AKI, 36% versus 13% (adjusted OR 2.32).[205] Forty-seven out the remaining 159 study patients (30%) developed AKI later in their hospital stay with a mortality of 16/47 (34%). As compared to mortality in burn victims who would be discharged without AKI, 6/112 (5%), late AKI conferred an unadjusted OR for death of 10.9.

The RIFLE criteria have been shown to provide incremental prognostic value regarding mortality in several settings of AKI.[263–267] In eight studies examining the utility of the RIFLE criteria following severe burns, mean mortality across categories was 42% with a median of 35%.[195] Mortality increased in a stepwise manner, both across studies with fixed serum creatinine cutoffs as well as across RIFLE stages.[195,201,204] Although the RR for mortality with AKI was 6.17 in those studies employing the RIFLE criteria, there was not a statistically significant increase in mortality for patients in the "R" category.[195] However, in 62 patients who developed RIFLE "R" or "I" within 24 hours of admission, 18 of whom progressed from their initial RIFLE stage to a more advanced one, Mosier et al. found mortality was significantly elevated in those who progressed versus those who stabilized or improved, 72% versus 20%.[205]

Despite refinements in continuous RRT techniques, the outlook for patients requiring dialysis remains harrowing and the need for dialysis is the strongest predictor of death in burn victims with AKI.[233] In a study of 1,360 burn patients admitted over 13 years to an ICU, mortality was found to be 6.9% in patients without AKI, 34.4% with AKI without RRT, and 62.5% in patients requiring dialysis.[233] Mortality in burn patients requiring RRT ranges across reported studies from 63% to 100%.[200,203 205,233,261,268–270] The median mortality in studies defining AKI by the need for RRT is 80%.[195]

Although need for RRT is the dominant risk factor for death in burn patients with AKI, multiple additional associations been reported including sepsis/MOF,[205,208,217] TBSA%,[190,233] RIFLE "F,"[190,208] age,[233] IHI,[190] serum albumin on presentation,[203] and SOFA score.[208] Burn size >65% TBSA confers a dramatic RR of 14.2 on multivariable analysis for mortality.[203] In 76 diagnosed with AKI, Chrysopoulo et al. noted 64/67 (94%) nonsurvivors as compared to 4/9 (44%) survivors were diagnosed with sepsis (P < .001).[217] As with AKI at large,[271] the development of oligo/anuria following a severe burn is predictive of worse outcomes. Mustonen et al. examined 32 patients requiring RRT.[233] Although 20/20 (100%) nonsurvivors were oliguric and 18/20 (90%) were anuric, only 6/12 (50%) survivors were oliguric and 2/12 anuric (17%). Mortality rates have alternatively been reported as higher in early AKI[200,233] and in late AKI[217] with another study finding them nearly identical.[205] With early AKI having larger TBSA percentage burns and higher rates of RML whereas late AKI involves more prevalent comorbidities and sepsis, both findings are plausible and the question awaits resolution in larger, multicenter studies.

It is interesting to note that in several studies only more severe AKI as evidenced by RIFLE "I" or "F" is associated with increased mortality.[190] This finding stands in clear distinction to that seen in multiple other populations where even

minor AKI confers an elevated risk for mortality.[163,272–275] It may be that other risk factors for mortality in burns such as TBSA percentage burned and IHI are so overwhelmingly impactful that the association between milder forms of AKI and death is overshadowed. Similarly, although TBSA percentage burned is an extremely strong predictor of mortality in multivariable models, once AKI develops, survival is similar across ranges of TBSA percentage burns. Especially in the late period, AKI is often associated with sepsis and MOF. It is possible that once such devastating complications have arisen, the power of TBSA percentage burned as a predictor of mortality is overwhelmed.

Encouragingly, there has been a strong trend toward decreasing mortality overall ($r = -0.5$; $P < .001$) and in those patients requiring RRT ($r = -0.6$; $P = .001$).[195] No prospective studies have evaluated long-term proteinuria, hypertension, or renal function in this patient population. Full inpatient renal recovery, variably defined, has been reported in multiple studies for many if not all survivors of burn associated AKI.[201,261,262] However, evidence is now overwhelming that even mild AKI is associated with increased risk for long-term CKD.[276–278] In light of this data, such optimistic findings seem premature and the long-term renal outcomes in survivors of postburn AKI await eliciting in future longitudinal studies.

Conclusion

AKI following burns is highly prevalent and conveys a grim prognosis. As the etiology is frequently multifactorial, interventions to reduce the incidence and severity of AKI must necessarily involve multiple targets. The prognosis following the onset of AKI has improved, even for those patients requiring dialysis, but remains poor. It remains to be seen whether the continued application and refinement of specialized burn therapies and RRT techniques will appreciably impact prevalence and mortality. Early, aggressive fluid resuscitation, control of infection, timely excision of eschars, and minimizing nephrotoxic medications remain the cornerstones of prevention for this devastating complication of an already horrific injury.

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CHAPTER 39

Role of the Kidneys in Hypertension

Joey P. Granger • Eric M. George

Over 40 years ago Guyton and Coleman[1–3] proposed that if an increase in arterial pressure could produce sustained elevations in sodium and water excretion through a mechanism of renal pressure natriuresis and diuresis, then this system would have a near infinite gain for the long-term control of arterial pressure by regulating blood volume. Thus, whenever arterial pressure is elevated, renal arterial pressure would enhance the excretion of sodium and water until blood volume is reduced sufficiently to return arterial pressure to control values. According to the renal–body fluid system concept, hypertension can develop only when something impairs the excretory ability of the kidney and shifts the relation between sodium excretion and arterial pressure toward higher levels.[1–4] Although there is strong theoretical and experimental evidence that the kidney is a major determinant of the long-term control of arterial pressure, the initial abnormality of the kidney need not be intrinsic to the development of hypertension.[4–6] A shift of pressure natriuresis can occur as a result of intrarenal abnormalities such as enhanced formation of angiotensin II or genetic defects that enhance renal sodium transport mechanisms. In other instances, the altered kidney function is caused by extrarenal disturbances, such as increased sympathetic nervous system activity or excessive formation of antinatriuretic hormones such as aldosterone.[4–6]

RENAL-BODY FLUID FEEDBACK SYSTEM FOR LONG-TERM BLOOD PRESSURE REGULATION

According to the renal–body fluid feedback mechanism for long-term control of arterial pressure, extracellular fluid volume is determined by the balance between intake and excretion of salt and water by the kidneys (Fig. 39.1). A temporary higher intake than output would lead to an increase in extracellular volume and arterial pressure. If the excretory ability of the kidney is not impaired, the increase in arterial pressure raises sodium excretion and extracellular fluid volume would then decrease, thereby reducing venous return and cardiac output until blood pressure returns to normal and fluid intake and output are reestablished. Conversely, when sodium output exceeds intake and extracellular fluid volume and blood pressure fall below normal, the kidneys retain sodium and water until arterial pressure is restored to the normal set-point. Thus, according to the renal–body fluid feedback mechanism concept, the set-point for long-term blood pressure control is the arterial pressure at which sodium and water intake and output are at equilibrium.[4–7]

A key component of this mechanism for regulating salt and water balance is pressure natriuresis/diuresis, which is the effect of increased arterial pressure to raise sodium and water excretion. An important feature of pressure natriuresis is that various hormonal and neural control systems can amplify or blunt the pressure natriuresis mechanism.[5,7,9–11] For example, in most individuals, chronic increases in sodium intake are associated with only small changes in arterial pressure. The lack of significant increases in arterial pressure in response to elevations in sodium intake results in decreased formation of antinatriuretic hormones and/or increased formation of natriuretic factors, which enhance the effectiveness of pressure natriuresis and allow sodium balance to be maintained with little or no increase in arterial pressure. On the other hand, excessive activation of antinatriuretic systems or abnormalities in natriuretic systems can reduce the effectiveness of pressure natriuresis, thereby necessitating greater increases in arterial pressure to maintain sodium and water balance. Thus, excessive activation of antinatriuretic systems or abnormalities in natriuretic systems impairs the excretory ability of the kidney and shifts the relation between sodium excretion and arterial pressure toward higher levels and resets the set-point for long-term blood pressure control (Fig. 39.2).

Although total peripheral resistance and cardiac output are determinants of arterial pressure, one prediction of the renal–body fluid feedback mechanism is that if the pressure natriuresis mechanism is not impaired, a primary increase in total peripheral resistance or increases in cardiac pumping ability would not result in long-term alterations in arterial pressure.[2–6] For instance, an increase in total peripheral resistance would result in an immediate elevation in arterial

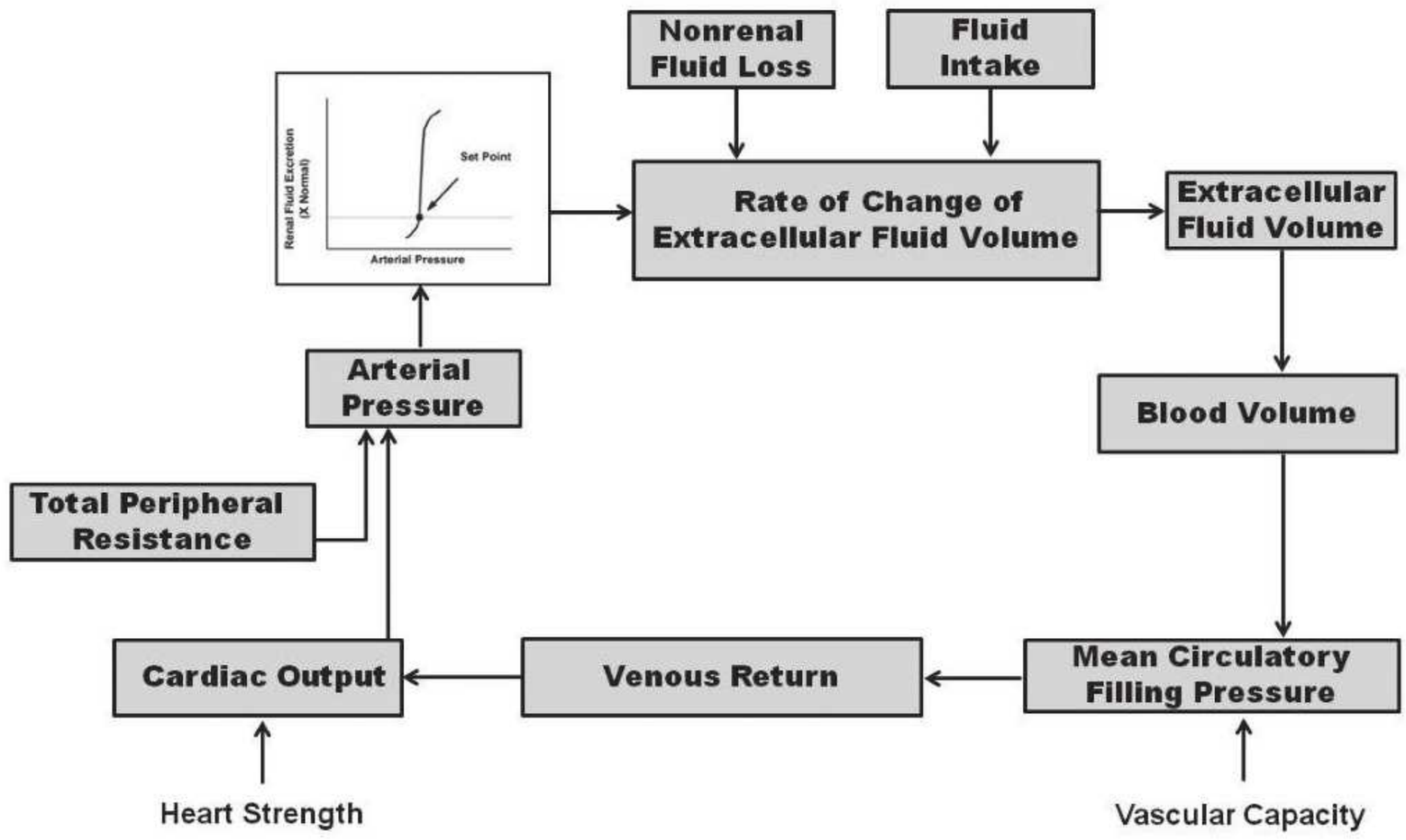

FIGURE 39.1 Basic renal–body fluid feedback mechanism for long-term regulation of blood pressure and body fluid volumes. (Redrawn from Granger JP, Hall JE. Role of the kidney sodium and fluid excretion in hypertension. In: Lip GYP, Hall JE, eds. *Comprehensive Hypertension*. New York: Elsevier; 2007.)

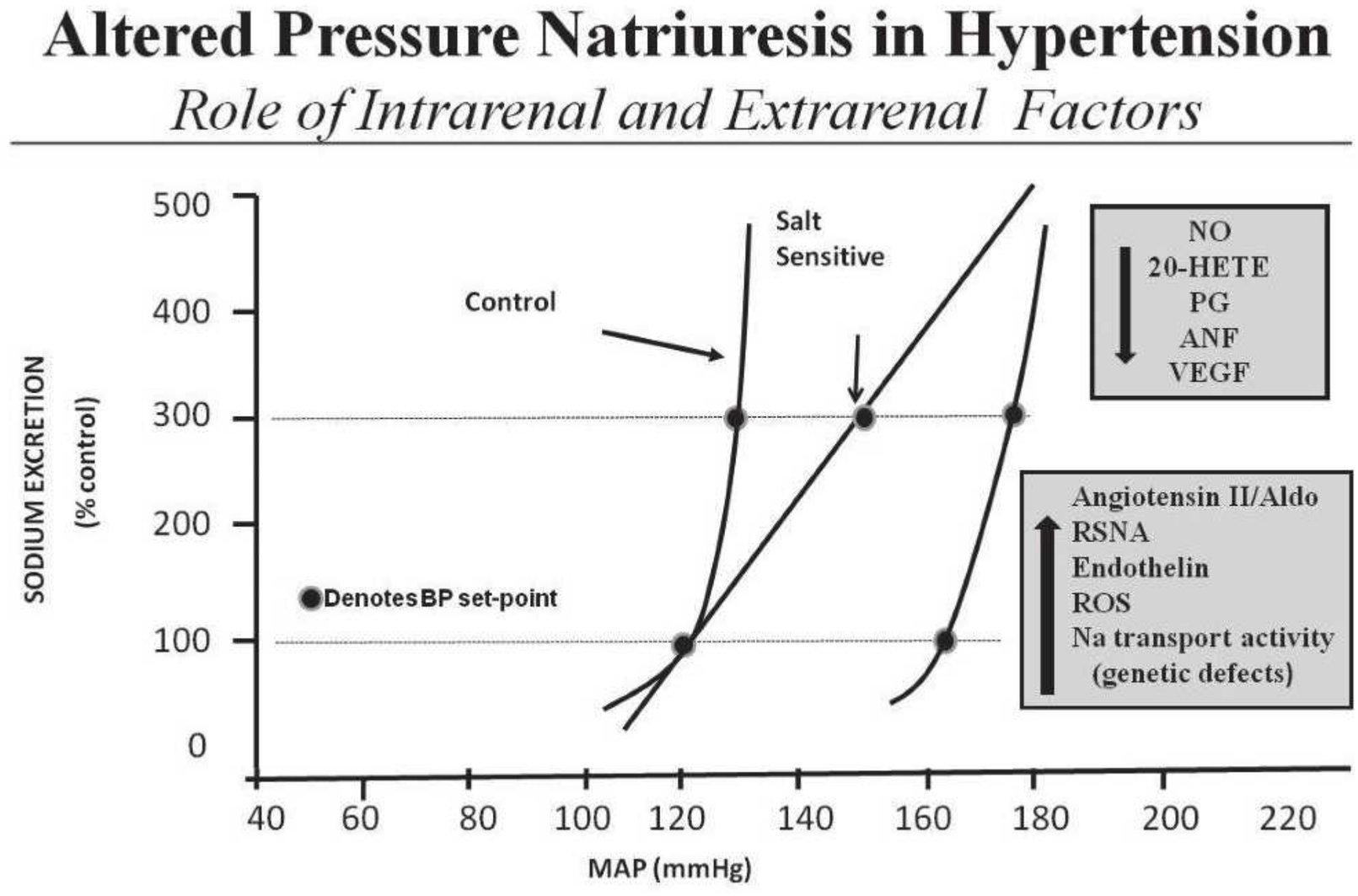

FIGURE 39.2 Steady-state relationships between arterial pressure and urinary sodium excretion and sodium intake for control subjects with normal kidneys and for subjects with a rightward hypertensive shift in the pressure-natriuresis relationship. A shift in the pressure natriuresis relationship can occur as a result of *intrarenal* abnormalities such as enhanced formation of angiotensin II (ANGII), reactive oxygen species (ROS), inflammatory cytokines and endothelin (via ET_A receptor activation), or decreased synthesis of nitric oxide (NO), natriuretic prostanoids (PG), vascular endothelial growth factor (VEGF), or even genetic defects that enhance renal sodium transport mechanisms. In other instances, the altered kidney function is caused by *extrarenal* disturbances, such as increased sympathetic nervous system (SNS) activity or excessive formation of antinatriuretic hormones such as aldosterone (Aldo) or decreased atrial natriuretic peptide (ANP).

pressure. The increase in arterial pressure would increase sodium and water excretion, via pressure natriuresis. As long as fluid excretion exceeds fluid intake, extracellular fluid volume will continue to decrease, reducing venous return and cardiac output, until blood pressure (BP) returns to normal and fluid balance is reestablished. Thus, according to the renal–body fluid volume control system concept, primary increases in total peripheral resistance or increases in cardiac pumping do not result in long-term alterations in arterial pressure and hypertension can develop only when something impairs the excretory ability of the kidney and shifts the relation between sodium excretion and arterial pressure toward higher levels.[2–6]

Although hypertension is a result of a reduction in the kidney's ability to excrete sodium and water (reduced pressure natriuresis), the hypertension may not necessarily be associated with increases in extracellular fluid volume. Indeed, many forms of hypertension are associated with increased total peripheral resistance and reduced rather than increased extracellular fluid volume. This occurs when a vasoconstrictor, such as norepinephrine, has a potent extrarenal vasoconstrictor effect to increase total peripheral resistance but a relatively weak antinatriuretic action. The antinatriuretic effect of norepinephrine shifts pressure natriuresis and the set-point for arterial pressure to a higher level. However, because norepinephrine has a more powerful extrarenal vasoconstrictor effect, arterial pressure initially increases above the renal set-point for sodium balance and causes transient natriuresis and decrease in extracellular fluid volume. Eventually arterial pressure stabilizes at a point where sodium intake and output are balanced. Thus, sodium retaining actions of norepinephrine are masked by peripheral vasoconstriction which raises BP above the renal set-point at which sodium balance is maintained causing increased renal excretion and decreased extracellular fluid volume. It is important to reiterate that the maintenance of high blood pressure chronically depends on norepinephrine's renal actions because norepinephrine's extrarenal effects to increase total peripheral resistance would in itself, as pointed out previously, only result in a transient increase in blood pressure.

PRESSURE NATRIURESIS: A KEY FACTOR IN MAINTAINING SODIUM BALANCE IN HYPERTENSION

Another important prediction of the renal–body fluid feedback control system concept is that an increase in BP in hypertensive states is an essential compensatory mechanism that allows sodium balance to be maintained in the face of an underlying sodium retaining defect.[5–7] The importance of the pressure-natriuresis mechanism in the regulation of sodium excretion can best be illustrated under conditions in which a sodium-retaining abnormality exists within the kidney and an increase in arterial pressure occurs to compensate for this abnormality. Such a condition is illustrated in the hypertension caused by aldosterone excess. To determine the importance of the pressure-natriuresis mechanism in achieving sodium balance during aldosterone-induced hypertension, Hall and colleagues[12] examined the long-term effects of aldosterone on sodium excretion and arterial pressure in normal dogs and in dogs in which renal artery pressure was prevented from increasing with an electronically servocontrolled aortic occluder. In dogs in which renal artery pressure was permitted to increase during chronic aldosterone infusion, sodium excretion decreased markedly on the first day and then returned to control levels on days 2 to 3 of aldosterone infusion as arterial pressure increased (Fig. 39.3). In contrast, in dogs in which renal artery pressure was prevented from increasing, sodium excretion decreased on the first day and remained below sodium intake for the 7 days of aldosterone infusion. The sustained sodium retention resulted in dramatic increases in cumulative sodium balance and systemic arterial pressure. The results from this study clearly demonstrated that an increase in renal arterial pressure is essential in allowing the kidneys to escape from the chronic sodium-retaining actions

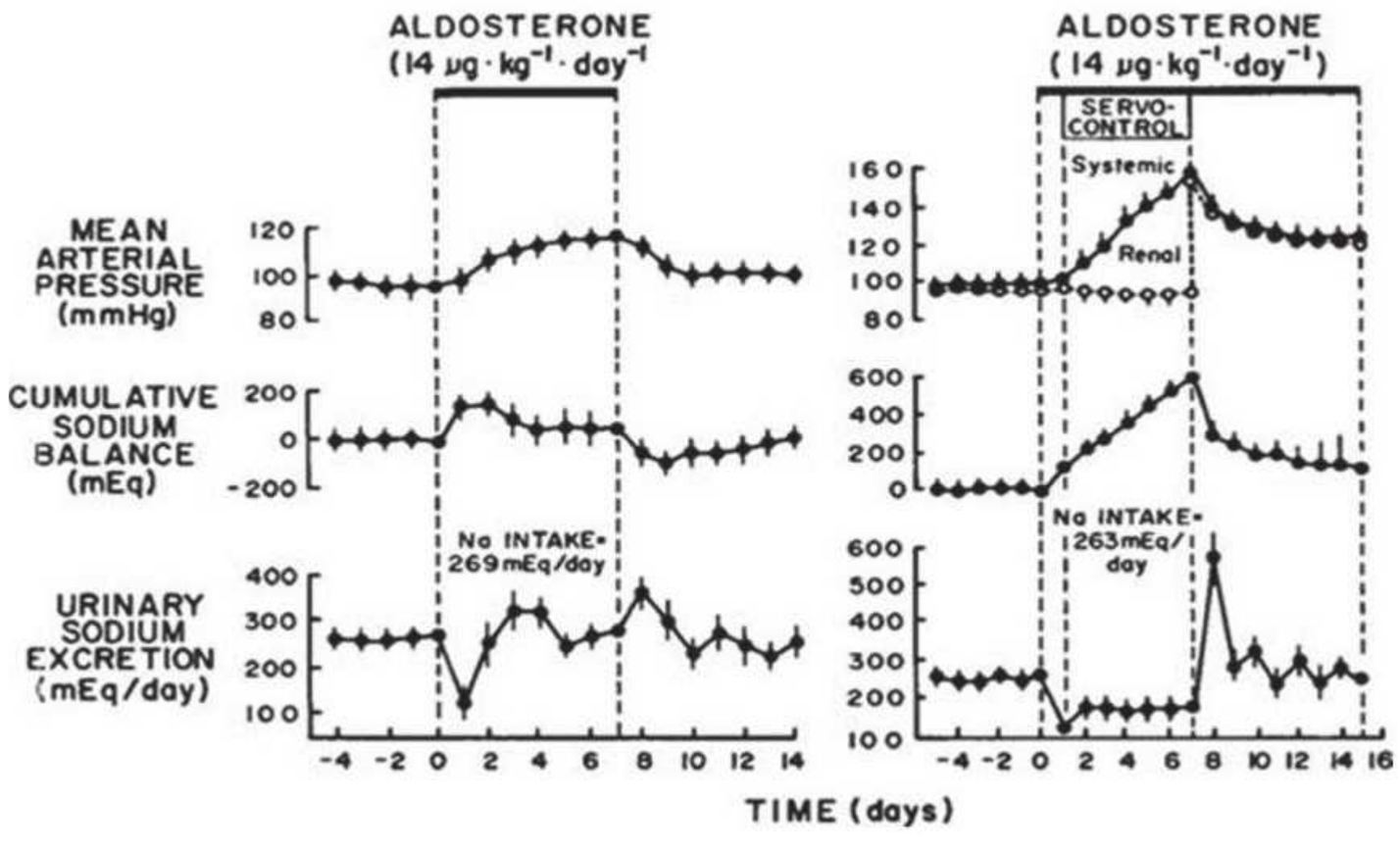

FIGURE 39.3 Effects of chronic aldosterone infusion on sodium excretion when renal perfusion pressure was allowed to increase (*left panel*) and was servo-controlled (*right panel*). Notice that when renal perfusion pressure was prevented from increasing, "escape" from sodium retention did not occur and cumulative sodium balance and systemic arterial pressure continued to increase. (Redrawn from Hall JE, Granger JP, Smith MJ, et al. Role of renal hemodynamics and arterial pressure in aldosterone "escape." *Hypertension*. 1984, 6(suppl I): I-183–I-192.)

of aldosterone and to achieve normal sodium balance. Similar findings were reported from the same group during chronic administration of other sodium-retaining hormones, such as angiotensin II and norepinephrine.[13,14] Thus, it appears that during pathophysiologic conditions associated with excess levels of sodium-retaining hormones, such as aldosterone or angiotensin II, the pressure-natriuresis mechanism is important for the maintenance of sodium balance.[15,16]

MECHANISM UNDERLYING RENAL PRESSURE NATRIURESIS

The ability of the kidney to alter urine flow and sodium excretion in response to acute changes in renal perfusion pressure has been of interest to investigators for many years.[17–19] Changes in sodium excretion in response to changes in renal perfusion pressure are thought to be due to alterations in tubular reabsorption of sodium, because glomerular filtration rate (GFR) and the filtered load of sodium are usually well autoregulated.[20] Thus, a decrease in renal perfusion pressure results in an increase in tubular reabsorption of sodium and a decrease in sodium excretion. In contrast, increases in renal perfusion pressure lead to decreases in tubular reabsorption of sodium and increases in sodium excretion, a phenomenon commonly referred to as pressure natriuresis.

The specific intrarenal mechanism responsible for the decrease in tubular reabsorption in response to increases in renal perfusion pressure appears to be related to increases in hemodynamic factors such as medullary blood flow and renal interstitial hydrostatic pressure (Fig. 39.4).[20–25] The mechanism whereby renal interstitial hydrostatic pressure (RIHP) increases in the absence of discernible changes in whole kidney renal blood flow and peritubular capillary hydrostatic and/or oncotic pressures may be related to increases in renal medullary flow as a result of nitric oxide-induced reductions in renal medullary vascular resistance.[26] Preventing RIHP from increasing in response to increases in renal perfusion pressure markedly attenuates pressure natriuresis.[24] Furthermore, direct increases in RIHP, comparable to increases measured in response to increases in renal perfusion pressure, have been shown to significantly decrease tubular reabsorption of sodium in the proximal tubule and increase sodium excretion.[20–24] The exact mechanism whereby RIHP influences tubular reabsorption is unknown but may be related to alterations in tight junction permeability to sodium in proximal tubules, redistribution of apical sodium transporters, and/or release of renal autacoids such as prostaglandin E2.[27–31]

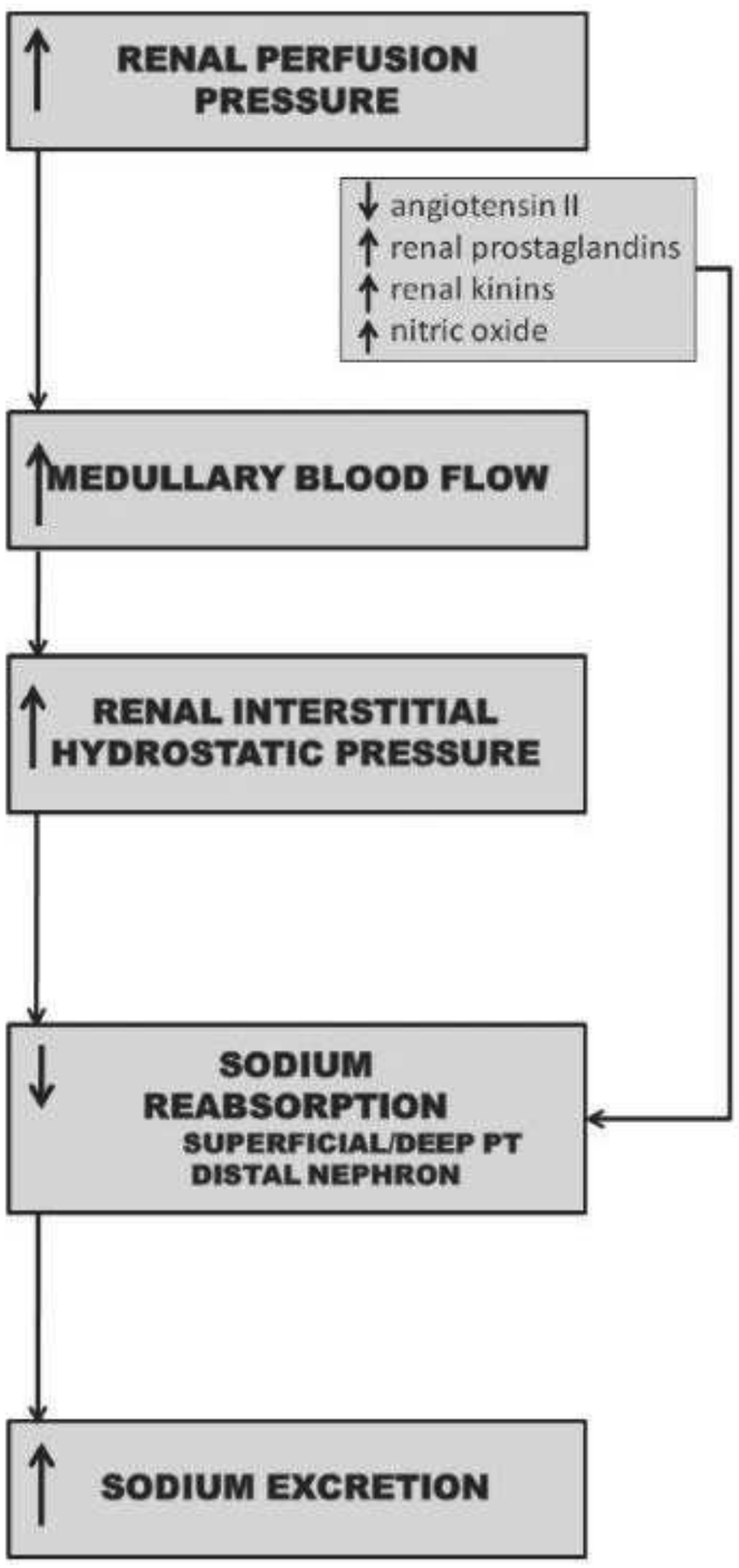

FIGURE 39.4 Mechanisms whereby increases in renal perfusion pressure enhance sodium excretion.

MECHANISMS OF IMPAIRED RENAL-PRESSURE NATRIURESIS IN HYPERTENSION

Several lines of evidence support an important role for the kidneys in the development and maintenance of hypertension. Some of the strongest evidence for a key role of the kidneys in hypertension derives from renal cross-transplantation studies.[4] Transplantation of a kidney from a hypertensive donor into a normotensive recipient has been demonstrated to produce sustained elevations in arterial pressure in numerous genetic models of hypertension (e.g., SHR, Dahl).[32] Of particular relevance to human hypertension is the study of Curtis et al.[33] demonstrating that BP returns to normal levels in hypertensive patients who were recipients of kidneys from normotensive donors. Another observation that points toward abnormal kidney function as a key factor in causing hypertension is that almost all forms of experimental hypertension are caused by perturbations to the kidneys that alter renal hemodynamics or tubular reabsorption and reduce the kidney's ability to excrete sodium and water. For example, constriction of the renal arteries (e.g., Goldblatt hypertension), compression of the kidneys (e.g., perinephritic hypertension), or administration of sodium-retaining hormones (e.g., ANG II) are all associated with either initial reductions in renal blood flow and GFR or increases in renal tubular reabsorption prior to development of hypertension.[7,34] Further evidence

supporting an important role for the kidneys in the development and maintenance of hypertension is that in all known monogenic forms of human hypertension, the common pathway to hypertension appears to be increased renal tubular reabsorption caused by mutations that directly increase renal electrolyte transport (e.g., Liddle or Gordon syndromes) or the synthesis and/or activity of antinatriuretic hormones (e.g., glucocorticoid remediable aldosteronism).[8]

Although specific abnormalities of kidney function are difficult to identify in most patients with primary hypertension, the one aspect of kidney function that is abnormal in all types of experimental and clinical hypertension is renal pressure natriuresis.[4,5,7,15,16,34] A shift in the pressure natriuresis relationship can occur as a result of intrarenal abnormalities such as enhanced formation of angiotensin II, reactive oxygen species (ROS), inflammatory cytokines, and endothelin (via ET_A receptor activation) or decreased synthesis of nitric oxide and natriuretic prostanoids or even genetic defects that enhance renal sodium transport mechanisms (Fig. 39.2). In other instances, the altered kidney function is caused by extrarenal disturbances, such as increased sympathetic nervous system (SNS) activity or excessive formation of antinatriuretic hormones such as aldosterone. The remaining portion of this chapter discusses how these intra- and extrarenal factors impair renal pressure natriuresis and lead to chronic hypertension.

The Renin-Angiotensin System

The renin-angiotensin system (RAS) plays a critical role in the long-term regulation of blood pressure and is involved in the pathogenesis of various forms of hypertension including renovascular hypertension and human essential hypertension.[10,15,34] Although the RAS has many components, its most important effects on renal hemodynamics and sodium reabsorption are exerted by angiotensin II (ANGII) via an ANGII type 1 (AT1) receptor (Fig. 39.5). ANGII also acts on ANGII type 2 receptors to cause renal vasodilation and natriuresis; however, the relative importance of this antihypertensive pathway is equivocal.[35–37] More recently, fragments of ANGII such as ANG 1–7 have also been suggested to have physiologic effects within the kidney, often opposing the actions of ANGII.[35–37] An isoform of angiotensin-converting enzyme (ACE), known as ACE2, appears to be involved in the formation of angiotensin 1–7.[35–37] Although experimental findings on Ang 1–7 are equivocal, ANG 1–7 has been shown to induce renal vasodilation, an effect that is thought to occur independently of binding to AT_1 or AT_2 receptors

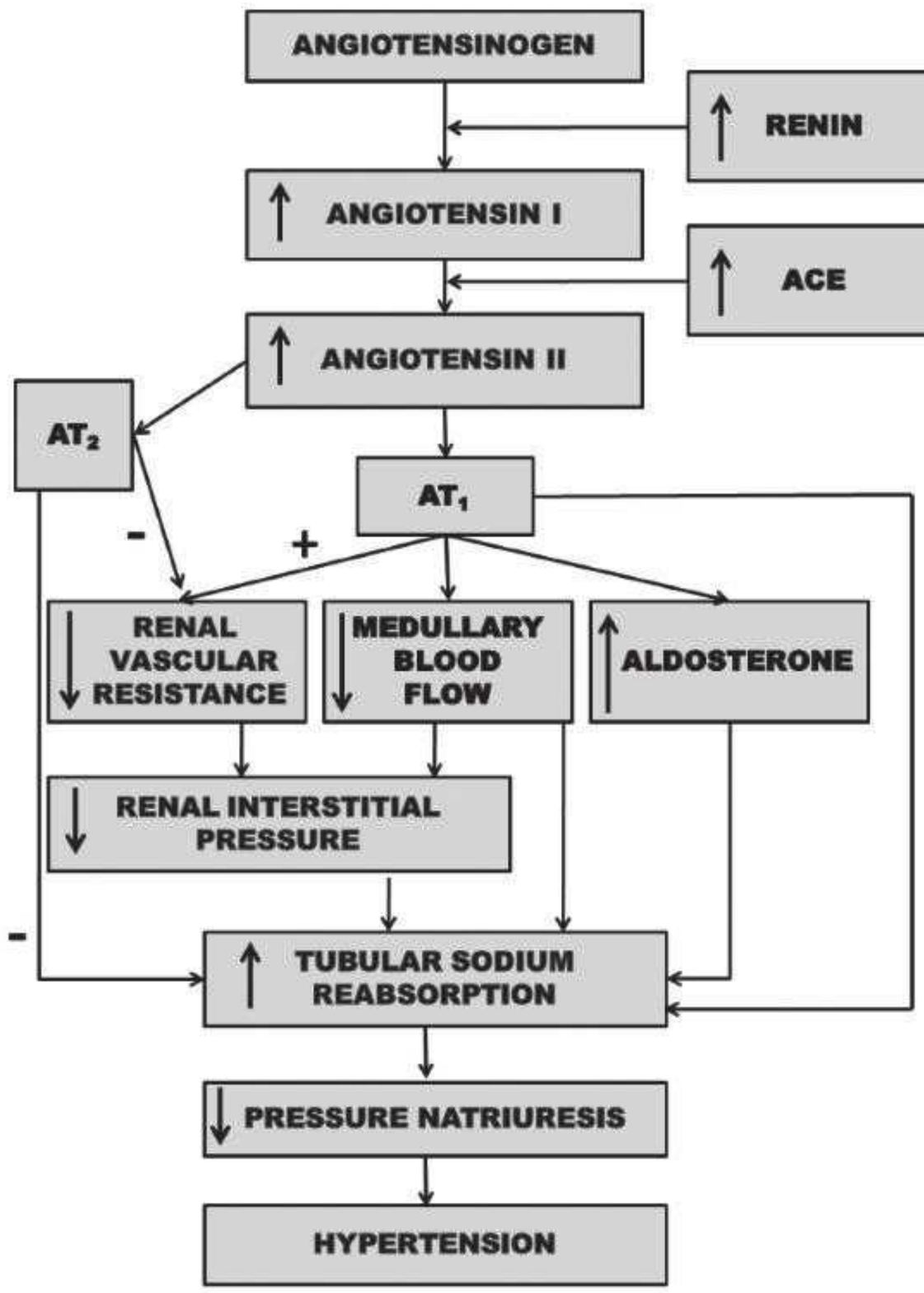

FIGURE 39.5 Renal mechanism whereby activation of the renin-angiotensin system reduces pressure natriuresis relationship and leads to hypertension.

but rather through a G protein–coupled Mas receptor.[35–37] Although ANG 1–7, ACE2, and the Mas receptor have all been detected within the kidney and an imbalance of these peptides, enzymes, or receptors have been reported to occur in various cardiovascular and renal diseases, the physiologic and pathophysiologic importance of ANG 1–7 has yet to be fully elucidated.[35–37]

The RAS, via AT1 receptor, plays an important role in maintaining sodium balance and a relatively normal pressure as sodium intake is altered from low to high levels.[9] As sodium intake is increased to high levels, ANGII levels are suppressed allowing sodium excretion to increase to match sodium intake. Conversely, when sodium intake is restricted ANGII levels increase and sodium excretion is reduced to match the low sodium intake. Thus, with a fully functional RAS, sodium balance is maintained in response to changes in sodium intake without the need to invoke significant changes in blood pressure. An inability to suppress the RAS in response to increases in sodium intake could be one potential mechanism for salt-sensitive hypertension in humans.[7,10]

The importance of the RAS in controlling blood pressure during changes in sodium intake is highlighted by the study of Hall and colleagues[9] where they reported that blockade of the RAS, with ANGII receptor blockers (ARB) or ACE inhibitors, increases renal excretory capability so that sodium balance can be maintained at reduced BPs (Fig. 39.6). However, blockade of the RAS also reduces the slope of pressure natriuresis and makes BP salt-sensitive.[9] Inappropriately high levels of Ang II reduce renal excretory capability and impair pressure natriuresis, thereby reducing the slope and necessitating increased blood pressure to maintain sodium balance.

The effect of ANGII to reduce renal pressure natriuresis and cause hypertension is the result of its effects to directly or indirectly stimulate sodium transport (Fig. 39.5). Infusion of a physiologic dose of ANGII is usually accompanied by an increase in renal vascular resistance and filtration fraction which favors tubular reabsorption.[10,38,39] In addition, acute administration of an ANGII antagonist or converting enzyme inhibitor under conditions where BP is not dramatically reduced results in a natriuresis that is associated with increases in renal blood flow and a reduction in filtration fraction.[10] ANGII has been shown to reduce peritubular capillary pressure, whereas converting enzyme inhibition increases peritubular capillary and renal interstitial hydrostatic pressure.[10] Thus, alterations in Starling forces across the peritubular capillaries provide an important mechanism whereby the RAS affects the tubular reabsorption of sodium.

Alterations in medullary blood flow may be another renal hemodynamic mechanism whereby ANGII could influence tubular reabsorption of sodium.[7,10,40] Ang II-induced constriction of efferent arterioles of the juxtamedullary nephrons or pericytes of descending vasa recta would decrease inner medullary blood flow. This, in turn, would enhance sodium reabsorption by increasing medullary interstitial tonicity. In support of this mechanism are studies demonstrating that ANGII, at a dose that does not alter whole-kidney GFR or renal blood flow, markedly reduces medullary blood flow and sodium excretion.[10] Studies by Cowley and associates have also shown that inner medullary blood flow is reduced in sodium-depleted dogs, a condition associated with enhanced activity of the RAS.[40] Moreover, ANGII blockade has been shown to improve medullary blood flow and enhance pressure natriuresis in various animal models of hypertension.[7,10,40]

Experimental evidence also suggests an important direct tubular action of ANGII.[10,38,39] Various studies suggest that increased proximal tubule transport in response to physiologic concentrations of ANGII is mediated by changes in HCO_{3-} coupled with Na transport.[38] This effect of ANGII has been attributed to activation of Na/H exchange. In addition to a proximal tubule effect on sodium transport, evidence also indicates ANGII may have direct actions on more distal parts of the nephron.[10,38,39]

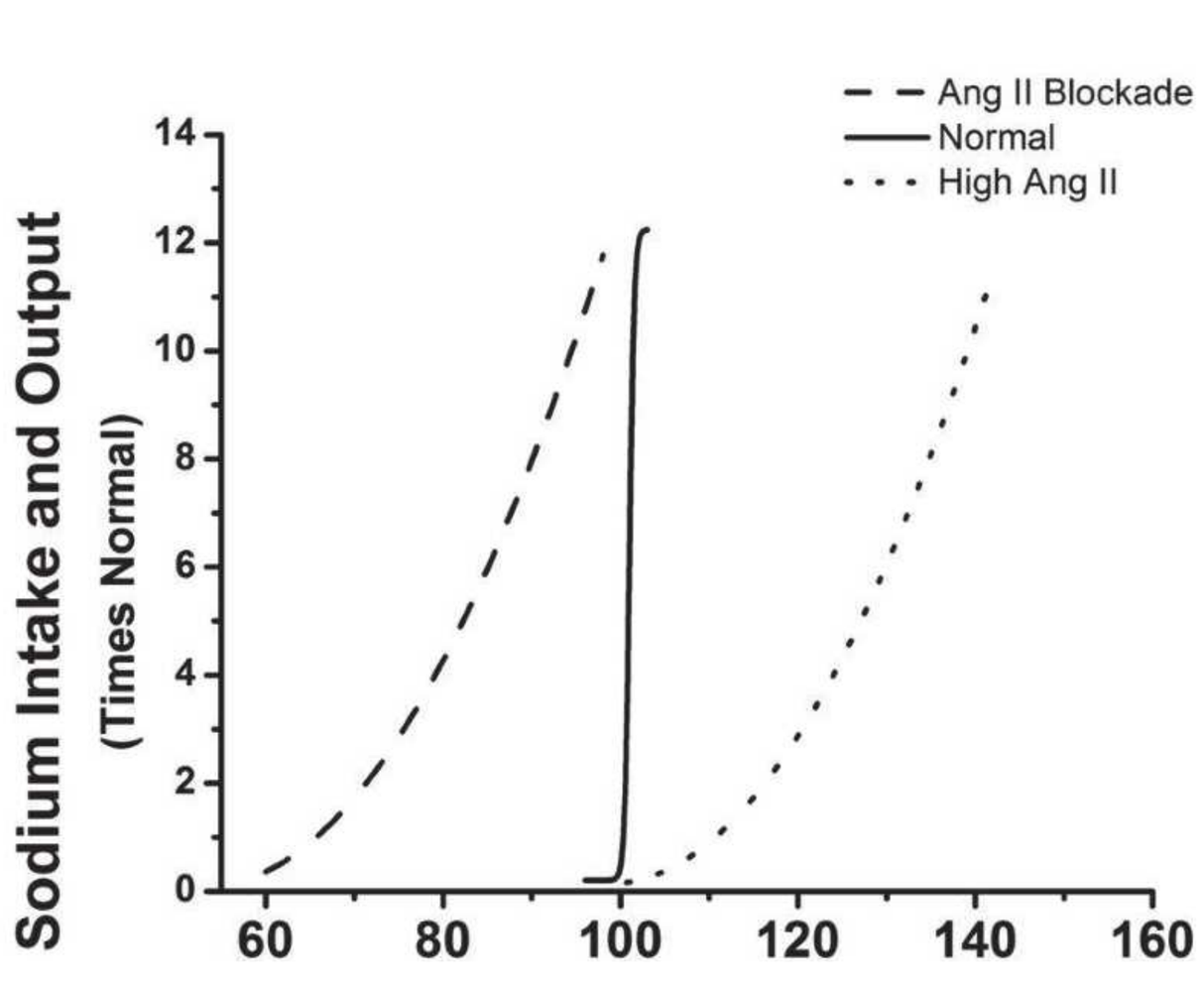

FIGURE 39.6 Effect of changes in mean arterial pressure during chronic changes in sodium intake after angiotensin-converting enzyme (ACE) inhibition, or when angiotensin II was infused at a constant low dose (5 ng/kg/min) to prevent angiotensin II from being suppressed when sodium intake was raised. (Redrawn from data in Hall JE, Guyton AC, Smith MJ Jr, et al. Blood pressure and renal function during chronic changes in sodium intake: Role of angiotensin. *Am J Physiol.* 1980;239:F271–F280.)

Although AT1 receptors are prominently expressed in the kidney, they are also expressed in the heart, blood vessels, adrenal glands, and the brain.[10] Because AT1 receptors are ubiquitously expressed, dissecting the quantitative importance of each individual organ system, including the kidney, in the long-term regulation of blood pressure has been difficult. Utilizing a combined gene targeting with renal cross transplantation approach, Coffman and colleagues examined the role of AT1 receptors in the kidney and their contribution to the development of ANGII-induced hypertension.[41–44] They found that ANG II causes hypertension primarily through effects on AT1 receptors in the kidney associated with reduced urinary sodium excretion, independent of actions of the sympathetic nervous system or aldosterone. When AT1 receptors are eliminated from the kidney, the extrarenal AT1 receptors are not sufficient to induce hypertension (Fig. 39.7). Thus, despite the fact that ANGII activates extrarenal vascular receptors and increases total peripheral resistance, when AT1 receptors are eliminated from the kidney, ANGII does not alter the pressure natriuresis relationship or increase arterial pressure.

More recently Coffman and colleagues reported that abrogation of AT1 receptors in the proximal tubule alone reduces proximal fluid reabsorption, alters expression of key sodium transporters, improves pressure-natriuresis, and significantly attenuates ANGII hypertension.[42] Collectively, the findings of Coffman and colleagues highlight the critical role of the kidney in the pathogenesis of ANGII-dependent hypertension. In addition, they suggest that the major mechanism of action of RAS inhibitors in hypertension is attenuation of ANG II effects in the kidney.

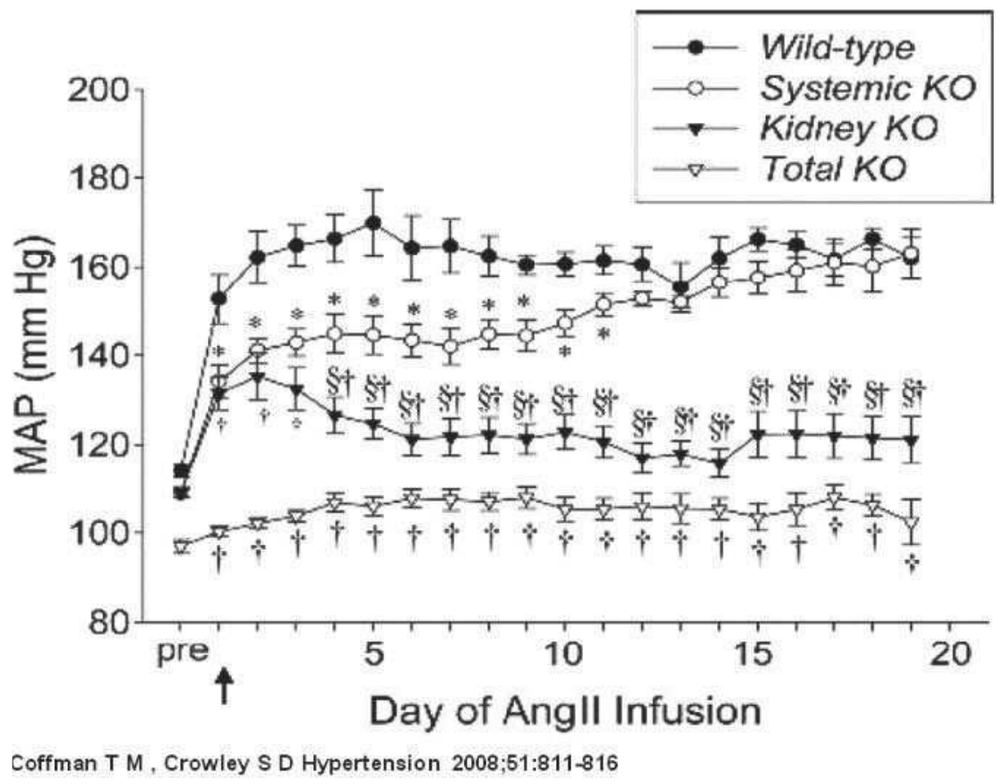

FIGURE 39.7 Blood pressures in cross-transplanted mice during 21 days of AngII infusion. Blood pressure response to AngII in systemic knockout (KO) recapitulates that of the wild-type group by day 12 of AngII infusion. Absence of renal AT_{1A} receptors in kidney KO animals ameliorates AngII–induced hypertension. Total KO blood pressure shows minimal response to AngII infusion. (Coffman TM, Crowley SD. Kidney in hypertension: Guyton redux. *Hypertension*. 2008;51(4):811–816.)

Aldosterone

Aldosterone also plays an important role in the chronic regulation of BP via its sodium-retaining actions on the kidney.[10,45–47] Aldosterone alters the renal pressure natriuresis relationship by enhancing sodium transport in the distal tubules and cortical collecting ducts. The sodium retaining effect of aldosterone is due to binding of aldosterone to the intracellular mineralocorticoid receptor and activation of transcription by target genes. These target genes, in turn, stimulate synthesis or activation of the sodium-potassium ATP-ase pump on the basolateral epithelial membrane and activation of amiloride-sensitive sodium channels on the luminal side of the epithelial membrane.[47,48]

As sodium intake is increased to high levels, aldosterone levels are suppressed allowing sodium excretion to increase to match sodium intake. Conversely, when sodium intake is restricted aldosterone levels increase and sodium excretion is reduced to match the low sodium intake. Thus, a change in aldosterone production in response to changes in sodium intake is another important hormone in the maintenance of sodium balance. An inability to suppress aldosterone production in response to increases in sodium intake therefore is another potential mechanism for salt-sensitive hypertension in humans.[46] In addition to primary hyperaldosteronism, excess activation of the mineralocorticoid receptor by aldosterone has also been implicated in several forms of human hypertension including renovascular hypertension, patients with resistant hypertension, and obesity-related hypertension.[46–51]

The Sympathetic Nervous System

Another antinatriuretic system that can reduce the renal pressure natriuresis relationship and cause chronic hypertension is the renal sympathetic nervous system.[52–56] The kidneys receive extensive sympathetic innervation and increases in renal sympathetic nerve activity reduce sodium excretion by increasing tubular reabsorption or decreasing the filtered load of sodium via alpha adrenergic receptor activation (Fig. 39.8).[52] Renal nerves can act directly on the tubule to increase sodium reabsorption or indirectly by increasing renal vascular resistance and reducing medullary blood flow and renal interstitial pressure. In addition, increased renal sympathetic nerve activity can enhance tubule reabsorption by activating the RAS.

Excessive activation of the renal SNS has been implicated in the pathogenesis of several experimental and genetic forms of hypertension.[52–56] Evidence for a role of the renal nerves in hypertension derives from animal studies showing that renal denervation attenuates or delays the development of hypertension in several forms of experimental hypertension.[56] One particular experimental form of hypertension that is mediated via enhanced renal sympathetic nerve activity is obesity-related hypertension.[56,57] Obesity is often associated with increased sympathetic activity.[56,57] To determine the role of renal nerves in mediating the sodium retention

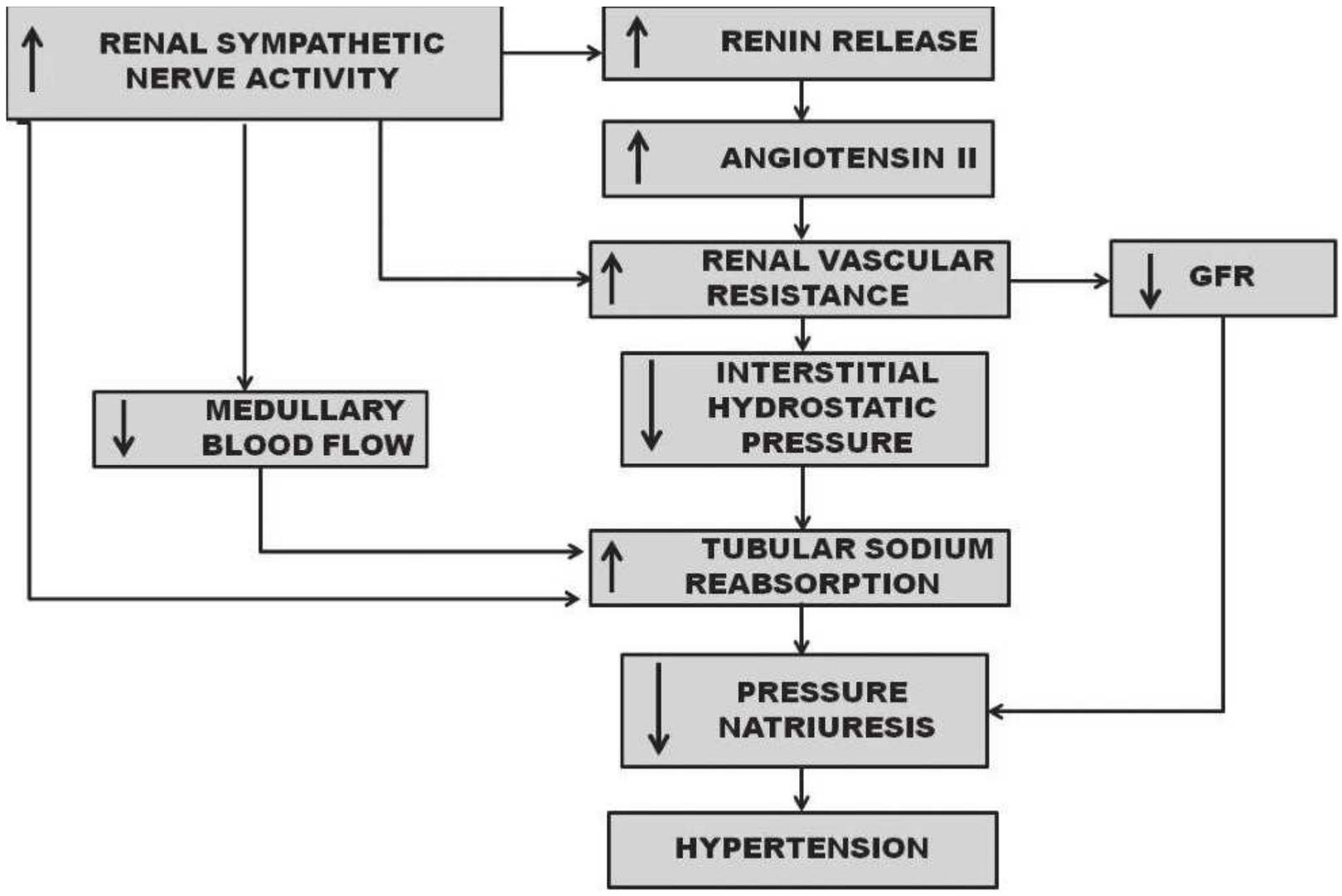

FIGURE 39.8 Renal mechanisms whereby activation of the sympathetic nervous system reduces pressure natriuresis relationship and leads to hypertension.

and hypertension associated with obesity, Kassab and colleagues[58] determined the hemodynamic and renal excretory responses to a high-fat diet in control and bilaterally renal-denervated dogs (Fig. 39.9). In response to a high-fat diet, body weight increased similarly (about 40%) in the control and bilaterally renal-denervated groups. Arterial pressure increased by 15% in the control group but in sharp contrast, 5 weeks of a high-fat diet in the bilaterally renal-denervated group did not significantly increase arterial pressure. Furthermore, after 5 weeks of a high-fat diet, cumulative sodium retention was 455 ± 85 mmol in the control group and only 252 ± 47 mmol in the bilaterally renal-denervated

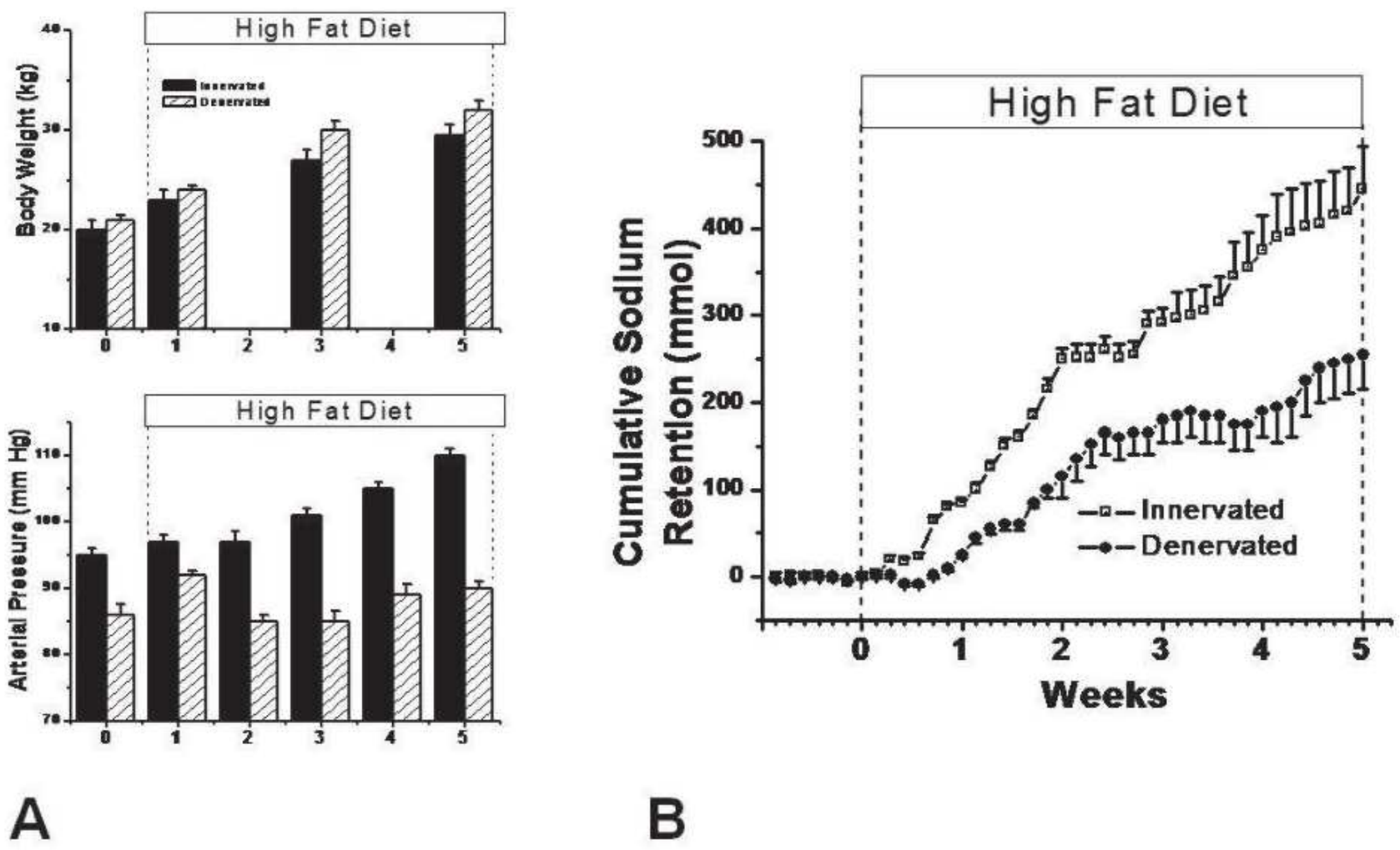

FIGURE 39.9 Changes in body weight and arterial pressure **(A)** and cumulative sodium balance **(B)** in response to a high-fat diet in dogs with innervated and denervated kidneys. (Redrawn from Kassab S, Kato T, Wilkins FC, et al. Renal denervation attenuates the sodium retention and hypertension associated with obesity. *Hypertension*. 1995;25:893–897.)

group. Similar increases in GFR and renal plasma flow occurred in both groups in response to the high-fat diet, indicating that the sodium retention in response to a high-fat diet was due to enhanced sodium reabsorption.[58] The results of this study indicate that the renal nerves play an important role in mediating the sodium retention and hypertension associated with obesity.

Although there is growing evidence for a role of the renal SNS in the development of several animal models of hypertension, the importance of renal nerves in the pathogenesis of human hypertension has yet to be fully elucidated.[56] Application of the norepinephrine spillover methodology in humans has demonstrated activation of the sympathetic nervous outflow to the kidneys in humans with essential hypertension.[56] Renal norepinephrine spillover, on average, is elevated two- to threefold in both normal weight patients with essential hypertension and in obesity-related hypertension.[56] The most compelling evidence for a role of renal nerves in human hypertension are the recent findings that ablation of the renal sympathetic nerves with a radiofrequency-emitting catheter inserted percutaneously significantly reduces BP in patients with resistant hypertension.[59] The level of BP reduction achieved in the resistant hypertensive patients undergoing renal nerve ablation was a mean reduction of 24/10 mm Hg at 3 months and 29/16 mm Hg at 12 months (Fig. 39.10). In a more recent study, the Symplicity HTN-2 trial,106 patients with treatment-resistant hypertension were assessed and renal denervation resulted in impressive reductions in mean office-based measurements of BP (32/12 mm Hg at 6 months), whereas BP remained almost unchanged in the control group.[60] Home and ambulatory measurements of BP followed a similar pattern; the corresponding reductions were 20/12 mm Hg and 11/7 mm Hg with renal denervation, whereas no significant reductions were observed in the control group. It is interesting to note that the average body mass index (BMI) of patients in the study was in the obesity range. Although these data support a potential role for renal nerves in patients with resistant hypertension, it remains unclear as to the relative importance of destruction of renal afferent versus efferent nerves in the antihypertensive effect achieved by the radiofrequency ablation procedure.[56]

The Endothelin System

Endothelin-1 (ET-1) is derived from a 203 amino acid peptide precursor, preproendothelin, which is cleaved after translation to form proendothelin.[61–65] Proendothelin is cleaved in the presence of a converting enzyme to produce the 21 amino acid peptide, ET-1. ET-1 receptor binding sites have been identified throughout the body with the greatest numbers of receptors in the kidneys. ET-1 can either elicit a prohypertensive, antinatriuretic effect by activating endothelin type A (ET_A) receptors and causing renal vasoconstriction or an antihypertensive, natriuretic effect via endothelin

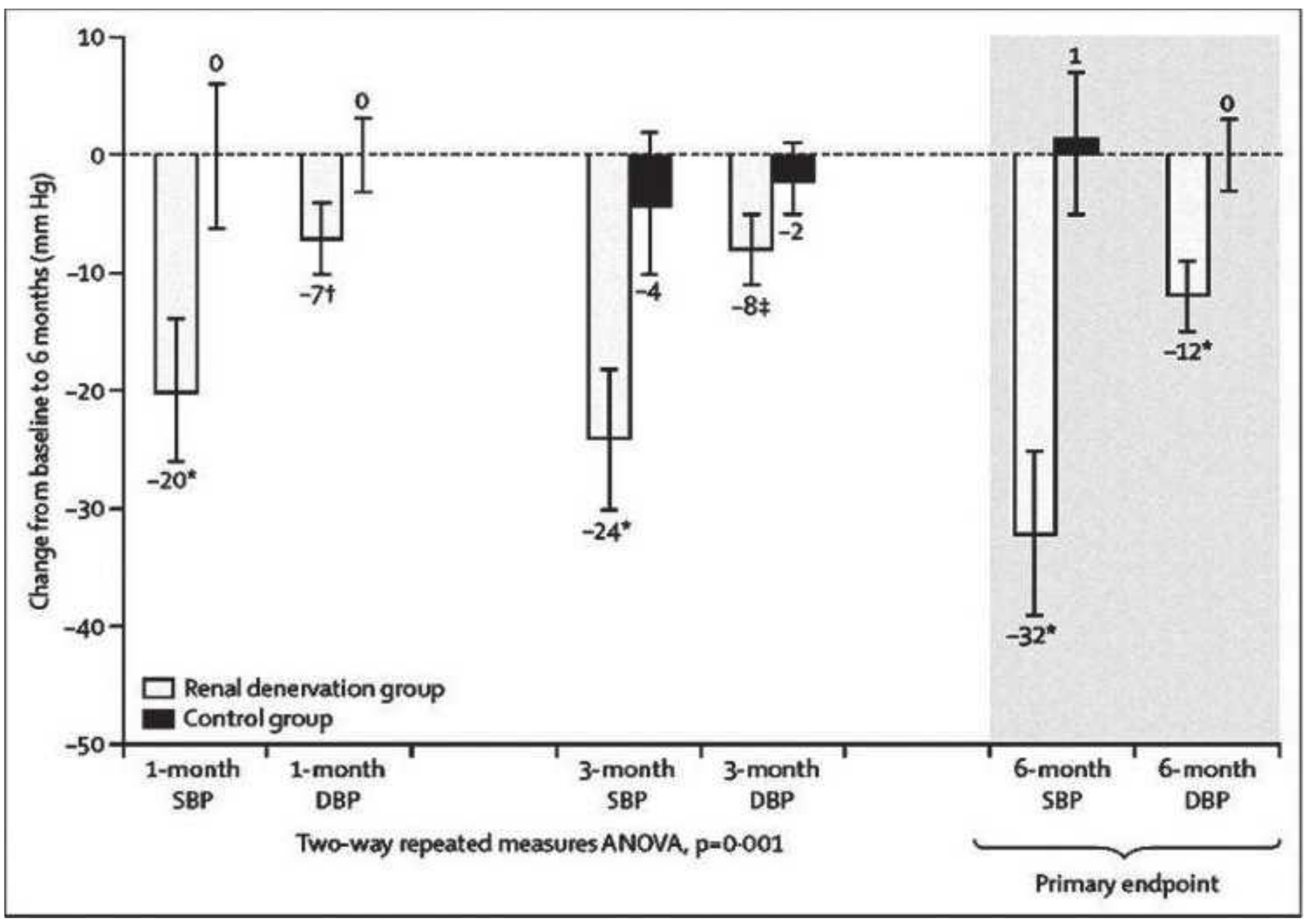

FIGURE 39.10 Blood pressure (BP) lowering effect of radiofrequency ablation of the renal sympathetic nerves for 18 months (M) in patients who were resistant to the usual antihypertensive drugs. *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure. (Krum H, Whitbourn R, Sobotka P, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof- of-principle cohort study. *Lancet*. 2009;373:1275–1281.)

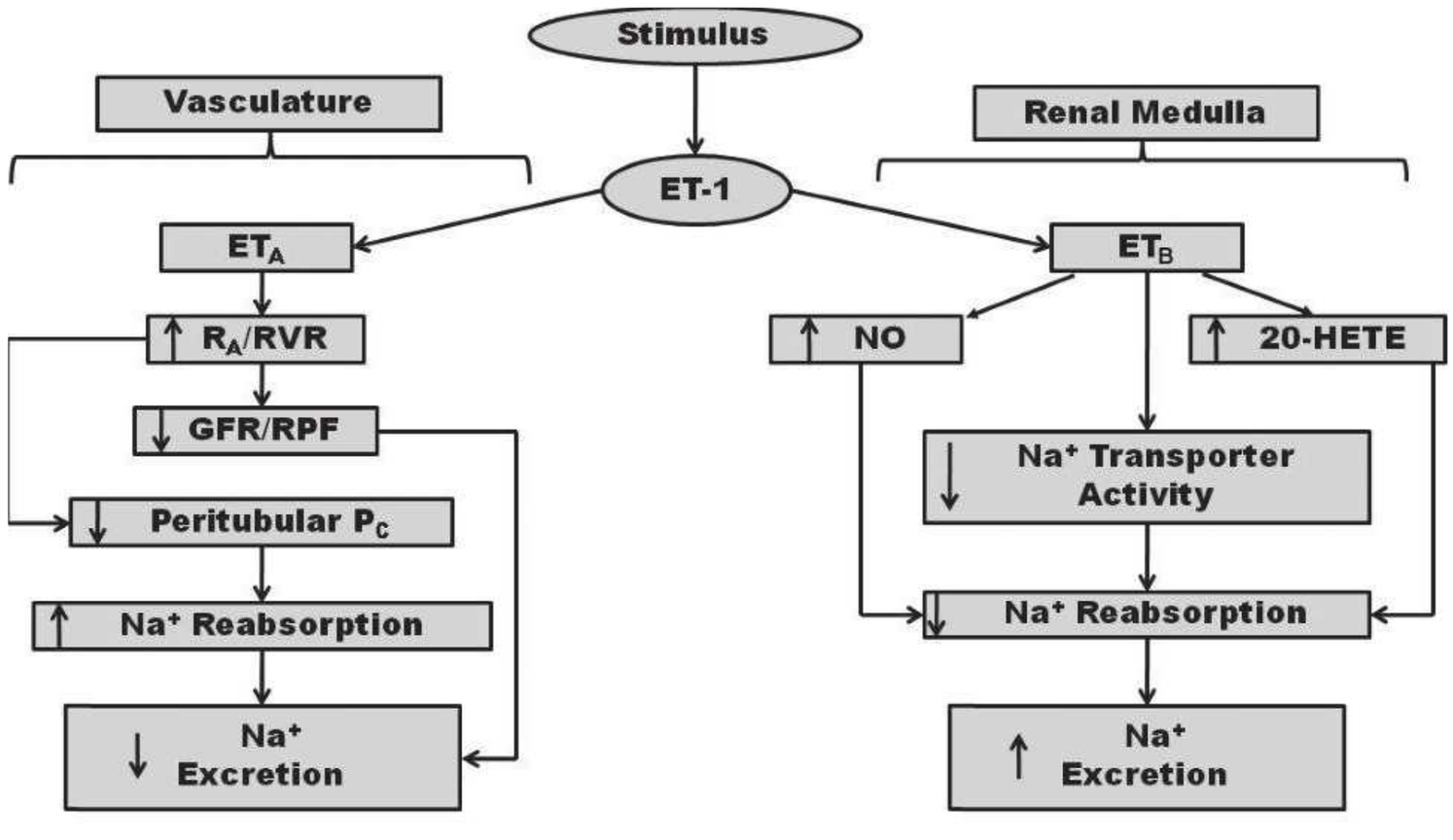

FIGURE 39.11 Summary of the pro- and antihypertensive actions of endothelin-1 (ET-1). The ability of ET-1 to influence blood pressure regulation and renal pressure natriuresis is highly dependent on where ET-1 is produced and which renal ET receptor type is activated. ET-1 can elicit a prohypertensive, antinatriuretic effect by activating ET_A receptors in the kidneys. Activation of renal ET_A receptors increases renal vascular resistance (RVR), which decreases renal plasma flow (RPF) and glomerular filtration rate (GFR), and enhances sodium reabsorption by decreasing peritubular capillary hydrostatic pressure (Pc). The net effect of renal ET_A receptor activation would be increased sodium retention and blood pressure. Conversely, ET-1 can elicit an antihypertensive, natriuretic effect via ET_B receptor activation. Activation of the renal ET_B receptor leads to enhanced synthesis of nitric oxide (NO) and prostaglandin E2 (PG) and suppression of the renin-angiotensin system. The net effect of renal ET_B receptor activation would be decreases in sodium retention and blood pressure.

type B (ET_B) receptor activation (Fig. 39.11). Thus, the ability of ET-1 to influence BP regulation and renal pressure-natriuresis is highly dependent on where ET-1 is produced in the kidney and which renal ET receptor type is activated.[66]

ET-1, via ET_A receptor activation, exerts a variety of actions within the kidney that, if sustained chronically, could contribute to the development of hypertension and progressive renal injury.[66] ET-1 decreases GFR and renal plasma flow.[66,67] Long-term effects of ET-1 on the kidney include stimulation of mesangial cell proliferation and extracellular matrix deposition as well as stimulation of vascular smooth muscle hypertrophy in renal resistance vessels.[68] Previous studies have indicated that expression of ET-1 is greatly enhanced in animal models of severe hypertension with renal vascular hypertrophy and in models of progressive renal injury.[66–68] In addition, treatment with endothelin receptor antagonists attenuated the hypertension and small artery morphologic changes and improved kidney function in these models.[66–68]

Several lines of evidence suggest that ET-1 may play an important role in salt-sensitive forms of hypertension. Dahl salt-sensitive (DS) rats placed on a high-sodium diet are characterized by an attenuated pressure natriuresis; development of hypertension and extensive renal lesions in the form of glomerulosclerosis, renal arteriolar, and tubular injury; and progressive renal failure in late phases of the disease.[69,70] Prepro-ET-1 mRNA and vascular responsiveness to ET-1 are increased in the renal cortex of DS rats compared with Dahl salt-resistant (DR) rats and a positive correlation between ET-1 generation in the renal cortex and the extent of glomerulosclerosis has been reported in DS hypertensive rats.[69,70] Acute infusion of a nonselective ET_A-ET_B receptor antagonist directly into the renal interstitium improves renal hemodynamic and excretory functions in DS rats but not in DR rats.[69,70] Moreover, chronic blockade of ET_A receptors attenuates the hypertension and proteinuria and ameliorates the glomerular and tubular damage associated with high salt intake in DS rats (Fig. 39.12). An interesting unanswered question that emerges is whether the beneficial effect of the ET_A blockade in reducing renal injury is mediated through reducing BP or through direct renal mechanisms.

Although ET-1 clearly plays a significant role in the pathogenesis of some forms of experimental hypertension, especially salt-sensitive models, its role in human hypertension has yet to be fully elucidated. Selective ET_A receptor blockade in hypertensive patients with chronic kidney disease produces a significant reduction in BP, suggesting an upregulation of the ET-1 system in chronic kidney disease–associated hypertension.[71] Krum and colleagues reported that bosentan, a nonselective ET receptor antagonist, reduces BP in patients with essential hypertension as much as did enalapril 20 mg.[72] In another study, 6 weeks of darusentan, a selective ET_A receptor antagonist, lowered both systolic and diastolic BP.[73] Bakris et al.[74] and Weber et al.[75] also showed that darusentan reduced mean 24-hour systolic BP more than placebo in patients with treatment-resistant

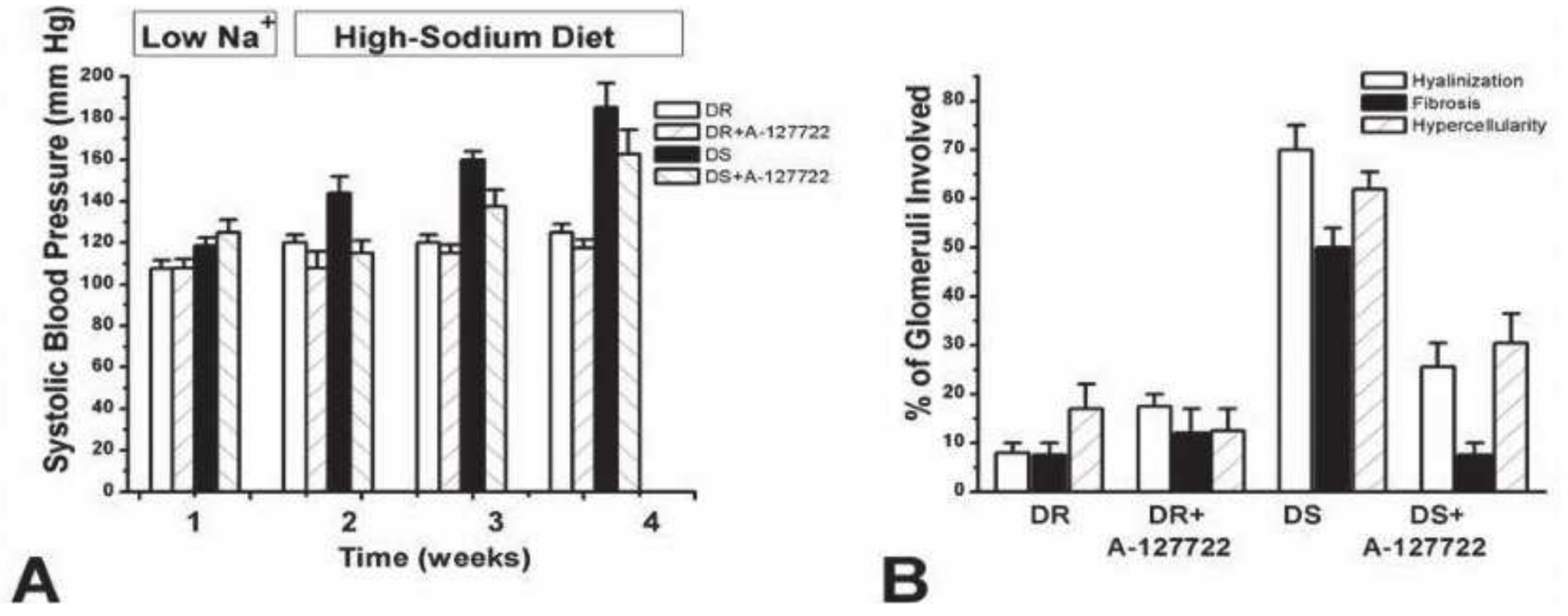

FIGURE 39.12 **A:** Systolic pressures in DS and DR control rats and in those chronically treated with a selective endothelin type A receptor antagonist, A-127722. **B:** Histopathologic changes within the glomeruli of DS and DR rats and in those chronically treated with A-l27722 . Data are expressed as percentage of glomeruli involved with fibrosis, hyalinization, or hypercellularity. (Redrawn from data in Kassab S, Miller M, Novak J, et al. Endothelin-A receptor antagonism attenuates the hypertension and renal injury in Dahl salt-sensitive rats. *Hypertension*. 1998;31:397–402.)

hypertension. Although these clinical studies suggest a potential role for ET-1 in several forms of human hypertension, the importance of ET-1 in human hypertension deserves further investigation.[76–78]

Although much attention has been given to the role of ET-1 in the pathophysiology of cardiovascular and renal disease acting via an ET_A receptor, recent studies indicate an important physiologic role for ET-1 in the regulation of sodium balance and arterial pressure via ET_B receptor activation.[68] The most compelling evidence that the endothelin system may play a significant role in the regulation of sodium balance and arterial pressure are the reports that transgenic animals deficient in ET_B receptors develop a severe form of salt-sensitive hypertension.[79,80] Additional evidence comes from studies indicating that pharmacologic antagonism of ET_B receptors produces significant hypertension in rats.[81]

Although systemic ET_B receptor blockade produces significant hypertension that is salt-sensitive, the physiologic mechanisms involved in mediating the hypertension are still unknown. Because ET_B receptors are located on multiple cell types through the body, including endothelial cells and renal epithelial cells, both intrarenal and extrarenal mechanisms may mediate the hypertension produced by chronic disruption of the ET_B receptor. However, several recent studies suggest that the renal endothelin system plays a major role in controlling BP under high sodium intake conditions. To examine the role of endothelial cell ET_B receptors in salt-sensitive hypertension, Bagnall and colleagues generated an endothelial cell-specific ET_B receptor in knockout mice using a Cre-loxP approach.[82] They reported that ablation of ET_B receptors exclusively from endothelial cells produces endothelial dysfunction in the absence of hypertension. In contrast to models of total ET_B receptor ablation, the BP response to a high-salt diet was unchanged in endothelial cell-specific ET_B receptor knockouts compared to control mice. These important findings suggest that nonendothelial cell ET_B receptors are important for regulation of BP.

There is growing evidence to suggest that ET-1, acting through the renal medullary ET_B receptors, is involved in the regulation of sodium balance and BP under normal physiologic conditions. The kidney is an important site of ET-1 production, and ET_B receptors are expressed at important renal sites of ET-1 synthesis, particularly in the renal medulla.[68] Some of the first studies using synthetic ET-1 demonstrated that nonpressor doses of ET-1 produced significant natriuresis and diuresis.[68] It is now known that ET_B receptors are located in various parts of the nephron, including the proximal tubule, medullary thick ascending limb, collecting tubule, and the inner medullary collecting duct.[68] The highest concentration of ET_B receptors appears to be on the inner medullary collecting duct in the renal medulla.[68] Activation of ET_B receptors has been reported to inhibit sodium and water reabsorption along various parts of the nephron.[68] Taken together, these data indicate that ET-1, via ET_B receptors, may influence the renal handling of sodium and water. The exact mechanism whereby ET_B receptor activation inhibits sodium reabsorption is unclear but could involve other autocoid factors such as nitric oxide, PGE2, and/or 20-HETE.

For the renal ET-1 system to be an important control system for the regulation of sodium balance, the production of renal ET-1 should change in response to variations in sodium intake. Moreover, blockade of ET_B receptors should result in a salt-sensitive form of hypertension. Although there is ample data showing that ET-1 can influence sodium reabsorption, there is a paucity of data in the literature examining the relationship between sodium intake and renal production of ET-1. A recent study by Pollock and Pollock,[81] however, has shown a positive correlation between sodium intake and renal excretion of ET-1. The most convincing

evidence for a role of the renal ET-1 in controlling sodium excretion and arterial pressure during chronic changes in sodium intake is the result of several recent studies. Gariepy and colleagues demonstrated that rats deficient in ET_B receptor expression display salt-sensitive hypertension.[80] Likewise, Pollock and Pollock reported that chronic pharmacologic blockade of the ET_B receptor in rats resulted in hypertension that was sensitive to dietary sodium intake.[80] Moreover, Ohuchi et al. reported elevation in BP by genetic and pharmacologic disruption of the ET_B receptor in mice.[83]

In a recent report by Ge and colleagues,[84] disruption of the ET_B receptor in the collecting duct cells of mice was found to produce significant hypertension that was salt-sensitive. Collecting duct ET_B knockout mice on a normal sodium diet were hypertensive.[84] Collecting duct ET_B knockout mice on a high-sodium diet had worsened hypertension, reduced urinary sodium excretion, and excessive weight gain.[84] Similar findings were found in mice with combined ET_B and ET_A receptor knockout in the collecting duct cells (Fig. 39.13).[85] These findings provide strong evidence that the collecting duct ET_B receptor is an important physiologic regulator of renal sodium excretion and blood pressure.

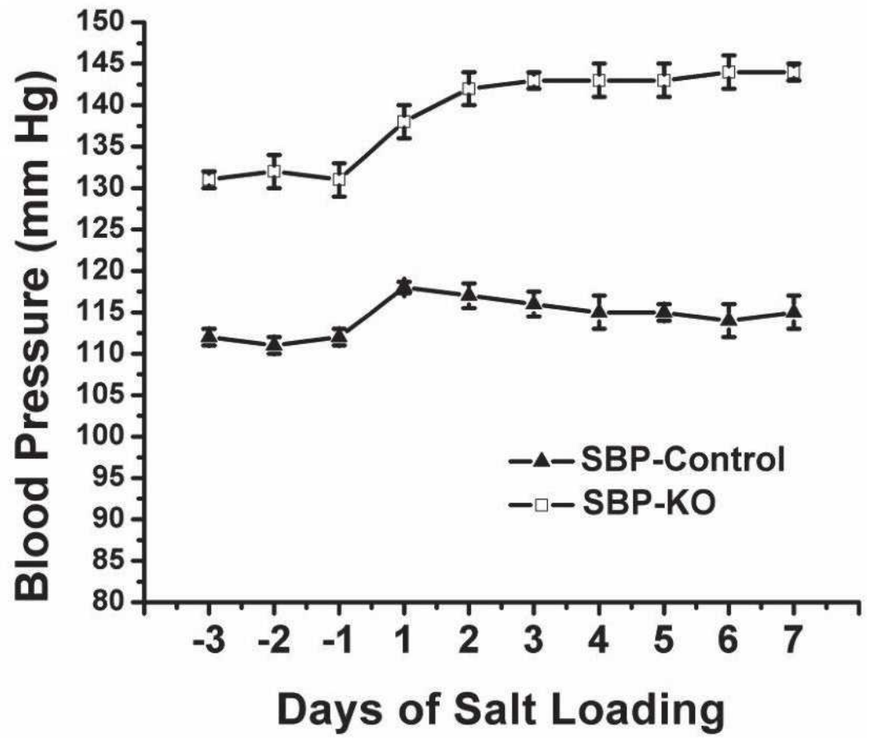

FIGURE 39.13 Collecting duct knockout of the endothelin B and A receptors (CD ETB/ETA KO) causes a hypertensive shift in the pressure natriuresis relationship and salt-sensitive hypertension. (Redrawn from Ge Y, Bagnall A, Stricklett PK, et al. Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. *Am J Physiol Renal Physiol.* 2008;295(6):F1635–1640.)

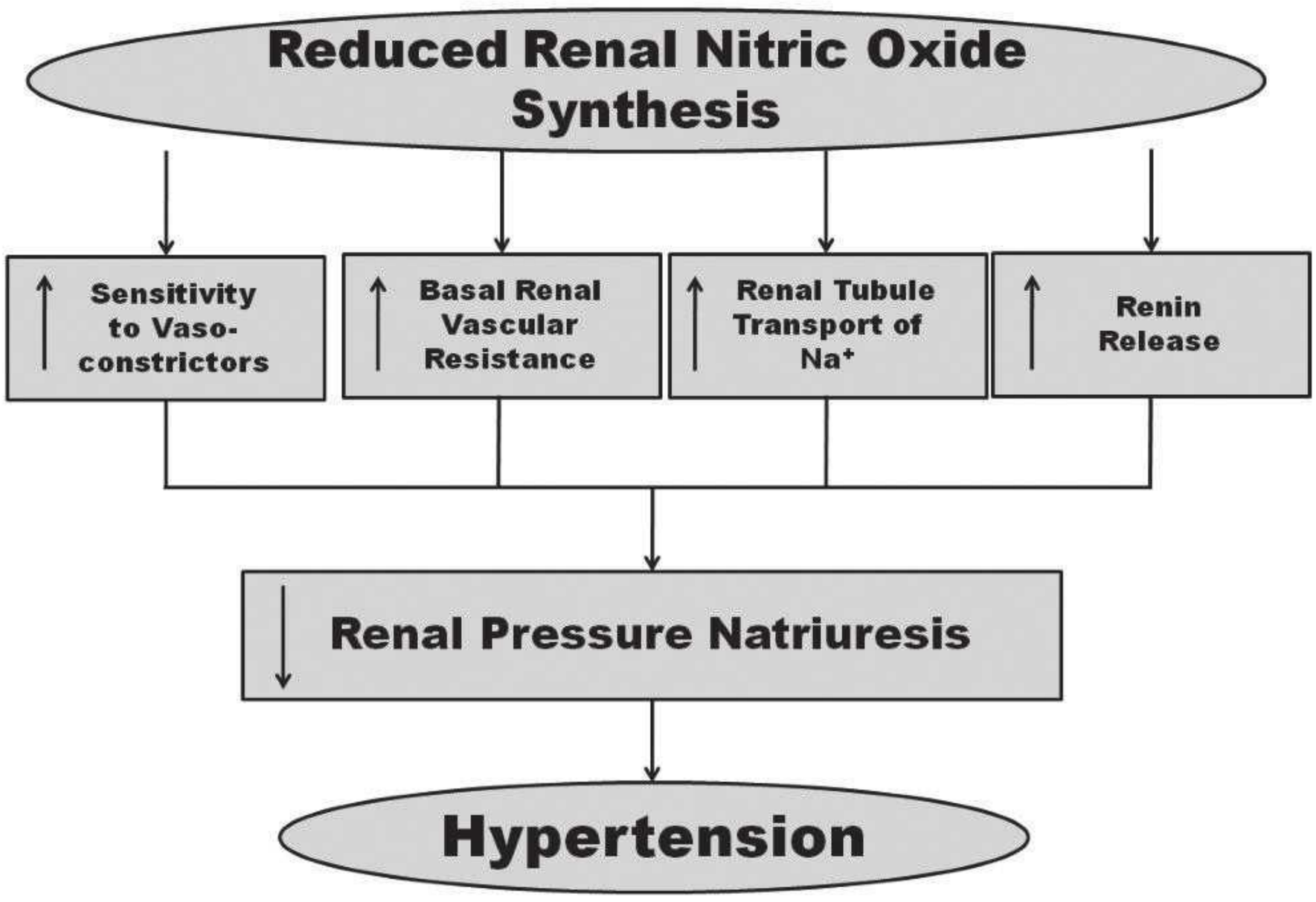

FIGURE 39.14 The renal effector mechanisms whereby reductions in NO synthesis decrease pressure natriuresis and increase blood pressure. A reduction in endothelial derived nitric oxide (EDNO) synthesis leads to a decrease in renal sodium excretory function by directly increasing basal renal vascular resistance, enhancing the renal vascular responsiveness to vasoconstrictors such as ANGII or norepinephrine, or activating the renin-angiotensin system. Reductions in NO synthesis also reduce sodium excretory function either through direct effects on tubular transport or through changes in intrarenal physical factors such as renal interstitial hydrostatic pressure or medullary blood flow.

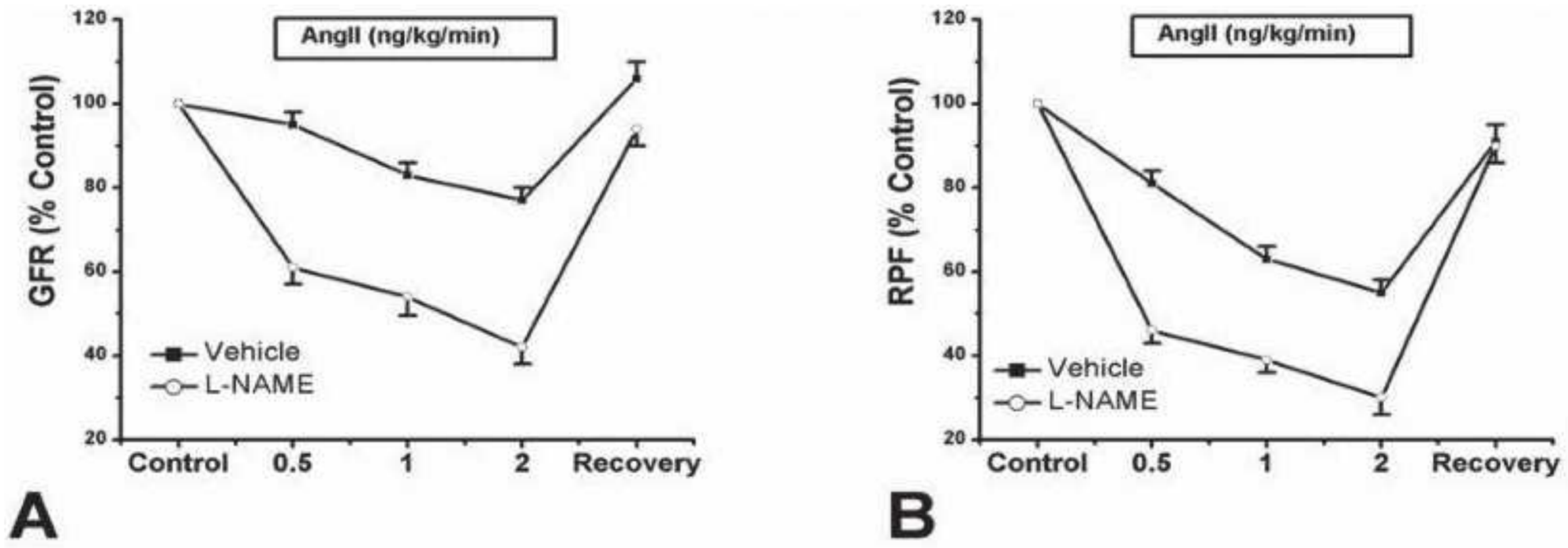

FIGURE 39.15 Glomerular filtration rate (GFR) and renal plasma flow (RPF) in response to intrarenal infusions of ANGII in control dogs (vehicle) and in dogs pretreated intrarenally with a nitric oxide synthesis inhibitor, L-NAME. (Redrawn from Ge Y, Bagnall A, Stricklett PK, et al. Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. *Am J Physiol Renal Physiol.* 2008;295(6):F1635–1640.)

Nitric Oxide

All components of the nitric oxide (NO) system are located within the kidney and pharmacologic or genetic disruption of this system results in a sustained hypertension associated with reductions in renal hemodynamics and pressure-natriuresis.[85–87] The magnitude of the increase in BP is also dependent on the dietary sodium intake.[85–87] These findings have led to the concept that NO is not only important in the long-term regulation of sodium balance and BP but also to the notion that abnormalities in NO production result in altered pressure natriuresis and a salt-sensitive form of hypertension.

The renal effector mechanisms whereby reductions in NO synthesis alter pressure natriuresis can be divided into hemodynamic and tubular components each of which may be modulated by processes that are intrinsic and extrinsic to the kidney (Fig. 39.14). For example, reductions in NO synthesis could lead to a decrease in renal sodium excretory function by directly increasing basal renal vascular resistance or by enhancing the renal vascular responsiveness to vasoconstrictors such as AngII or norepinephrine (Fig. 39.15).[87–90] Reductions in NO synthesis also reduce sodium excretory function either through direct effects on tubular transport or through changes in intrarenal physical factors such as renal interstitial hydrostatic pressure or medullary blood flow.[85,86,91,92] Consistent with this hypothesis are observations that the acute infusion of an NO synthase (NOS) inhibitor directly into the renal medulla significantly reduces papillary blood flow, renal interstitial hydrostatic pressure (RIHP), and decreases urinary sodium and water excretion without affecting GFR or systemic pressure.[91–93] Chronic medullary interstitial infusion of NOS inhibitors in conscious rats results in sustained reductions in medullary blood flow, sustained sodium and water retention, and hypertension which are reversed when the infusion is discontinued (Fig. 39.16). These findings demonstrate that

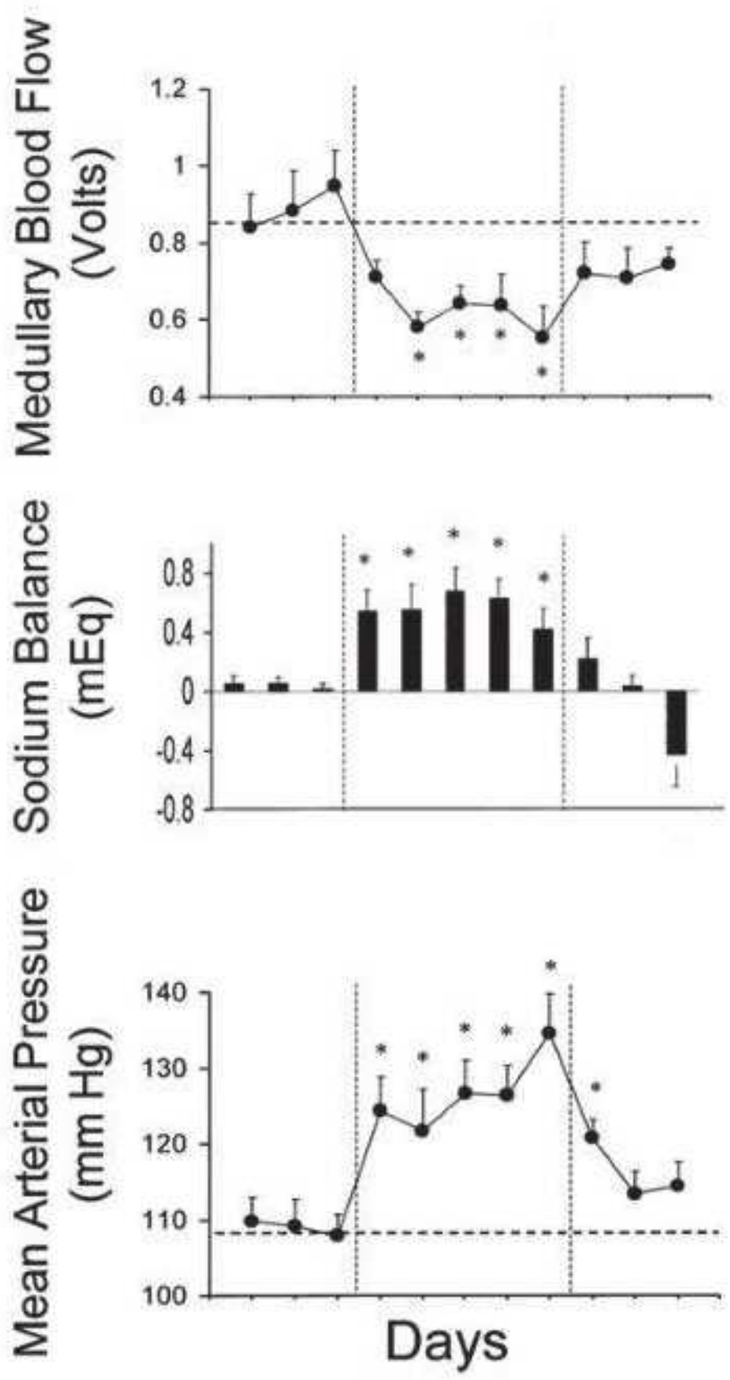

FIGURE 39.16 Chronic effect of renal medullary interstitial infusion of the nitric oxide synthase inhibitor L-NAME (8.6 mg · kg^{-1} · day^{-1}) on renal medullary blood flow (*top*), daily sodium balance (*middle*), and mean arterial blood pressure (*bottom*) in conscious Sprague-Dawley rats. Vertical dashed lines indicate the L-NAME infusion period. *Significant difference from control ($P < 0.05$). (Mattson DL, Lu SH, Nakanishi K, et al. Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol Heart Circ Physiol.* 1994;266:H1918–H1926.)

reductions in medullary blood flow may be another important mechanism whereby inhibition of NO in the kidney leads to a hypertensive shift in pressure natriuresis.[91]

Inhibition of NO synthesis may have direct effects on renal tubule transport.[86,94] NO has direct effects on sodium uptake in cultured cortical collecting duct cells by altering apical sodium channels.[86] Sodium transport in the cortical collecting duct in vivo is mediated through changes in cyclic GMP. Micropuncture studies have shown NOS inhibitors decrease proximal tubule reabsorption in anesthetized rats.[86] This effect has been attributed to antagonism of Ang II–mediated sodium transport. An effect of NO on proximal reabsorption has also been inferred from changes in lithium clearance induced during inhibition of NO production.[86] Thus, NO can affect sodium reabsorption via direct effects on tubular transport or indirectly via alteration in medullary blood flow or renal interstitial hydrostatic pressure.

Another mechanism whereby NO synthesis inhibition may reduce pressure natriuresis is via activation of the RAS.[85] Inhibition of NO production enhances renin release from rat cortical kidney slices.[85] Inhibitors of NO synthesis also increase plasma renin activity. Intrarenal inhibition of NO increased renin release in dogs, an effect that is dependent on the macula densa mechanism.[95]

Several lines of evidence suggest that NO may play an important role in the regulation of sodium balance and in pathogenesis of salt-sensitive hypertension.[84,94] An increase in renal NO production or release as evidenced by increased urinary excretion of NO metabolites or the NO second messenger, cyclic GMP, has been reported to be essential for the maintenance of normotension during a dietary salt challenge. Prevention of this increase in renal NO production results in salt-sensitive hypertension.[84,85,94]

There is also ample in vitro evidence demonstrating that NO synthesis is impaired in some vascular beds in human essential hypertension. The extent to which these observations reflect effects of the hypertensive process or reflect important mechanisms for the pathogenesis of the hypertensive condition remains unclear.

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) elicits an antihypertensive, natriuretic effect via its receptors (NPR-A). ANP is a 28 amino acid peptide synthesized and released from atrial cardiocytes in response to stretch.[96,97] Once ANP is released from the atria, it enhances sodium excretion through extrarenal and intrarenal mechanisms.[96] ANP increases GFR while having no effect on renal blood flow.[96,98] However, increased GFR is not a prerequisite for ANP to enhance sodium excretion. A deficiency in ANP production or a defect in its receptors may reduce pressure natriuresis and lead to hypertension by enhancing tubular sodium reabsorption either directly by enhancing the active tubular transport of sodium or indirectly via alterations in medullary blood flow, physical factors, and intrarenal hormones (Fig. 39.17).

ANP also has actions at several sites of the RAS cascade.[96,98] Intrarenal or intravenous infusion of ANP reduces the renin secretion rate, presumably by a macula densa mechanism because ANP failed to reduce renin secretion in nonfiltering kidneys. The reduction in renin secretion would decrease intrarenal levels of AngII, which could contribute to ANP-induced natriuresis. When intrarenal levels of AngII were prevented from decreasing the natriuretic effects of ANP were blunted.[96]

ANP also decreases aldosterone release from the adrenal zona glomerulosa cells.[96] Two mechanisms for ANP-induced suppression of aldosterone release have been suggested: (1) a direct action on adrenal glomerulosa cells and (2) reduced circulating levels of AngII due to suppressed renin secretion under in vivo conditions.[96] Although the suppression

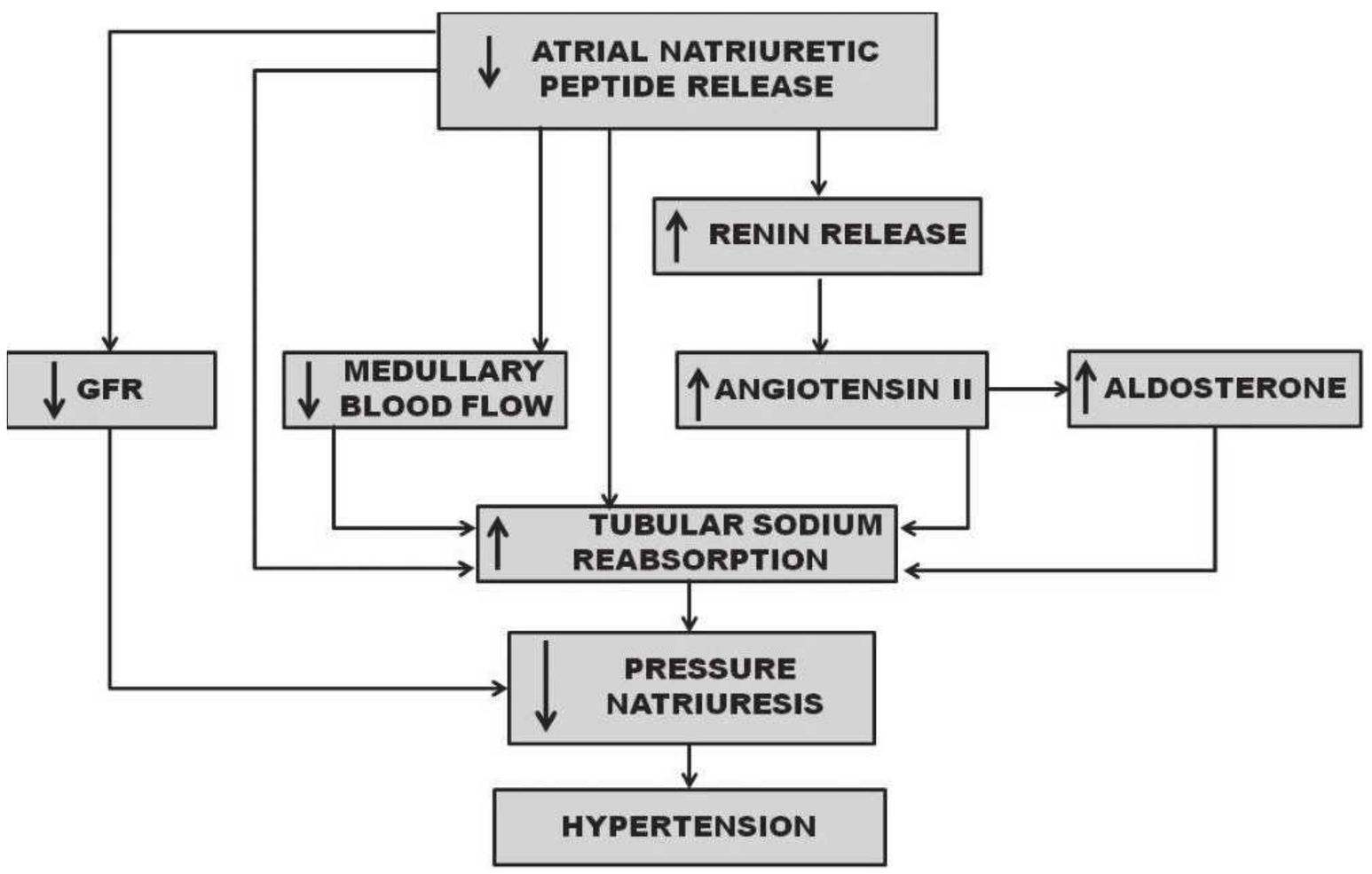

FIGURE 39.17 Renal mechanisms whereby reductions in atrial natriuretic peptide synthesis or atrial natriuretic peptide receptor defects reduce pressure natriuresis relationship and lead to hypertension.

of aldosterone release would not play a role in mediating the acute natriuretic responses to ANP, decreases in circulating levels of aldosterone could contribute to the long-term actions of ANP on sodium balance and arterial pressure regulation.

Plasma levels of ANP are elevated in numerous physiologic conditions associated with enhanced sodium excretion.[96,97] Acute saline of blood volume expansion consistently elevates circulating levels of ANP. Some, but not all, investigators have reported that chronic increases in dietary sodium intake raise circulating levels of ANP. Several studies have reported that infusions of exogenous ANP at rates that result in physiologically relevant plasma concentrations, comparable to those observed during volume expansion, have significant renal and cardiovascular effects.[96,97] Infusion of ANP at a rate that causes a twofold increase in plasma ANP elicits significant natriuresis, especially in the presence of other natriuretic stimuli, such as high renal perfusion pressure.[96] Long-term physiologic elevations in plasma ANP also shift the renal-pressure natriuresis relationship and reduce arterial pressure.[98]

The development of genetic mouse models that exhibit chronic alterations in expression of the genes for ANP or its receptors (NPR-A, NPR-C) have also provided compelling evidence for a role of ANP in chronic regulation of renal pressure natriuresis and BP.[99] Transgenic mice overexpressing ANP gene are hypotensive relative to the nontransgenic littermates, whereas mice harboring functional disruptions of the ANP or NPR-A genes are hypertensive. The ANP gene knockout mice develop a salt-sensitive form of hypertension in association with failure to adequately suppress the RAS (Fig. 39.18). These findings suggest that genetic deficiencies in ANP or natriuretic receptor activity could play a role in the pathogenesis of salt-sensitive hypertension.[100] Indeed, common genetic variants at the NPPA-NPPB locus associated with circulating natriuretic peptide concentrations may contribute to interindividual variation in blood pressure and hypertension.

Arachidonic Acid Metabolites

Cyclooxygenase metabolizes arachidonic acid into prostaglandin (PG) G_2 and subsequently to PGH_2, which is then further metabolized by tissue-specific isomerases to PGs and thromboxane.[101,102] Although the kidney produces many types of PGs with multiple functions, the major renal prostaglandin controlling sodium excretion is probably PGE_2.[101,102] However, production of other arachidonate acid metabolites, such as prostacyclin, thromboxane, and 20-HETE, may also influence renal-pressure natriuresis and BP regulation. The largest production of PGE_2 occurs in the medulla with decreasing synthesis in the cortex. PGE_2 is synthesized and rapidly inactivated and, once synthesized, is released and not stored. Once released, PGE_2 influences sodium transport by several intrarenal mechanisms.

Despite numerous reports that PGs may contribute to the natriuresis of acute physiologic perturbations, the importance of endogenous renal PGs in the long-term

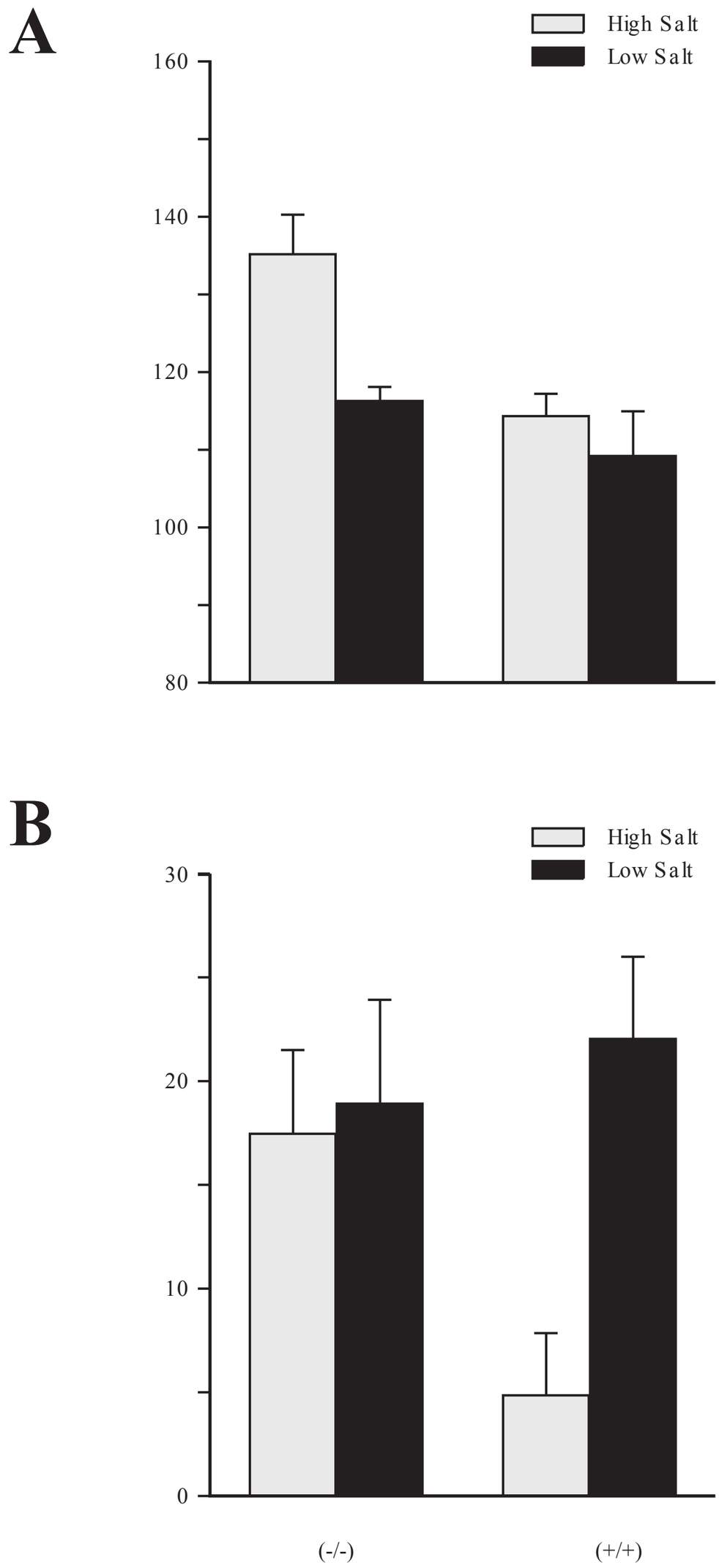

FIGURE 39.18 Mean arterial pressure (MAP) and plasma renin activity (PRA) in atrial natriuretic peptide knockout mice fed on HS or LS diets for 3 to 4 weeks. (Redrawn from Melo LG, Veress AT, Chong CK, et al. Salt-sensitive hypertension in ANP knockout mice: potential role of abnormal plasma renin activity. *Am J Physiol.* 1998; 274: R255–R261.)

regulation of sodium balance remains unclear.[101] Increases in dietary sodium intake have little or no effect on urinary PG excretion. In addition, nonspecific cyclooxygenase inhibitors do not affect the sodium excretory or BP responses to chronic alterations in dietary sodium intake. Thus, it appears that endogenous renal PGs may not play a major role

in regulating sodium excretion during chronic changes in sodium intake.[101]

Although long-term administration of PG synthesis inhibitors has very little effect on volume and/or arterial pressure regulation under normal physiologic conditions, renal PGs may be important in pathophysiologic states associated with enhanced activity of the RAS.[101] In vitro and in vivo studies indicate that renal PGs protect the preglomerular vessels form excessive AngII-induced vasoconstriction.[101] In the absence of this protective mechanism in pathophysiologic states the renal vasculature could be exposed to the potent vasoconstrictor actions of AngII. This could lead to significant impairment of renal hemodynamics, reduced excretory function, and hypertension.

It is now known that there are at least two distinct cyclooxygenases, COX-1 and COX-2.[53] COX-1 is called the constitutive enzyme because of its wide tissue distribution, whereas COX-2 has been termed as inducible because of its more restricted basal expression and its upregulation by inflammatory and/or proliferative stimuli.[101] COX-2 inhibition has been shown to decrease urine sodium excretion and induce mild to moderate increases in arterial pressure. In addition, nonsteroidal anti-inflammatory drugs (NSAIDs) in general and COX-2 inhibitors in particular can aggravate preexisting hypertension. Moreover, blockade of COX-2 activity can have deleterious effects on renal blood flow and GFR.[101] In addition to physiologic regulation of COX-2 expression in the kidney, increased renal cortical COX-2 expression is seen in experimental models associated with altered renal hemodynamics and progressive renal injury. Thus, NSAIDs can cause acute kidney injury in patients with compromised renal hemodynamics.

In addition to renal PGs generated via the COX pathway, other eicosanoids that inhibit tubular sodium transport are produced by cytochrome P450 (CYP) monooxygenase metabolism of arachidonic acid.[102] CYP enzymes metabolize arachidonic acid primarily to 20-HETE and EETs. 20-HETE is a potent constrictor of renal arterioles that may have an important role in autoregulation of renal blood flow and tubuloglomerular feedback (Fig. 39.19).[102] 20-HETE and EETs also inhibit sodium reabsorption in the proximal tubule and thick ascending loop of Henle (TALH). Compelling evidence suggests that the renal production of CYP metabolites of arachidonic acid is altered in genetic and experimental models of hypertension and that this system contributes to the resetting of pressure natriuresis and the development of hypertension. In the SHR, the renal production of 20-HETE is increased and inhibitors of the formation of 20-HETE decrease arterial pressure.[102] Blockade of 20-HETE synthesis also reduces BP or improves renal function in deoxycorticosterone acetate (DOCA)-salt, angiotensin II-infused, and Lyon hypertensive rats.[102] In contrast, 20-HETE formation is reduced in the thick ascending limb

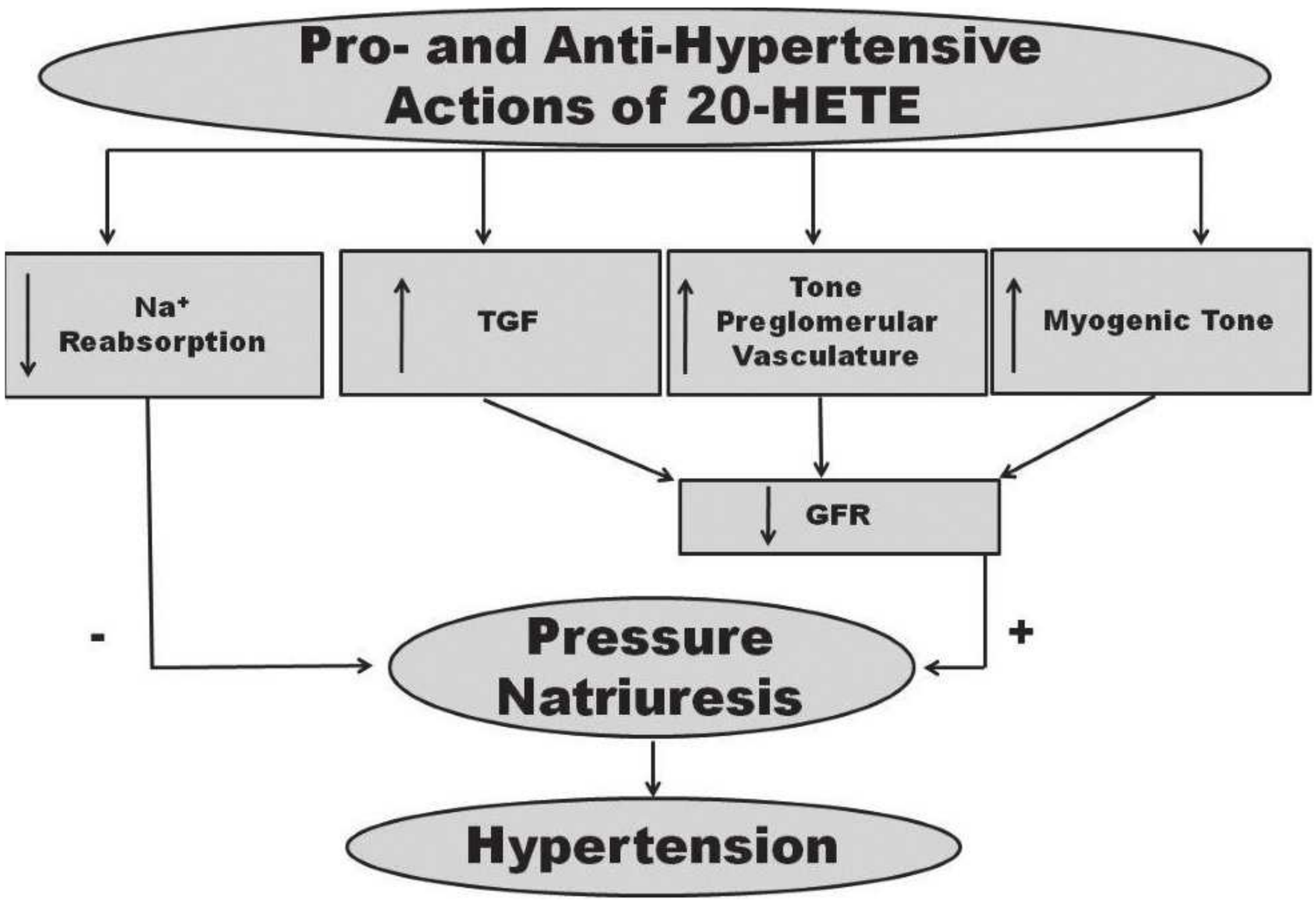

FIGURE 39.19 Summary of the pro- and antihypertensive actions of 20-HETE. 20-HETE produced in the renal tubules inhibits sodium transport and lowers blood pressure. In the renal vasculature and glomerulus, 20-HETE is a constrictor that lowers glomerular filtration rate, promotes sodium retention, and increases arterial pressure. In the peripheral circulation, 20-HETE increases vascular tone and increases blood pressure. *TGF*, tubuloglomerular feedback; *TPR*, total peripheral resistance.

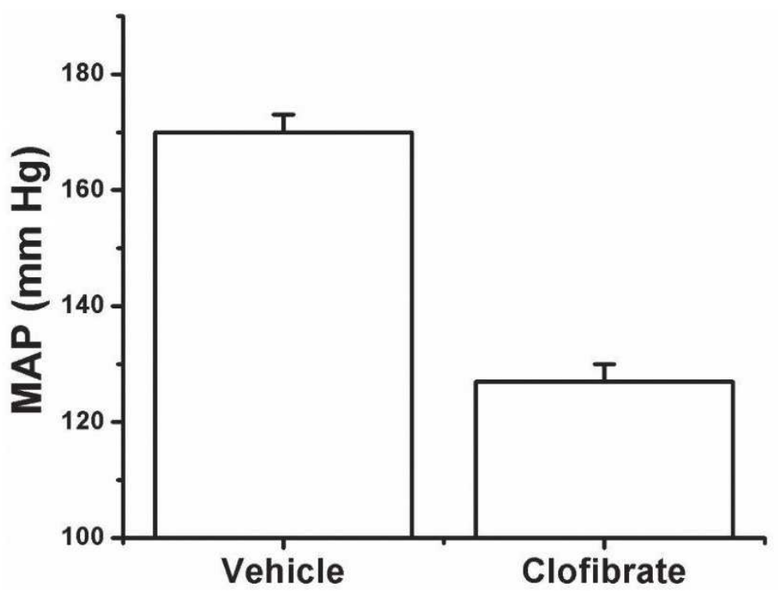

FIGURE 39.20 Left: effect of induction of the renal production of 20-HETE and expression of CYP4A protein with clofibrate on mean arterial pressure (MAP) of Dahl S rats fed a high-salt diet for 4 weeks. (Redrawn from Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev.* 2002;82:131–185.)

of Dahl S rats and this contributes to elevated sodium reabsorption.[102] Enhanced 20-HETE synthesis improves pressure-natriuresis and lowers BP in Dahl S rats (Fig. 39.20) whereas inhibitors of 20-HETE production promote the development of hypertension in Lewis rats.[102]

Studies in humans also suggest that CYP metabolites may play a role in sodium homeostasis. Urinary 20-HETE excretion is regulated by salt intake and is differentially regulated in salt-sensitive versus salt-resistant subjects.[103,104] Moreover, there appears to be a strong negative relationship between the excretion of 20-HETE and body mass index (BMI), suggesting that some factor related to obesity may be responsible for decreased synthesis or excretion of this eicosanoid in hypertension.[103,104] These observations support the possibility that impaired renal production of 20-HETE could contribute to impaired renal-pressure natriuresis in human hypertension, especially when associated with obesity. However, further mechanistic studies are needed to test the importance of 20-HETE in human hypertension.

Oxidative Stress

Recent studies suggest that ROS may play a role in the initiation and progression of cardiovascular dysfunction associated with diseases such as hyperlipidemia, diabetes mellitus, and hypertension.[105–108] In many forms of hypertension, the increased ROS are derived from NAD(P)H oxidases, which could serve as a triggering mechanism for uncoupling endothelial NOS by oxidants.[105,106]

ROS produced by migrating inflammatory cells and/or vascular cells have distinct functional effects on each cell type.[105] These effects include endothelial dysfunction, renal tubule sodium transport, cell growth, migration, inflammatory gene expression, and matrix regulation. ROS, by renal hemodynamics and renal tubule cell function (Fig. 39.21),

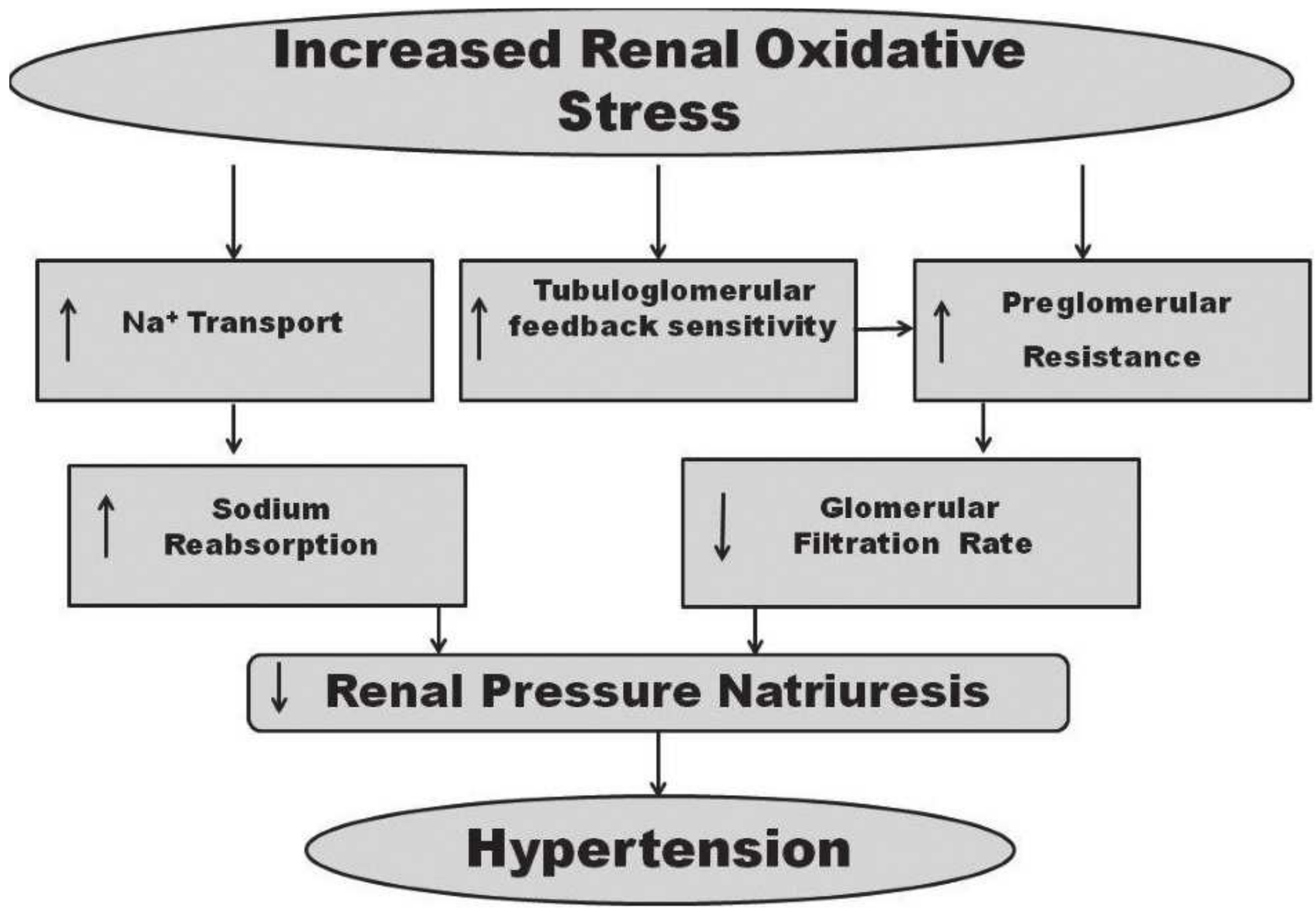

FIGURE 39.21 Renal mechanisms whereby reactive oxygen species impair pressure natriuresis and increase blood pressure. An increase in renal oxidative stress impairs renal pressure natriuresis by increasing renal vascular resistance or enhancing tubuloglomerular feedback, both of which decrease the glomerular filtration rate. Renal oxidative stress also reduces sodium excretion by direct effects to increase renal tubular reabsorption.

can play a role in altering renal pressure natriuresis and BP regulation.[105,108–111]

Growing experimental evidence supports a role for ROS in various animal models of sodium-sensitive hypertension.[105–111] The Dahl salt-sensitive (S) rat has increased vascular and renal superoxide production and increased levels of H_2O_2. The renal protein expression of superoxide dismutase (SOD) is decreased in the kidney of Dahl S rats, and long-term administration of Tempol, a superoxide mimetic, significantly decreases arterial pressure and renal damage. Another salt-sensitive model, the stroke-prone spontaneously hypertensive rat (SP-SHR), has elevated levels of superoxide and decreased total plasma antioxidant capacity. Superoxide production is also increased in the deoxycorticosterone acetate (DOCA)-salt hypertensive rat. Treatment of the DOCA-salt rats with apocynin, an NADPH oxidase inhibitor, decreases aortic superoxide production and arterial pressure.

The importance of oxidative stress in human hypertension is unclear. An imbalance between total oxidant production and the antioxidant capacity in human hypertension has been reported to occur in some but not all studies.[105,106] The equivocal findings in human studies are most likely due to difficulty of assessing oxidative stress in humans. Moreover, most recent human studies have found that vitamin E and C supplementation has little or no effect on BP.[105,106] However, the relatively low doses of vitamin E and C used in many of these studies are weak antioxidants. Thus, further clinical studies are necessary to determine the quantitative role of oxidative stress in human hypertension.

Inflammatory Cytokines and the Immune System

Although inflammation and the immune system were first associated with hypertension over four decades ago, growing evidence over the last 5 years have shown that both innate and adaptive immunity directly contribute to hypertension and renal injury.[112,114–116] Inflammatory cells, including macrophages and T cells, have been reported to accumulate in the kidney of hypertensive animals.[116] Additional support for a role for cytokines in hypertension are findings that plasma levels of proinflammatory cytokines correlate with increased BP in human hypertension and in some experimental animal models of hypertension.[117,118] Moreover, several studies have demonstrated that chronic increases in plasma cytokines, comparable to concentrations observed in the hypertension associated with hypertension preeclampsia, cause significant and sustained increases in BP.[117,118]

Lee and coworkers[119] found that hypertension caused by chronic ANGII excess may depend, at least in part, on

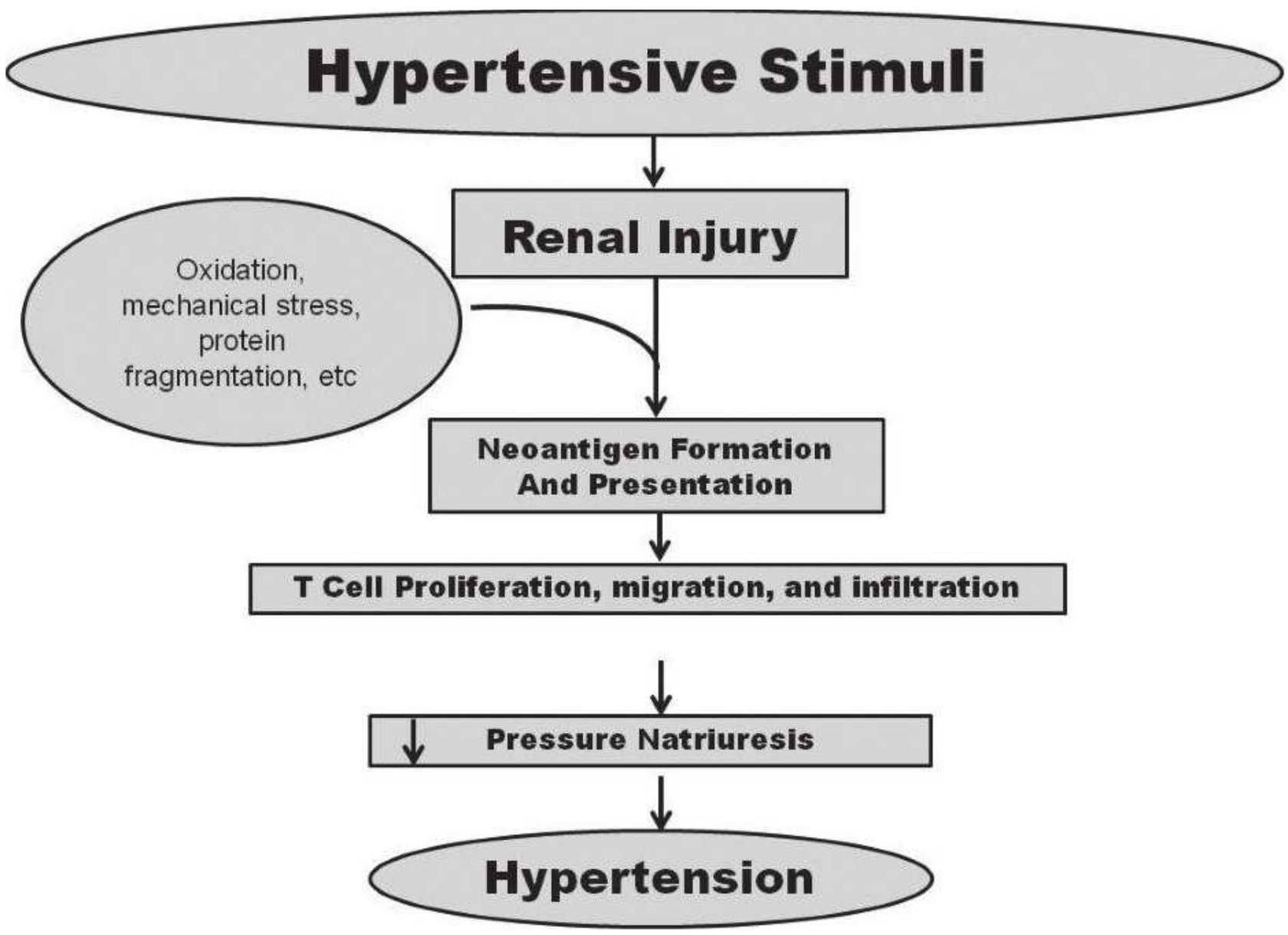

FIGURE 39.22 Proposed role of T cells and inflammation in progression of chronic hypertension. Initial hypertensive stimuli leads to renal injury, neoantigen formation, and eventual T cell activation within the kidney. T cell–derived signals promote entry of other inflammatory cells such as macrophages which result in renal vasoconstriction and sodium reabsorption, thereby increasing the severity of hypertension. (Redrawn from Granger JP, Hall JE. Role of the kidney sodium and fluid excretion in hypertension. In: Lip GYP, Hall JE, eds. *Comprehensive Hypertension*. New York: Elsevier; 2007.)

the presence of interleukin 6 (IL-6). Mice with knockout of IL-6 had significantly lower BP than wild-type mice during 2 weeks of ANGII infusion. Although these findings demonstrate a significant role for IL-6 in mediating the chronic hypertensive response to ANGII in mice, the importance of inflammatory cytokines in the pathogenesis and progression of the various forms of human hypertension is unclear and is currently an area of active investigation.

Several recent studies have demonstrated that T cells play an important role in the progression of hypertension.[115,120] Harrison and colleagues proposed that hypertensive stimuli lead to renal injury, neoantigen formation, and eventual T cell activation within the kidney.[120] T cell–derived signals promote entry of other inflammatory cells such as macrophages which result in renal vasoconstriction and increased sodium reabsorption, thereby increasing the severity of the hypertension (Fig. 39.22). Supporting this concept is a recent report that RAG-1–/– mice, which lack T cells and B cells, do not develop the degree of hypertension in response to ANGII infusion as the wild type mice, an observation that was attributed to lack of T cells (Fig. 39.23).[121] Moreover, chronic ANGII infusion was associated with a greater number of activated T cells as well as increased Rantes, a chemotoxic protein, in the vasculature and perivascular fat. These observations were confirmed by Crowley et al. using a model very similar to the RAG-1–/– mice.[121] They reported that ANGII hypertension, renal injury, left ventricular hypertrophy, and cardiac fibrosis were prevented in SCID mice lacking T cells.

Although there is growing evidence that the immune system may play a role in the progression of hypertension, the mechanisms by which hypertension stimulates an immune response remain unclear, but might involve the formation of neoantigens that activate adaptive immunity. Moreover, although findings in experimental models of hypertension are intriguing, the importance of the immune system in the pathogenesis of essential hypertension in humans remains to be determined.

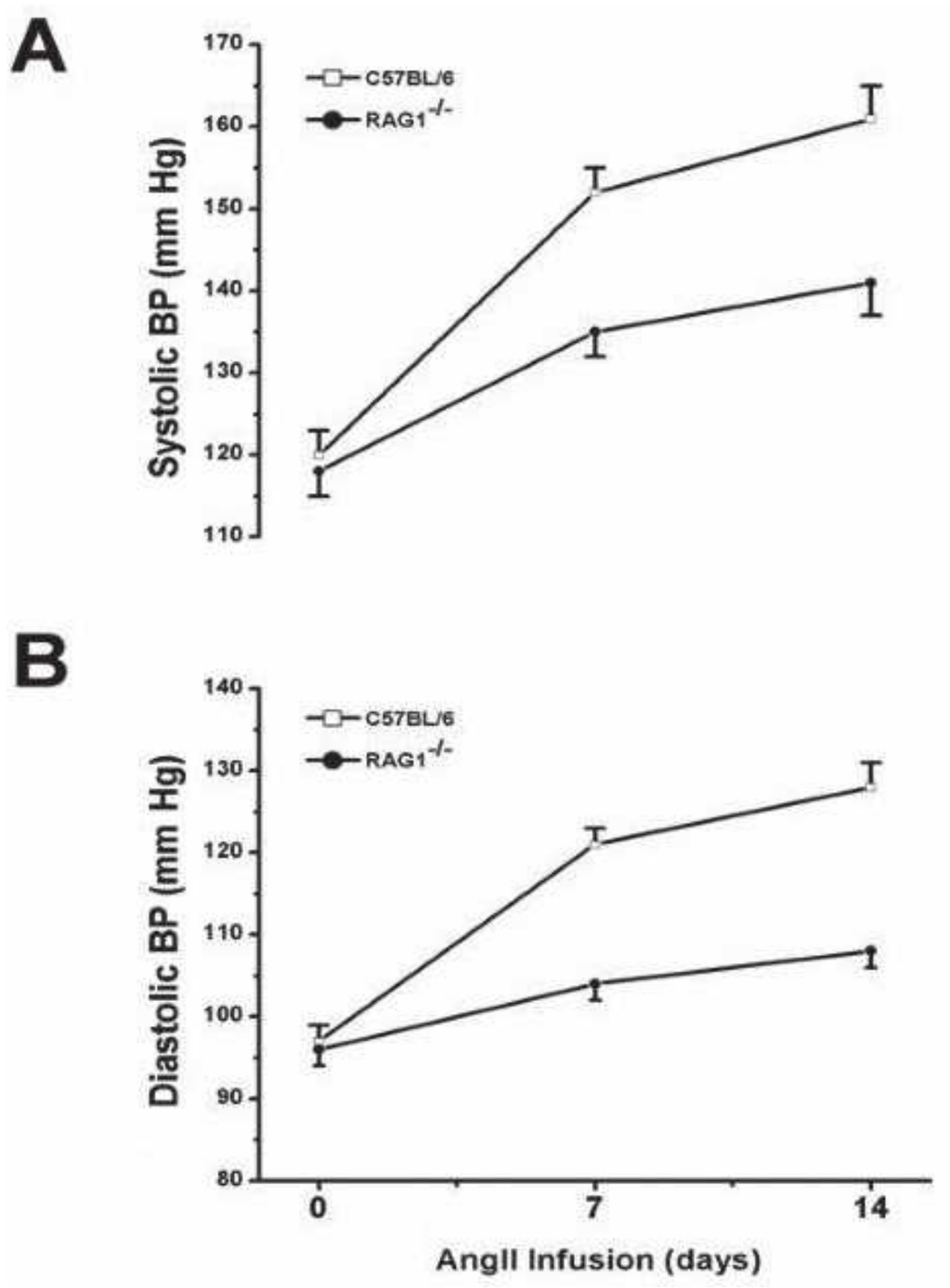

FIGURE 39.23 C57BL/6 and RAG-1–/– mice were treated for 14 days with 490 ng/min/kg angiotensin II, which was administered subcutaneously via osmotic minipump. (Redrawn from Guzik TJ, Hoch NE, Brown KA, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med*. 2007;204:2449–2460.)

Vascular Endothelial Growth Factor

Although vascular endothelial growth factor (VEGF or VEGF-A) is known to regulate angiogenesis and arteriogenesis, experimental studies over the last decade have suggested that VEGF may also affect renal function and BP regulation.[123,124] VEGF belongs to a family of secreted glycoproteins, including VEGF-B, C, D, and placenta growth factor (PlGF). VEGF signaling is mediated via two receptors, VEGFR1/Flt1 and VEGFR2/Flk1. An array of VEGF pathway inhibitors have been developed to block formation of tumor blood vessels and cause tumor regression. Although hypertension appears to be one of the most common side effects of VEGF inhibitors, the mechanisms underlying the increase in BP in response to VEGF pathway inhibitors have not been fully elucidated.[125,126] Because the endothelium is a major target for the actions of VEGF, it is likely that decreases in the production of endothelium-derived relaxing factors such as NO or enhanced production of vasoconstricting factors such as endothelin play a role in the hypertensive response to drugs that block the VEGF pathway (Fig. 39.24). Facemire and colleagues reported that administration of a specific antibody against the major VEGF receptor, VEGFR2, to normal mice caused a rapid and sustained increase in BP that was associated with reductions in expression of endothelial and neuronal NO syntheses in the kidney (Fig. 39.25).[127] They also reported that L-NAME administration abolished the difference in BP between the vehicle- and anti-VEGFR2–treated groups.[127] These findings suggest that VEGF, acting via VEGFR2, plays a critical role in influencing basal levels of BP control by enhancing NOS expression and NO activity. Moreover, the results suggest that reducing NO production and/or availability may be one mechanism underlying hypertension caused by anti-angiogenic agents targeting VEGF.[122]

Another important endothelial-derived factor that may play a role in mediating the hypertension produced by VEGF inhibition is the vasoconstrictor ET-1.[122] A study by Murphy et al. suggests an important role of ET-1 in mediating the hypertension in a model of preeclampsia that has elevated levels

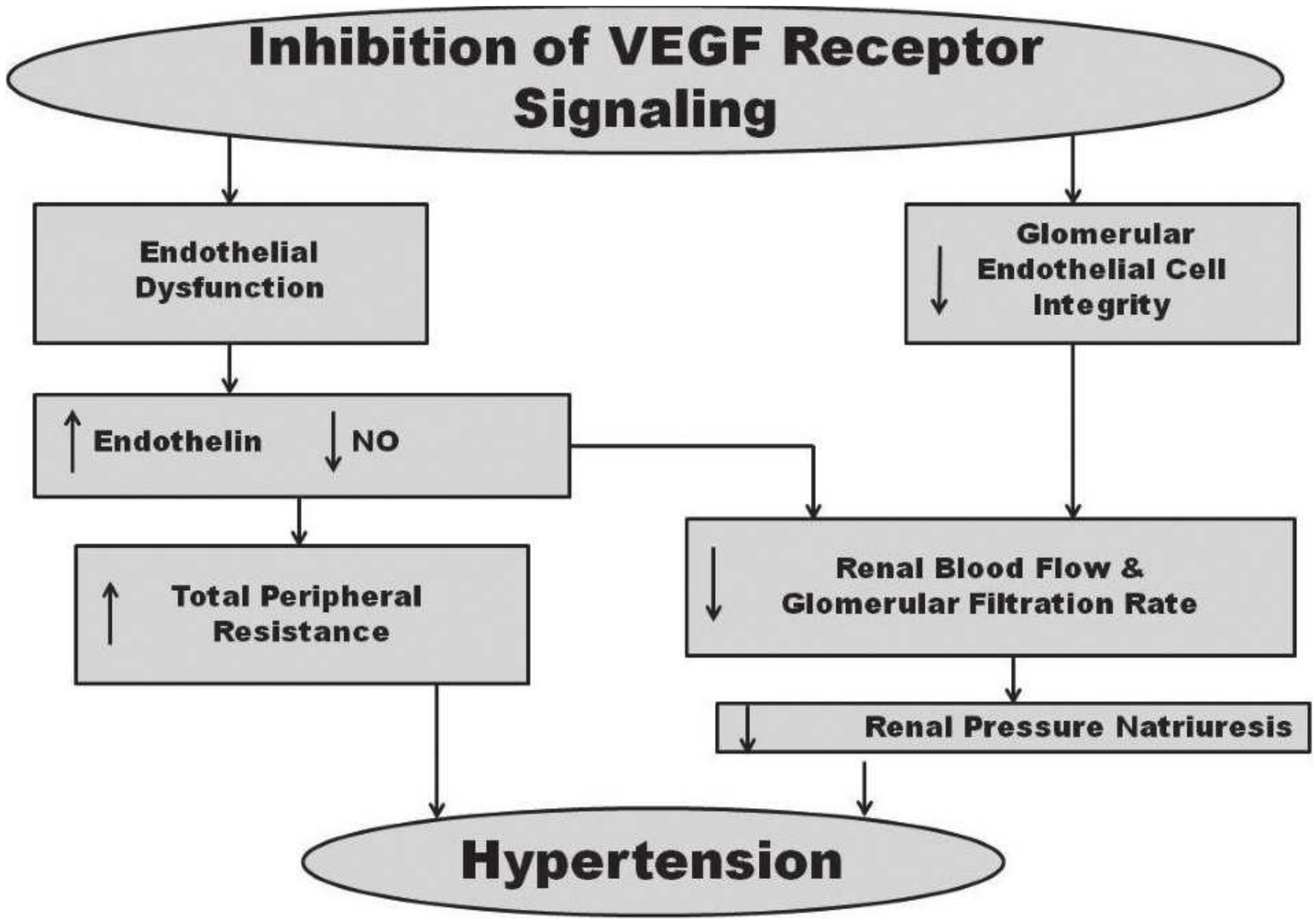

FIGURE 39.24 Potential mechanisms whereby inhibitors of vascular endothelial growth factor (VEGF) receptor signaling raise blood pressure. Blockade of VEGF receptors results in endothelial dysfunction leading to decreased production of endothelium-derived relaxing such as nitric oxide and prostaglandin or enhanced production of vasoconstrictor factors such as thromboxane and endothelin. Inhibitors of VEGF signaling may also result in alterations in glomerular structure and function. These changes may elevate blood pressure by reducing renal blood flow and GFR and impairing the kidney's ability to excrete sodium and water (depicted by a decrease in the pressure natriuresis relationship).

of the soluble VEGF receptor antagonist, sFlt-1.[128] Kappers et al. also reported that sunitinib, an inhibitor of tyrosine kinases including the VEGF receptor, induces a reversible rise in BP in patients and in rats associated with activation of the endothelin-1 system and generalized microvascular dysfunction.[129] Finally, deBeers and colleagues recently reported that VEGF inhibition with sunitinib in pigs results in endothelin-mediated hypertension.[130] Thus, another potential mechanism whereby VEGF blockade could increase BP is by enhancing ET-1 synthesis.[122,131]

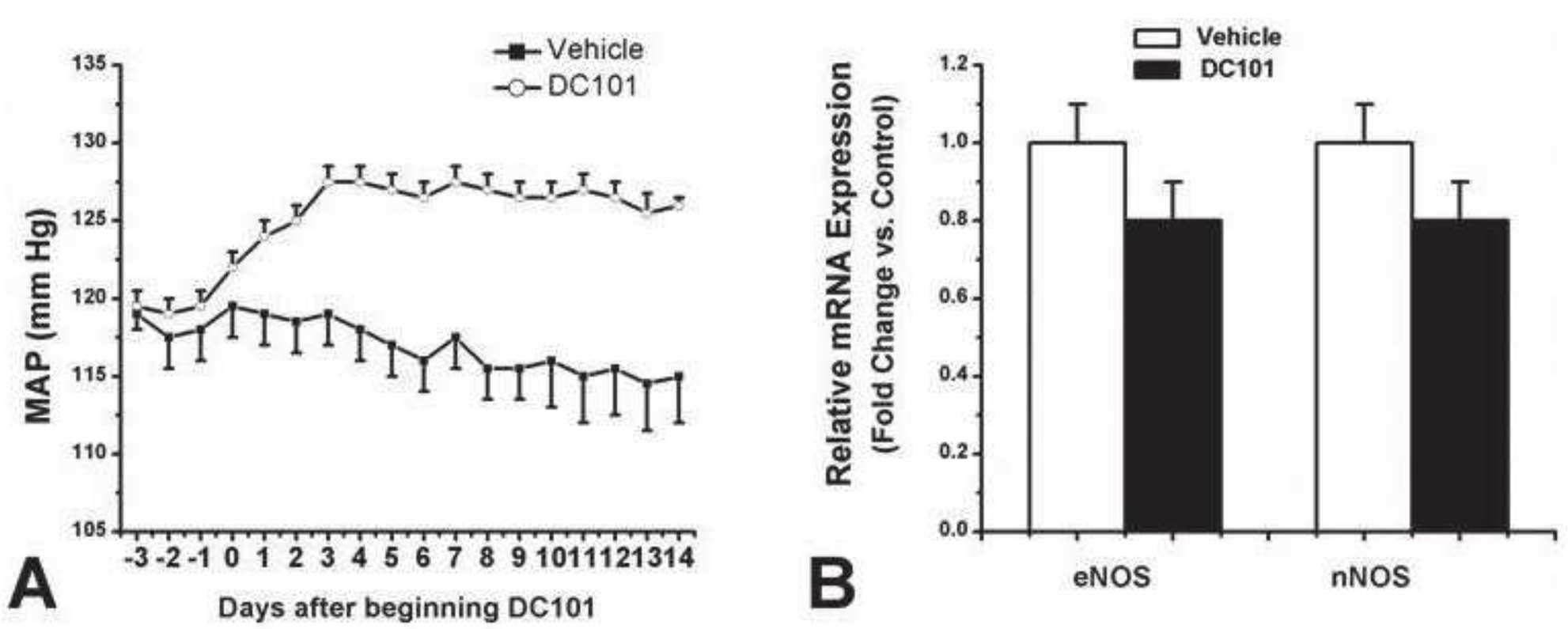

FIGURE 39.25 Effect of vascular endothelial growth factor receptor 2 inhibition on mitogen-activated protein and nitric oxide synthase mRNA expression in kidney in mice. (Redrawn from Facemire CS, Nixon AB, Griffiths R, et al. Endothelial growth factor receptor 2 (VEGFR2) controls blood pressure by regulating nitric oxide synthase expression. *Hypertension*. 2009;54(3):652–658.)

Although it is thought that the blockade of VEGF-A signaling pathway plays a critical role in the hypertension produced by VEGF inhibitors, a study by Machnik and colleagues suggests a role for VEGF-C.[132] The authors propose that macrophages regulate salt-dependent volume and BP by a VEGF-C dependent buffering mechanism. They suggest that VEGF-C, which is produced by macrophages, stimulates lymphatic vessel growth, creating a third fluid compartment that buffers the increased total body sodium and volume and buffers the high BP in response to increases in sodium intake.[132] This novel mechanism could potentially serve as an additional extrarenal mechanism that prevents changes in BP in response to increases in sodium intake. Moreover, loss or abnormalities in this putative sodium buffering pathway could be another potential mechanism for salt-sensitive hypertension. Indeed, Machnik and colleagues reported that macrophage depletion or inhibition of VEGF-C signaling increased BP in response to a high-sodium diet. The authors suggested that increase in BP was due to a decrease in lymphatic vessel growth and a reduction in the fluid compartment.[132] However, the fact that macrophage depletion or inhibition of VEGF-C signaling caused a chronic increase in BP indicates that inhibition of VEGF-C signaling also reduces the kidney's ability to excrete sodium and water. Future studies will be necessary to discern the renal mechanisms whereby macrophage depletion or inhibition of VEGF-C signaling alters the pressure natriuresis relationship.

CONCLUSION

Experimental and theoretical evidence strongly support a central role for the kidneys in the long-term regulation of body fluid volume and arterial pressure. A pivotal part of the renal–body fluid feedback control system for long-term BP regulation is the renal-pressure natriuresis mechanism. Increases in renal perfusion pressure lead to significant increases in sodium and water excretion, an effect that is thought to be mediated by increases in medullary blood flow and renal interstitial pressure. Renal pressure natriuresis is abnormal in all types of experimental and clinical hypertension. Hypertension is an important compensatory mechanism that allows maintenance of sodium balance when renal pressure natriuresis is impaired. Impaired renal pressure natriuresis and chronic hypertension can be caused by factors that either reduce GFR and/or increase tubular reabsorption. A shift of pressure natriuresis can occur as a result of intrarenal abnormalities such as enhanced formation of angiotensin II, ROS, and endothelin (via ET_A receptor activation) or decreased synthesis of NO or natriuretic prostanoids. In other instances, the altered kidney function is caused by extrarenal disturbances, such as increased SNS activity or excessive formation of antinatriuretic hormones such as aldosterone.

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CHAPTER

40 Hypertension in Chronic Kidney Disease

Peter N. Van Buren • Robert D. Toto

INTRODUCTION

The prevalence of hypertension in patients with chronic kidney disease (CKD) exceeds that of the general population. Although hypertension is believed to be the etiology of kidney disease in many of these patients, hypertension is often the consequence of kidney disease stemming from any etiology. The pathophysiology of hypertension in CKD is related to multiple factors, including expanded extracellular volume from sodium retention, activation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS), and imbalances in vasoconstrictor and vasodilator substances that regulate peripheral vascular resistance. Untreated or poorly controlled hypertension in CKD patients is associated with adverse outcomes including deterioration of renal function, development of left ventricular hypertrophy (LVH), and increased mortality. Recent clinical trials have investigated the role of various antihypertensive treatments and blood pressure targets in preventing these outcomes. The purpose of this chapter is to review the epidemiology, pathophysiology, and management of hypertension in patients with CKD. Because diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in the United States, the unique aspects of its pathophysiology and the treatment of hypertension in this setting warrant a separate discussion. Although mentioned briefly herein, the reader is referred to a recent extensive review of this topic.[1]

EPIDEMIOLOGY

Chronic Kidney Disease

Hypertension is a prevalent comorbidity associated with CKD. The prevalence of hypertension in the overall U.S. population, according to the National Health and Nutrition Examination Survey (NHANES) data from 2007 to 2008, is 29%.[2] Data published by the United States Renal Data System (USRDS) according to NHANES data collected from 1999 to 2006 indicate that the prevalence of hypertension in the non-CKD population is 23.3%.[3] In contrast, the prevalence of hypertension in the CKD population is much higher. The prevalence of hypertension increases with each stage of CKD and is estimated to be 35.8%, 48.1%, 59.9%, and 84.1% in patients with stage I, II, III, and IV/V CKD, respectively. Although the awareness of hypertension among patients with stage III and IV CKD was similar to that in the non-CKD population, the failure to control hypertension (defined as blood pressure [BP] 130/80 mm Hg for those with CKD and 140/90 mm Hg for those without CKD) is higher among the CKD patients.

The Chronic Renal Insufficiency Cohort (CRIC) is a National Institutes of Health (NIH) sponsored prospective observational study among 3,612 patients with an estimated glomerular filtration rate (GFR) of 20 to 70 mL per minute designed to better understand factors responsible for CKD progression and cardiovascular disease.[4] Although the sample population for CRIC is smaller than NHANES, the detailed ascertainment of individual demographics, comorbidities, medication use, and laboratory analysis provides useful information for understanding the relationship between CKD and hypertension control in a population that is established in the health care system. In this population, 85.7% of patients are hypertensive based on the definition of a BP >140/90 mm Hg or with the use of an antihypertensive medication. The percentage of patients from CRIC that are aware of their diagnosis of hypertension (98.9%) and who are treated for hypertension (98.3%) is higher than in the NHANES data. However, the control of hypertension in CRIC patients is still suboptimal, with 67.1% having BP >130/80 mm Hg and 46.7% having BP >140/90 mm Hg. The CRIC data indicate that older age, African American race, and a greater amount of proteinuria are risk factors for a failure to control BP to either 140/90 or 130/80 mm Hg.[5] Overall, the epidemiologic evidence from NHANES and CRIC identifies that hypertension is a significant burden for patients with CKD and the health care providers responsible for managing these patients.

The long-term consequences of uncontrolled hypertension highlight the significance of this disease in CKD patients. Studies have reported that elevated systolic BP increases the incidence of CKD,[6,7] the progression of CKD,[8] and the incidence of ESRD.[9] Furthermore, hypertension is reported to be the second leading cause of ESRD in the

United States based on USRDS data.[10] However, it has been difficult to establish the independent effect of BP in CKD from effects related to the degree of baseline proteinuria.[11]

Elevated BP is also a risk factor for cardiovascular events, including stroke and myocardial infarction among CKD patients; however, the exact relationship between BP and outcomes is not consistent among studies. A J-shaped relationship between cardiovascular morbidity and BP has been shown,[12] suggesting that the highest risk for an outcome occurs at the highest and lowest BP, whereas the lowest risk for an outcome occurs at an intermediate BP. In one longitudinal study of patients with stage III and IV CKD, systolic BP >130 mm Hg was a predictor for an incident stroke; however, those with systolic BP <120 mm Hg had a greater risk than those with a systolic BP between 120 and 129 mm Hg.[12] In contrast, a post hoc analysis of the Perindopril Protection Against Recurrent Stroke Study (PROGRESS), a prospective randomized placebo controlled trial of the effects of perindopril on stroke among patients with a prior history of cerebrovascular disease, CKD patients had a reduced risk for a recurrent stroke across all strata of systolic BP. Moreover, there was no increase in the risk for recurrent stroke in those who achieved a systolic BP <120 mm Hg compared to higher achieved BP levels.[13]

When considering mortality as an outcome, some observational data confirm the association with low BP and events.[14,15] One study showed an increased mortality risk in subjects with baseline systolic BP in the lowest quartile (<133 mm Hg) compared to the other quartiles, and another study showed the highest mortality risk with a systolic BP <110 mm Hg and >180 mm Hg in a cohort of CKD patients inclusive of both diabetic and nondiabetic CKD. The effect from the latter study was strongest in older patients with advanced CKD and without proteinuria, thus limiting the generalizability of this finding.

In summary, observational studies demonstrate an increased risk for cardiovascular morbidity and mortality at BP levels considered hypertensive for the general population. However, it is unclear if an aggressive reduction of BP translates into decreased cardiovascular morbidity and mortality in the CKD population. The evidence from randomized clinical trials on specific BP targets is discussed in the treatment section of the chapter.

Ambulatory Blood Pressure Measurements and Chronic Kidney Disease

Although CKD patients frequently have BP measured in an office setting, it is important to recognize some of the limitations that can arise in this context. Of utmost importance to the topic of hypertension is the relationship between home and clinic BP measurements. Home and ambulatory measurements better predict the presence of end organ damage such as proteinuria compared to clinic measurements.[16] Ambulatory BP measurements also predict which CKD patients with an elevated clinic BP have a greater risk for progression to ESRD or reaching the composite outcome of ESRD or death.[17] Thus, a comprehensive ascertainment of BP burden requires the consideration of more than measurements obtained in the office.

Hemodialysis Patients

ESRD patients on hemodialysis (HD) have an annual mortality rate close to 20%, with cardiovascular disease and infections accounting for the highest percentage of deaths.[18] Although the prevalence of hypertension in the HD population is near 90%,[19] a target BP to improve outcomes has yet to be identified. Early epidemiologic studies showed that for BP measurements obtained in the HD unit, low systolic BP and systolic BP in excess of 200 mm Hg were associated with the highest mortality, particularly in older patients and diabetics.[20,21] However, it has also been shown that uncontrolled hypertension with systolic BP in excess of 140 mm Hg results in the increased incidence of LVH, de novo ischemic heart disease, and de novo cardiac failure.[22] It must be considered that low systolic BP can be a manifestation of decreased cardiac output, resulting from the structural and functional consequences of long-standing uncontrolled hypertension, which would explain its association with increased mortality.

Similar to pre-ESRD CKD patients, the timing and location of BP measurements are also important considerations in HD patients. Home and ambulatory BP measurements during the interdialytic time period, in comparison to individual HD-unit measurements, are better predictors of mortality.[23] The significance of BP changes during HD treatments has also been recently investigated. Intradialytic hypertension, increases in BP from pre- to post-HD, has been associated with increased short-term (6 month) morbidity and mortality in prevalent HD patients and decreased 2-year survival in incident HD patients.[24,25] There is evidence that mechanisms responsible for the phenomenon include extracellular volume overload[26] or increased vasoconstriction related to intradialytic endothelin-1 surges as a manifestation of endothelial cell dysfunction.[27–29] Patients with intradialytic hypertension have also been shown to have increased ambulatory blood pressure and greater impairment in underlying endothelial cell function during the interdialytic time period.[30,31] Additional mechanisms that have been proposed, but that have yet to be confirmed, include increased activity of the RAAS and SNS, changes in electrolytes during HD, and removal of antihypertensive medications during the course of HD.[32]

PATHOPHYSIOLOGY

The etiology of hypertension in CKD is multifactorial and related to both increases in cardiac output and increased peripheral vascular resistance (Table 40.1). Positive sodium balance can affect either component (Fig. 40.1), and achieving sodium balance remains a primary target of managing hypertension in patients with CKD. Multiple other mechanisms, including activation of the SNS and RAAS, endothelial cell dysfunction related to imbalances in vasodilator and vasoconstrictor substances, and increased oxidative stress, can modify the effects of each other and result in hypertension, particularly when these systems are disrupted in the context of CKD. A comprehensive summary of these processes is outlined in Figure 40.2.

40.1 Etiology of Hypertension in Chronic Kidney Disease

Extracellular volume overload
Increased renin-angiotensin-aldosterone system activity
Increased sympathetic nervous system activity
Endothelial cell dysfunction
    Increased endothelin-1 release
    Accumulation of asymmetric dimethylarginine
    Decreased production of nitric oxide
Oxidative stress
Increased vasopressin release
Hypertensinogenic drugs (erythropoietin)

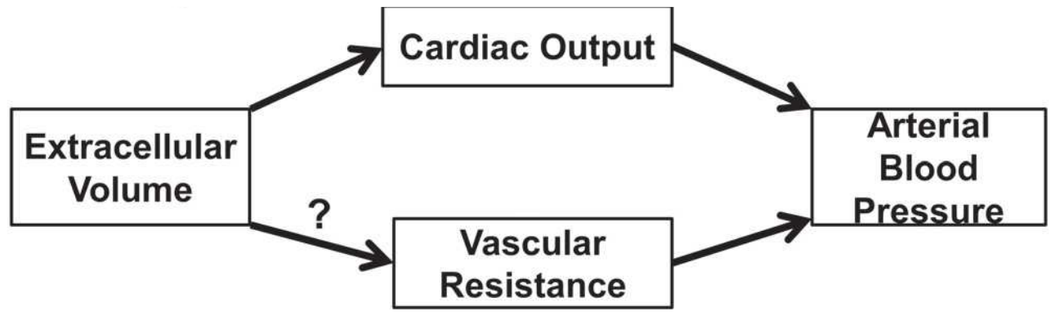


FIGURE 40.1 Blood pressure is directly related to cardiac output and vascular resistance. Extracellular volume increases cardiac output and, potentially, vascular resistance in patients with chronic kidney disease and end-stage renal disease. The achievement of euvolemia and a reduction in vascular resistance remain the primary target of blood pressure reduction in these patient populations.

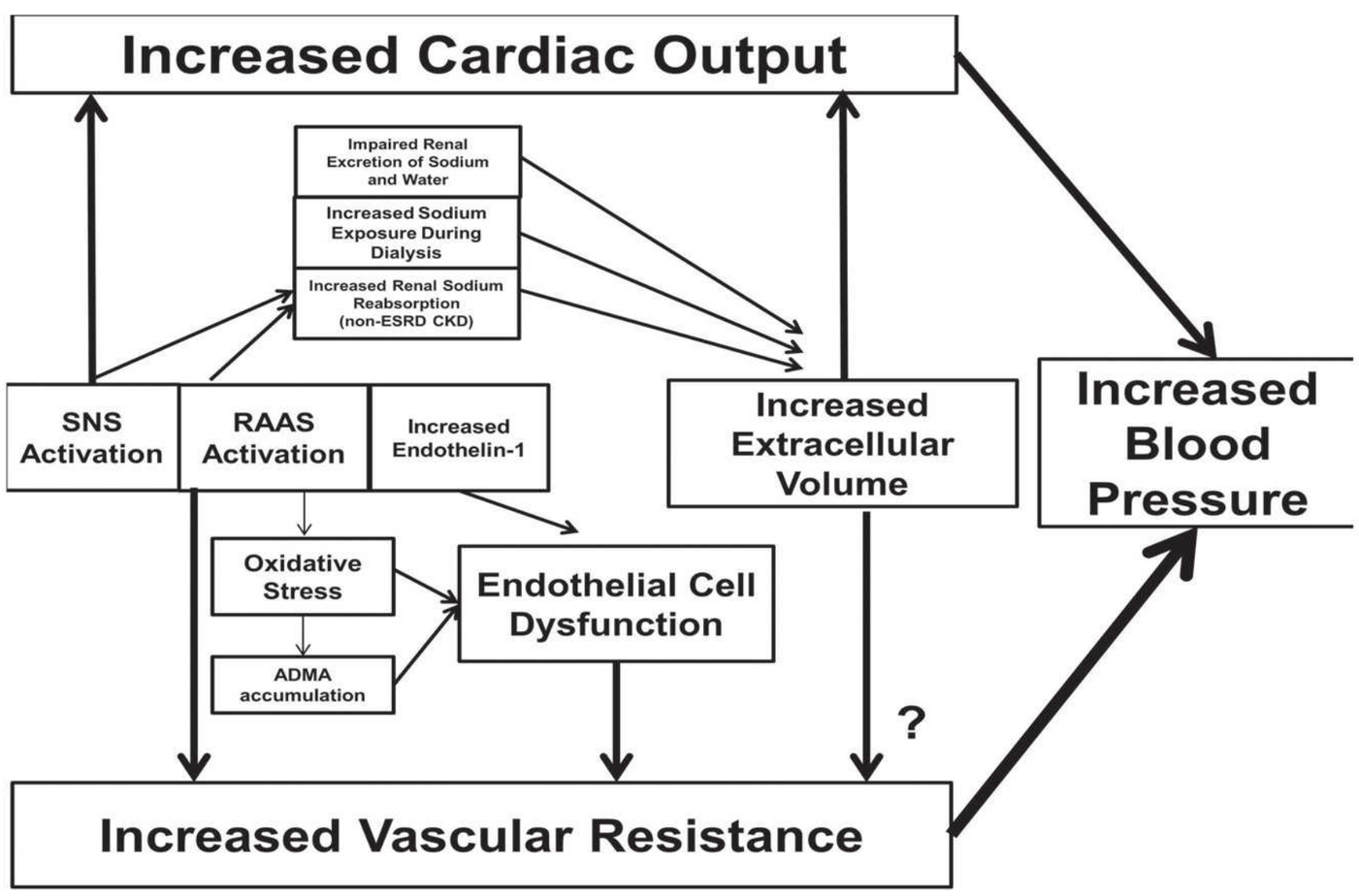


FIGURE 40.2 Both increased cardiac output and increased vascular resistance contribute to increased blood pressure in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD). Increased cardiac output results primarily from increased extracellular volume. Activation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) contributes to renal sodium reabsorption in non-ESRD CKD patients. In both CKD and ESRD patients, the decreased renal excretion of sodium and water from the decreased glomerular filtration rate also contributes to increased extracellular volume. Finally, in ESRD patients on hemodialysis, the transfer of sodium from the dialysate to the plasma can promote thirst and interdialytic weight gain.

Vascular resistance can be increased by the activation of the SNS and the RAAS, as well as by enhanced vasoconstriction caused by endothelial cell dysfunction. Increased RAAS activity increases angiotensin (Ang) II, which binds to receptors on vascular smooth muscle cells and causes vasoconstriction. Ang II also activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and increases oxidative stress, which is believed to be responsible for the increase in the inhibitor of endothelial nitric oxide synthase, asymmetric dimethylarginine. Endothelial cell dysfunction refers to an imbalance in mediators released from the endothelial cells of which one of the results is increased vasoconstriction. An increase in asymmetric dimethylarginine (ADMA) interferes with the production of the vasodilator nitric oxide (NO). Reactive oxidative species also combine with NO to form peroxynitrite and to prevent NO that has already been produced from binding to its receptor. In the context of impaired production and function of NO, there is less opposition to the vasoconstrictive effects of endothelin (ET)-1. Extracellular volume overload has varying effects on vascular resistance, but there is evidence that there is a delayed increase in vascular resistance that follows volume-induced increases in blood pressure and acts to sustain hypertension.

Basic Concepts

BP in humans is determined both by the cardiac output and by peripheral vascular resistance. Cardiac output is dependent on the intravascular component of the extracellular space as well as the heart rate; and peripheral resistance is dependent on functions of the vascular endothelial and smooth muscle cells in response to the actions of various vasoactive mediators (both vasoconstrictors and vasodilators). Although alteration of either cardiac output or vascular resistance would initially be expected to affect BP in a concordant direction, a healthy kidney can adapt to short-term changes in BP to restore normotension. An increase in renal sodium excretion is the expected response to increased BP in healthy individuals. This pressure natriuresis allows for the restoration of extracellular volume and BP following an increase in cardiac output. However, in CKD, this homeostatic mechanism is impaired, and the kidney fails to sufficiently excrete sodium loads. In CKD and ESRD, it is hypothesized that an increase in cardiac output initiates the increase in BP, but ultimately, an increase in vascular resistance sustains the BP elevation.[33,34] Accordingly, addressing both volume status and the degree of peripheral vasoconstriction are necessary to control BP in these patient populations.

Sodium Balance and Volume Overload

Sodium balance is an important aspect in BP control in CKD and ESRD. Because of the kidney's ability to excrete sodium, healthy individuals can tolerate large amounts of sodium intake without significant increases in BP.[35] However, in the presence of kidney disease, BP is highly dependent on extracellular volume. Rats that have undergone 70% renal ablation develop severe hypertension on a high sodium diet, but hypertension is completely prevented in these animals while on a low sodium diet.[36] A lower hematocrit found in the animals on a high sodium diet suggested that extracellular volume overload was present and likely the mechanism responsible for the increased BP. Another experiment with a renal ablation model demonstrated that increased cardiac output is responsible for the initial increase in BP during acute salt loading, but increased peripheral resistance (which occurs after BP has already become increased) is responsible for the maintenance of elevated BP even after cardiac output has returned to normal.[37]

Chronic Kidney Disease Patients Not on Hemodialysis

Because CKD patients are limited in the amount of renal sodium excretion, BP and sodium balance are interrelated. Among CKD patients, those with more severe renal impairment experience greater increases in BP in response to a sodium load. Although the pressure natriuresis curve serves to maintain sodium balance in healthy individuals, CKD patients require much higher BP increases than healthy individuals to augment their renal sodium excretion. Similarly, the degree of blood volume expansion required to induce a natriuresis is higher in patients with severe renal impairment compared to those with moderate renal impairment. Overall, these findings show that the effects on BP related to sodium intake ("salt sensitivity") are augmented with the progression of renal disease.[38] During the early stages of CKD, despite suboptimal renal sodium excretion, some patients remain normotensive despite an increase in cardiac output because of a reduction in peripheral resistance.[33] Although BP is sustained in the normotensive range, these patients, who are already limited in their sodium excretion because of reductions in GFR, fail to induce the necessary pressure natriuresis to re-establish sodium balance. Consequently, further increases in sodium loading result in increased cardiac output, peripheral resistance, and BP.

When subjects with CKD increase dietary sodium ingestion from 20 to 120 mEq per day, they experience an increase in BP that is not seen even in healthy subjects who increase their dietary sodium ingestion to levels as high as 1200 mEq per day.[39] Although both groups have a similar suppression of RAAS at moderate amounts of sodium ingestion, plasma renin activity (PRA) and angiotensin (Ang) II decreased drastically in healthy subjects during periods of high sodium ingestion (1200 mEq per day).[39] Although vascular resistance could not be measured directly in this study, increased vasoconstriction in the CKD subjects appeared to be the likely mechanism responsible for the increased BP and the reduced distribution of fluid into the interstitial space. Even under clinical conditions of pharmacologic RAAS blockade, salt balance has an important role in BP. In nondiabetic proteinuric subjects with creatinine clearance ranging from 33 to 110 mL per minute ingesting a low sodium diet, a reduction in BP and proteinuria during the chronic administration of fixed doses of angiotensin converting enzyme (ACE) inhibitors is reversed during periods of increased dietary sodium ingestion.[40] As further evidence of the importance of sodium intake in relation to BP and proteinuria in CKD patients, the coadministration of hydrochlorothiazide during a high salt intake period reduces both BP and proteinuria back to values seen during the low sodium diet.

Chronic Kidney Disease On Hemodialysis

In ESRD patients with little or no renal sodium excretion, sodium removal through ultrafiltration during HD is the primary means to maintain extracellular volume status because a pressure natriuresis is not possible. Consequently, compared to healthy controls, HD patients have a significantly higher cardiac output.[41] However, the presence of hypertension among HD patients is also highly dependent on elevations in vascular resistance.[41] Extracellular volume potentially impacts both of these parameters to influence BP. When subjected to sodium loading, HD patients typically respond with an increase in both BP and cardiac output. The pattern of vascular resistance following sodium loading is more variable, but increases typically do not occur until after the elevated cardiac output has already led to an

increase in BP.[34,42] In between HD treatments, during the interdialytic period, HD patients gradually gain weight with their routine dietary intake of sodium and water. The BP patterns during a typical interdialytic period show rhythmic oscillations superimposed on a general linear increase in BP over time.[43] This pattern is modified by interdialytic weight gain such that greater weight gain is associated with an increase in the slope of BP rise.[44] Subsequently, greater increases in interdialytic weight gain have been associated with increased pre-HD systolic BP at the next HD treatment.[45] However, that increased interdialytic weight gain is also associated with a greater reduction in BP during the course of that treatment (likely as a response to the ultrafiltration required to remove the interdialytic fluid gain).[45] Such evidence supports the hypothesis that extracellular volume (through either increases in cardiac output or vascular resistance) is primarily responsible for hypertension in this population. Consequently, one HD center has described a 98% success rate in withdrawing antihypertensive medications while using the ultrafiltration that can be achieved during a cumulative weekly dialysis time of 24 hours.[46] Thus, recognition and successful attainment of a patient's dry weight can facilitate the initial BP management in most, but not all, HD patients.

Therefore, HD treatment with adequate ultrafiltration should normalize BP in many HD patients. However, the basis for this practice assumes that interdialytic sodium and fluid intake is not excessive. An insufficient time on HD needed to completely restore normal extracellular fluid volume and achieve dry weight without inducing symptomatic hypotension limits this practice. A cross-sectional study found that major differences between ultrafiltration-sensitive and ultrafiltration-resistant HD patients were the pre- and post-HD atrial natriuretic peptide (ANP) levels between groups.[47] The ultrafiltration-sensitive group had reductions in ANP during HD (as well as lower pre-HD ANP), whereas the higher pre-HD ANP levels in the ultrafiltration-resistant group persisted despite similar amounts of ultrafiltration, suggesting that this group had remaining extracellular volume contributing to the elevated BP. It should be recognized, however, that extracellular volume overload may not always manifest itself overtly. In some cases, patients may have additional extracellular volume despite appearing euvolemic on clinical exam. The Dry Weight Reduction in Hypertensive Hemodialysis Patients (DRIP) study was a randomized clinical trial in which hypertensive HD subjects were randomized to either continue their current HD and ultrafiltration prescription or have their dry weight challenged during each HD treatment over several weeks by 0.1 kg per 10 kg dry weight until symptoms developed. The subjects randomized to additional ultrafiltration demonstrated significant decreases in ambulatory systolic BP after 4 weeks and 8 weeks.[48]

The dialysate used during HD is another important factor that may contribute to hypertension in ESRD patients. Although ultrafiltration effectively removes water and sodium concurrently through convection, there may be an additional exchange of sodium from the dialysate to the patient's plasma depending on the sodium concentration gradient between the two compartments. Directly programmable ultrafiltration enables greater convective sodium removal in shorter periods of time, but increases the risk of intradialytic hypotension related to the abrupt hemodynamic changes. Although the use of higher dialysate sodium concentrations may reduce the risk of intradialytic hypotension, it may increase the risk of hypertension.[49] In contrast, the use of individualized dialysate sodium concentrations (as opposed to standardized concentrations, which may exceed the pre-HD plasma sodium of the patient) have been associated with lower pre-HD systolic BP in the context of decreased thirst and interdialytic weight gain.[50]

The Renin-Angiotensin-Aldosterone System and Hypertension

The RAAS has local and systemic effects that control BP by altering renal sodium reabsorption and vascular resistance. Renin released from juxtaglomerular cells cleaves angiotensinogen to Ang I, which is ultimately converted to Ang II by the ACE. Ang II causes vasoconstriction upon binding to angiotensin type 1 (AT1) receptors in vascular smooth muscle cells (VSMCs). Ang II increases proximal tubular reabsorption of sodium and stimulates aldosterone release from the adrenal gland, which is responsible for further sodium reabsorption in the distal nephron. Although this proposed sequence applies to the levels and activity of RAAS components in the plasma, there is also local RAAS activity. Consequently, measurements of RAAS mediators in the plasma may not always identify a disruption of the axis at the tissue level.[51]

Early evidence for systemic RAAS activation in CKD stems from studies in patients with autosomal dominant polycystic kidney disease (ADPKD). It has been shown that PRA and plasma aldosterone were higher in hypertensive ADPKD patients compared to patients with essential hypertension, and plasma aldosterone is higher in normotensive ADPKD patients compared to healthy controls (despite similar creatinine clearance between the groups).[52] Autosomal dominant polycystic kidney disease patients also manifest an accentuated response to ACE inhibitors compared to unaffected family members, further supporting the role of RAAS in the hypertension seen in ADPKD even prior to the onset of significant renal impairment.[53] However, the findings from these studies may not be entirely generalizable to more heterogeneous groups of CKD patients because the proposed renal ischemia induced by large cyst formation in ADPKD does not necessarily apply to CKD from other etiologies. There is evidence that PRA and aldosterone are higher in hypertensive CKD patients compared to healthy controls, essential hypertension patients, or even normotensive CKD patients.[54,55] However, there is also evidence that CKD patients have lower PRA compared to healthy

controls, although the values were similar to subjects with essential hypertension and normal renal function.[56] Despite these conflicting findings, increased intrarenal RAAS activity has been described in patients with hypertension and varying etiologies of CKD, including immunoglobulin A (IgA) nephropathy, membranous nephropathy, and diabetic nephropathy.[57–59]

As mentioned previously, success in managing BP can be achieved in many ESRD patients by appropriately using ultrafiltration for volume removal.[46] However, there are HD patients who remain quite hypertensive despite achieving their estimated dry weight. Previous reports have shown both increased or normal PRA in groups of HD patients compared to controls, and PRA did not consistently correlate with BP.[60,61] However, increased PRA has been demonstrated in HD patients with hypertension that is ultrafiltration resistant compared to those whose BP responds to ultrafiltration.[61] Bilateral nephrectomy, a procedure previously used for ultrafiltration-resistant hypertension in ESRD patients, has been shown to reduce PRA, Ang I and II, along with reductions in BP in these ultrafiltration-resistant hypertensive patients.[62]

Renin and Aldosterone

Although the primary action of angiotensin converting enzyme (ACE) inhibitors and ARB is to decrease the production and action of Ang II via the inhibition of the ACE enzyme or Ang II receptor, a complete perspective on the role of RAAS in hypertension associated with kidney disease warrants a discussion of the other components of the RAAS, including aldosterone and renin. Following the use of an ACE inhibitor or ARB, serum aldosterone levels typically decrease. However, in up to 40% of patients receiving these medications, aldosterone levels can rebound to pretreatment levels through a process referred to as aldosterone escape.[63] Aldosterone increases BP via enhanced sodium reabsorption in the distal nephron, but it is also likely involved in vasoconstriction via interaction with the Ang II receptor.[64] Despite evidence that CKD patients experiencing aldosterone escape may have worse control of proteinuria, systemic BP does not seem to be different from CKD patients whose aldosterone levels remain depressed following ACE inhibitor or ARB treatment.[65,66] These studies included patients with IgA nephropathy who were normotensive and had creatinine clearance >50 mL per minute or patients with early diabetic nephropathy and hypertension; such investigations have not been performed in broader groups of CKD patients. Evidence regarding the potential benefit of mineralocorticoid receptor blockers is included in the Treatment section of this chapter.

In contrast to the expected decrease in aldosterone following ACE inhibitor or ARB use, renin levels and PRA are expected to increase as a result of the blockade in events downstream from the main actions of renin. It has been demonstrated in vivo that Ang II can be generated by enzymes other than ACE.[67] This presents a potential obstacle to complete RAAS blockade if renin levels are sufficiently elevated in the context of an ACE inhibitor or ARB administration, and the possible use of add-on therapy to ACE inhibitors or ARB with a direct renin-inhibitor is further discussed in the Treatment section.

Angiotensin Converting Enzyme 2

Understanding of the RAAS continues to expand, and attention has been drawn to another enzyme in this pathway. ACE2 is a monocarboxypeptidase homolog of ACE that decreases Ang II levels by (1) increasing the degradation of Ang II to Ang 1-7, a vasodilatory and antiproliferative mediator, and (2) converting Ang I to Ang 1-9. This latter process not only prevents the conversion of Ang I to Ang II via ACE, but the increase in Ang 1-9 acts as a substrate for additional enzymatic conversion to Ang 1-7. Infusion of human recombinant ACE2 (rACE2) does not alter BP significantly in normotensive mice.[68] However, rACE2 prevents BP increases induced by the infusion of Ang II when they are infused together. Consistent with the proposed mechanism of ACE2, Ang II levels were significantly lower and Ang 1-7 levels were higher in mice receiving Ang II plus rACE2 compared to Ang II alone. ACE2 is highly expressed in the kidney and is believed to play a role in the progression of CKD via local activity of the renal RAAS. Mice that have undergone 5/6 nephrectomy have significantly reduced renal ACE2 expression and a trend toward reduced renal ACE2 activity compared to sham-operated mice.[69] Although BP was similar in nephrectomy and sham-operated mice, there was greater proteinuria in the nephrectomy mice. The proteinuria was further increased following administration of the ACE2 inhibitor. The major implications of these studies are that there may be further opportunities to reduce Ang II levels beyond the currently implemented strategies.

Sympathetic Nervous System and Hypertension

The SNS has been studied extensively as a possible contributor to hypertension in CKD and ESRD patients. In CKD patients, there is evidence for multiple pathways that the SNS may affect to increase BP. In support of the previous discussion of the effects of salt balance on BP, renal sympathetic nerve stimulation increases proximal tubular reabsorption of sodium and water.[70] Additionally, the systemic effects of the SNS include increased cardiac output and vasoconstriction. Although evidence of activated SNS activity based on elevated catecholamine levels in CKD is inconsistent and possibly confounded by decreased renal clearance of these compounds, other estimates of SNS, such as muscle sympathetic nerve activity (MSNA), confirm the hyperactive state in CKD and ESRD. The mechanisms responsible for hyperactive SNS are thought to be related to increased renal nerve activity. The kidney possesses baroreceptors and chemoreceptors that increase renal nerve firing secondary to pressure changes or metabolites produced in response to ischemia

or uremia. Animal models of renal artery stenosis, arterial ligation causing partial renal ablation, or intrarenal phenol injection all reveal increased nerve activity.[71–73] Activation of renal nerves results in a centrally mediated hypertension via activation of the SNS, which can ultimately be interrupted by a blockade of neural signals. For example, Sprague-Dawley rats that have undergone a 5/6 nephrectomy experience an attenuation in hypertension following a dorsal rhizotomy with a concurrent reduction in the turnover of norepinephrine in the hypothalamic nuclei and locus coeruleus.[72] In humans, there is evidence for SNS activation even prior to overt renal impairment if a potential cause for renal ischemia is present. Hypertensive ADPKD patients have increased MSNA compared to controls despite preserved renal function in both groups.[74] Muscle sympathetic nerve activity was even higher in ADPKD patients with decreased GFR, but was not different between normotensive ADPKD patients and controls. Given the experimental association with bilateral renal ischemia, the possibility exists that the increased MSNA is related to increases in RAAS activation. One study confirmed the presence of increased MSNA in CKD patients, and showed that the administration of the ACE inhibitor enalapril caused a reduction in MSNA (relative to the decrease in BP it caused), whereas amlodipine increased MSNA.[75] In a small study including patients with various etiologies of nondiabetic CKD and matched control, MSNA changed similarly in the patients and controls with variability in volume status, but MSNA was persistently higher in the CKD patients.[76]

The evidence for the role of increased SNS activity in the etiology of hypertension in renal disease is best supported by the findings of Converse et al.[77] Twenty years ago it was established that ESRD patients had increased MSNA compared to healthy controls and other ESRD patients that had undergone bilateral nephrectomies. Higher mean arterial BP was also seen in the ESRD patients whose native kidneys were still present, and the hypothesis was that afferent signaling from the ischemic kidneys modulated an overall SNS response best managed by surgical removal of the kidneys. This is further supported by the fact that reversing the uremic state of ESRD patients with renal transplantation does not decrease MSNA, but a native kidney nephrectomy in the transplant recipient does.[78] The fact that nondiabetic HD patients have a normal arterial and cardiopulmonary baroreflex response weakens any suggestion that uremia-induced impairments in these reflexes might further contribute to abnormally elevated SNS activity in this population.[79]

Renalase

Adding to the evidence that CKD patients, particularly those with hypertension, have increased MSNA and circulating catecholamine levels is the recent discovery of a protein that may facilitate higher levels of catecholamines. Renalase is an amine oxidase that contributes to catecholamine degradation, which, in comparison to other common amine oxidases, can circulate in the plasma. Renalase is secreted into the circulation by the kidneys, and patients with ESRD on HD have nearly undetectable levels, supporting the role of adequate renal function to maintain its presence.[80] Although in CKD patients, there is no direct evidence how circulating renalase is responsible for hypertension, animal models demonstrate the development of hypertension following knockout of the renalase gene and a reduction in BP following the infusion of recombinant renalase in Sprague-Dawley rats.[80]

ENDOTHELIAL CELL DYSFUNCTION AND HYPERTENSION

Because the increase in peripheral vascular resistance also contributes to the elevated BP in patients with kidney disease, it is important to understand the mechanisms responsible for vasoconstriction. Blood vessels are lined with endothelial cells, which release mediators that exert their actions on VSMC receptors. The balance between vasoconstricting mediators and vasodilating mediators dictates the ultimate response of the VSMC and the amount of resistance. Endothelial nitric oxide synthase (eNOS) uses arginine as a substrate to produce the vasodilator nitric oxide (NO). This process is dependent on the presence of the cofactor tetrahydrobiopterin (BH4), and production of NO can be inhibited by the arginine analog asymmetric dimethylarginine (ADMA). One of the primary vasoconstrictive agents is endothelin-1 (ET-1), but the ultimate response of the vascular tone is dependent on which ET receptor is being bound. The interplay between all of these mediators is complex. Substances such as Ang II modify the activity of ET-1, and NO release is sensitive to the relative state of oxidative stress and inflammation.

Endothelin

ET-1 is a 21 amino acid mitogenic peptide that is produced ubiquitously, but to a large extent in vascular endothelial cells. Its original description demonstrated that it had more potent vasoconstrictive effects than other vasoconstrictive peptides including Ang II, vasopressin, and neuropeptide Y while having a longer lasting effect on vascular tone than the endothelial-derived relaxing factor NO.[81] Additionally, ET-1 has been shown to promote vascular cell hypertrophy and increase nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, resulting in oxidative stress and endothelial cell dysfunction.[82] There are two primary receptors for ET-1: ET-A and ET-B. ET-1 binding to ET-A and ET-B receptors found on VSMC causes vasoconstriction, whereas binding to ET-B receptors on the endothelial cells causes vasodilation, suggesting a mechanism for feedback inhibition to stimulation by ET-1. Because ET-1 has paracrine behavior and migrates from the endothelial cells to VSMC (away from the lumen), plasma levels of ET-1 have proven to be unreliable in establishing a clear causal relationship with hypertension. However, mRNA expression of ET-1 in the endothelium of resistance vessels is higher in patients with moderate-to-severe hypertension compared to those with mild hypertension

or controls.[83] Its role in hypertension in humans is further implicated by its effects on arterial tone. Following an infusion of ET-1, forearm blood flow decreases (increased tone) in both hypertensive and healthy humans, although the effect is greater in hypertensive patients. The pharmacologic inhibition of ET-A receptors alone or the combined inhibition of ET-A and ET-B receptors results in significant increases in forearm blood flow in the hypertensive patients, but not the healthy subjects.[84] The administration of a nonselective ET receptor blocker in a dose of >500 mg per day resulted in reductions in systolic and diastolic office and ambulatory BP as compared to placebo; the BP reduction from the drug was similar to that achieved with the ACE inhibitor enalapril.[85]

In patients with CKD, both systemic and local renal effects are proposed to contribute to hypertension in this population. Partial nephrectomy Sprague-Dawley rat models suggest that there is an imbalance of ET expression and degradation in the uremic state. In these animals, there were increased ET-1 levels (related to increased expression) in the endothelial cells of the thoracic aorta and renal cortex.[86] It was also found that the number of ET-B receptors was decreased in these locations, but there was a mild increase in ET-A receptor expression in the VSMC. Downregulation of ET-B receptors disables a mechanism for ET-1 degradation and can further contribute to the increased presence and action of ET-1.[87,88]

In CKD, there is also increased urinary and plasma levels of ET-1, independent of BP.[89,90] The renal excretion rate of ET-1 is increased in hypertensive CKD patients, but not in subjects with normal renal function who have increased plasma ET-1 levels accompanying essential hypertension. These findings suggest that renal production of ET-1 increases as renal function declines and contributes to hypertension in CKD.[91] Mechanisms through which ET-1 can increase BP include vasoconstriction, salt/water retention, and activation of the RAAS. ET-A receptor inhibitors have been shown to decrease systemic BP in CKD patients and controls.[92] They also increased renal blood flow and decreased renal vascular resistance in CKD patients, and this effect dissipated with a concurrent administration of an ET-B receptor inhibitor. These findings and the evidence that ET-A receptor antagonists reduce proteinuria in diabetic patients have already been further investigated in a clinical trial. The ASCEND study was a randomized double-blind placebo-controlled trial studying the effects of avosentan in patients with diabetic nephropathy.[93] Subjects were randomized to avosentan 25 mg daily, 50 mg daily, or placebo while being continued on current therapy with an ACE inhibitor or ARB. There were trends toward lower sitting and standing systolic BP in the low dose group, and this difference was statistically significant compared to placebo. However, the study was terminated early because of increased cardiovascular events in the avosentan group, particularly from congestive heart failure exacerbations and pulmonary edema. A smaller randomized placebo-controlled trial in 27 nondiabetic proteinuric CKD patients compared the effects of a selective ET-A receptor antagonist, sitaxsentan, with either the calcium channel blocker nifedipine or placebo.[94] Sitaxsentan significantly lowered BP and arterial stiffness compared to placebo. There was no difference in these outcomes compared to nifedipine, but proteinuria reduction was greater. Thus, the practical use of inhibiting various endothelin receptors remains to be fully established in CKD.

Nitric Oxide and Asymmetric Dimethylarginine

NO is one of the primary vasodilators released from the endothelium and counteracts the effects of the vasoconstrictors. NO also plays an important role in modifying renal blood flow and sodium excretion. Infusion of L-arginine, the substrate for NO production, decreases renal vascular tone in hypertensive patients who are either salt sensitive or salt resistant, but this effect is diminished in salt-sensitive patients with increasing sodium intake.[95] This suggests increased dietary sodium impairs NO release in salt-sensitive patients, and there is additional evidence that impaired renal response to arginine predicts an increased prevalence of end-organ damage in patients with salt-sensitive hypertension.[96] Furthermore, transition from a low sodium diet to a high sodium diet decreases plasma NO levels in salt-sensitive hypertensive patients compared to salt-resistant hypertensive patients where there was an inverse relationship between changes in plasma NO and BP changes.[97] These findings demonstrate that, in essential hypertension, there is impaired NO production and this impairment is associated with increased BP and long-term consequences related to uncontrolled BP.

Animal models of renal ablation have consistently identified a reduction in renal NO production, but not systemic NO production.[98,99] Differences in these studies may be related to the method of renal ablation and the consequential effects on systemic BP that could confound overall production of NO in the aorta.[98] In humans, total body NO production assessed by varying techniques has been noted to be lower in CKD patients and ESRD patients compared to healthy controls.[100–102] Although decreased NO synthase activity may be responsible for these findings, CKD patients were more hypertensive and had increased serum levels of the eNOS inhibitor ADMA.[101]

The association between ADMA and renal function has also been demonstrated in animal CKD models. Rats that have undergone a 5/6 nephrectomy have increased ADMA, and these levels correlate with increased BP.[103] Urinary ADMA excretion was increased in these animals, but the enzyme responsible for the metabolism of ADMA, dimethylarginine dimethylaminohydrolase (DDAH) was found to be decreased, suggesting that mechanisms beyond impaired renal excretion are responsible for ADMA accumulation in CKD. Further support for the role of ADMA in hypertension comes from evidence that transgenic mice overexpressing DDAH have lower BP and ADMA than controls, and that the administration of DDAH attenuates the increase in BP that

occurs following a 5/6 nephrectomy in animal models.[104,105] Infusion of ADMA into healthy humans causes an increase in systolic BP associated with decreased cardiac output, but increased vascular resistance.[106,107] In patients with nondiabetic CKD (IgA nephropathy and ADPKD) elevated ADMA levels can be seen even in the early stages of CKD prior to a significant reduction in GFR compared to healthy controls.[108] However, in this study, there were no significant differences in ADMA between hypertensive and normotensive CKD patients. Elevated ADMA has been confirmed in ESRD patients on HD where the increased levels were predictive of cardiac structure and function, as well as cardiovascular morbidity and mortality.[109–112] Cumulatively, these studies fail to consistently show a direct correlation with ADMA and BP in CKD and ESRD patients, but ADMA is associated with adverse events in this population.

Oxidative Stress

A common aspect related to many of the previously described mediators of hypertension in CKD is oxidative stress. Oxidative stress refers to an imbalance of reactive oxygen species (ROS) and naturally occurring antioxidant enzymes that favor an elevation of ROS. Chronic kidney disease is a state of both relative ROS excess and antioxidant depletion. Increased RAAS activity can generate ROS via the Ang II–induced activation of NADPH oxidase, which may be one explanation for the degree of oxidative stress seen in CKD and ESRD. Furthermore, it has been demonstrated that the enzyme responsible for the degradation of ADMA, DDAH, can be influenced by increased oxidative stress.[113] Following a 5/6 nephrectomy, Sprague-Dawley rats have increased BP that is attenuated by the administration of an antioxidant and lipid peroxidation inhibitor.[114] The BP increases again following withdrawal of the antioxidant. The levels of plasma malondialdehyde, a marker of lipid peroxidation, increased in the CKD animal models and correlated with BP. Again, malondialdehyde levels decreased when the animals were given the antioxidant, but increased when the antioxidant was withdrawn.

Additionally, ROS can interact with NO that has already been synthesized by eNOS and deplete the amount of NO available to induce vasodilation. This was shown in an experiment using a 5/6 nephrectomy in Sprague-Dawley rats where a diet fortified in the antioxidant vitamin E attenuated BP increases that occurred following the nephrectomy.[115] There was an increase in plasma and tissue nitrotyrosine, a measure of the effects of ROS on NO, in all CKD animals; this effect was reduced in the CKD animals receiving a diet high in vitamin E. Similarly, NO production from isolated vascular tissues was higher in the animals receiving vitamin E compared to the animals on a regular diet.

Numerous studies in humans with CKD and ESRD have aimed to determine if intervention with antioxidant therapy is capable of improving outcomes. A retrospective analysis of the Heart Outcomes Protection Evaluation (HOPE) study, which included nonproteinuric CKD subjects with serum creatinine (Cr) <2.3 mg per deciliter, found no difference in the composite of cardiovascular outcomes between subjects taking vitamin E 400 units daily or placebo.[116] Hemodialysis patients receiving antioxidants including vitamin E or N-acetylcysteine in randomized placebo-controlled trials showed improved cardiovascular outcomes, but there was no difference in mortality between these groups.[117,118]

Arginine Vasopressin

Experimental evidence suggests that vasopressin may also contribute to hypertension in CKD patients, although currently no therapy is aimed at decreasing its effects. Arginine vasopressin (AVP) is a peptide released from the hypothalamus in response to increases in plasma osmolarity. Receptors for vasopressin include the V1a, V1b, and V2 receptors. Traditionally, V1a receptors were believed to be responsible for vasoconstriction via VSMC, and V2 receptors responsible for water reabsorption through the insertion of aquaporin channels in the collecting duct of the nephron. Based on animal studies, the V1a receptor may mediate other effects on BP related to decreases in circulating blood volume, baroreflex sensitivity, and RAAS activity.[119,120] In CKD patients, AVP levels are higher and increase in response to osmolarity changes with a greater slope than in controls.[121] Vasopressin levels are also elevated in HD patients, and BP decreases following infusion of an AVP inhibitor in HD patients that have been saline loaded.[122,123]

Secondary Hyperparathyroidism

Secondary hyperparathyroidism is a complication of CKD that contributes to many comorbidities associated with CKD. The increased production and secretion of parathyroid hormone (PTH) is triggered in part as a response to the accumulation of serum phosphorus caused by decreased renal phosphorus excretion in CKD. Furthermore, in CKD, there is decreased renal 25-hydroxyvitamin D_3 1α-hydroxylase activity, which results in the reduced production of active vitamin D. Previous in vitro studies have investigated the role of secondary hyperparathyroidism in the etiology of hypertension, but recent clinical trials have added to the data available in human studies. One study in adult CKD patients demonstrated an association with increased BP and elevated serum PTH levels proposed to be related to higher cytosolic calcium in the subjects with elevated PTH levels.[124] This association was supported by the improvement in mean BP following treatment with the vitamin D analog alfacalcidol. Treatment with vitamin D also decreased PTH and cytosolic calcium levels along with the decrease in BP. It has since been demonstrated that although treatment with 1,25 dihydroxyvitamin D in spontaneously hypertensive rats (SHR) achieves a reduction in BP mediated through the attenuation of endothelium-dependent VSMC contraction, the administration of vitamin D had no effect on the amount of free cytosolic calcium,[125] suggestive of an effect downstream from the increase in calcium.

Although the exact mechanisms remain under debate, further evidence has been generated to support the use of active vitamin D treatment in CKD. The selective vitamin

D receptor activation with paricalcitol for a reduction of albuminuria in patients with type 2 diabetes (VITAL) study was a clinical trial where patients with diabetic nephropathy (mean estimated glomerular filtration rate: 39 to 42 mL per minute) were randomized to receive placebo versus 1 μg paracalcitol versus 2 mcg paricalcitol.[126] In this study there was a reduction in albuminuria following the administration of the larger dose of vitamin D compared to placebo. Additionally, there was a significant reduction in systolic BP in patients receiving vitamin D compared to placebo. The identification of inactive vitamin D deficiency and insufficiency in the general population and in CKD patients with secondary hyperparathyroidism has generated further interest in the effects of BP response to inactive vitamin D repletion/supplementation. A recent meta-analysis of the effects of vitamin D on the cardiovascular system among healthy individuals showed a trend toward reductions in systolic BP.[127]

Drug Related

Erythropoietin-Stimulating Agents

Beyond the disruption of endogenous mediators of hypertension that occurs in CKD patients, it is also important to consider the iatrogenic effects of commonly implemented interventions in this patient population. Anemia is another prevalent comorbidity in CKD. Though iron deficiency contributes to decreased hemoglobin levels in CKD and ESRD patients, the underlying erythropoietin deficiency has prompted widespread use of erythropoiesis-stimulating agents (ESAs) as commonly used therapeutic agents to correct anemia. Increases in BP in both previously hypertensive and nonhypertensive patients is one of the reported adverse effects of ESA use.[128] Despite the recognition that ESA administration increases BP, several meta-analyses have failed to consistently show significant differences in hypertension-related adverse events in ESA use versus nonuse or high or low target hemoglobin groups using ESA in CKD and ESRD patients.[129–131] Proposed mechanisms of ESA-induced hypertension include increased ET-1 release and increased sensitivity to Ang II and adrenergic stimuli.[132,133] Furthermore, acute and chronic ESA administration in pre-HD CKD patients resulted in impairment of flow-mediated dilatation as a measurement of endothelial cell function.[134] Current guidelines recommend treating hypertension that arises during treatment with an ESA as opposed to withholding ESA treatment in anemic patients.[135]

HYPERTENSION IN SPECIFIC ETIOLOGIES OF CHRONIC KIDNEY DISEASE

Because diabetic nephropathy and CKD attributed to hypertension are the two leading causes of ESRD in the United States, it is important to consider what aspects of the underlying disease processes make CKD-associated hypertension unique in these cases.

Hypertension in Diabetic Nephropathy

Diabetic nephropathy is the leading cause of ESRD in the United States. The timing of the onset of hypertension in patients with diabetic nephropathy is related to whether type 1 or type 2 diabetes is the underlying cause. The onset of hypertension correlates with the development of microalbuminuria in type 1 diabetics and is rarely present before that time. However, a "non-dipping" nocturnal BP pattern, where BP fails to decrease at night from the daytime values, predicts the onset of microalbuminuria in patients with type 1 diabetes and normoalbuminuria.[136] There is also evidence of genetic factors that determine whether hypertension will be present in type 1 diabetics because there is a higher prevalence of hypertension in the family members of patients with type 1 diabetes and microalbuminuria.[137] Conversely, hypertension is frequently already present in patients with type 2 diabetes before the onset of microalbuminuria.[138,139] The overlap between risk factors for hypertension and type 2 diabetes in patients with obesity and the metabolic syndrome may explain why hypertension occurs before renal disease in this population.

There are many similarities in the pathophysiology of hypertension in diabetic nephropathy and nondiabetic kidney disease. It should be recognized that in addition to the impact that impaired renal function has on sodium excretion, RAAS activation, SNS activation, endothelial cell dysfunction, and oxidative stress, the metabolic derangements present in diabetes further accentuate the role these factors have in causing increased vasoconstriction and BP.

Hypertensive Nephrosclerosis

Although diabetic nephropathy is the leading cause of ESRD in the United States, the second leading cause of ESRD is hypertensive nephrosclerosis, with an incidence rate of 100 per million in 2010 according to the USRDS.[3] Because hypertensive nephrosclerosis frequently presents with a normal urinary sediment and a lack of sonographic changes beyond decreased kidney size, many cases of CKD/ESRD attributed to hypertensive nephrosclerosis are never confirmed with a renal biopsy. Often, the identification of hypertension as the sole risk factor for CKD accounts for the designation of hypertension as the etiology. Kidney biopsies from nondiabetic hypertensive individuals with proteinuria and/or increased serum Cr revealed thickened and hyalinized arterioles with an exaggeration of arteriolar smooth muscle hypertrophy in excess of that typically seen from normal aging.[140] Additionally, both hypertrophic and sclerotic glomerular lesions can be seen in hypertensive nephrosclerosis with both autoregulatory dysfunction and ischemia possibly playing a role.[141] Because the number of patients in the general population who have a diagnosis of hypertension far exceeds the number of those who go on to develop kidney disease, there must be certain risk factors that predispose some individuals to hypertensive nephrosclerosis.

In particular, African Americans are more susceptible to renal disease than other races and often develop CKD at

younger ages. Additionally, the severity of hypertension does not correlate with renal function as well as in non-African American patients.[142] There are differences in the renal biopsy findings in African American and Caucasian patients with hypertensive nephrosclerosis, although the etiology of these differences remains unexplained.[143] Genetic variants that predominate in African Americans are sought after as possible explanations for the pattern of CKD in this population. An association between nonmuscle myosin heavy chain 9 (MYH9) gene polymorphisms and the pathologic diagnosis of focal segmental glomerulosclerosis and HIV nephropathy in African Americans has been detected.[144] In several cohorts of African American patients, it has also been shown that the presence of some MYH9 gene polymorphisms is associated with the presence of nondiabetic ESRD previously diagnosed as hypertensive nephrosclerosis.[145] Because MYH9 is expressed in podocytes, mesangial cells, and renal capillary beds, such evidence suggests the possibility that hypertension alone is not sufficient to cause progressive renal disease, but in fact, a genetic susceptibility that primarily affects the kidneys predisposes individuals to the onset of renal disease in the context of other inciting factors, including hypertension.

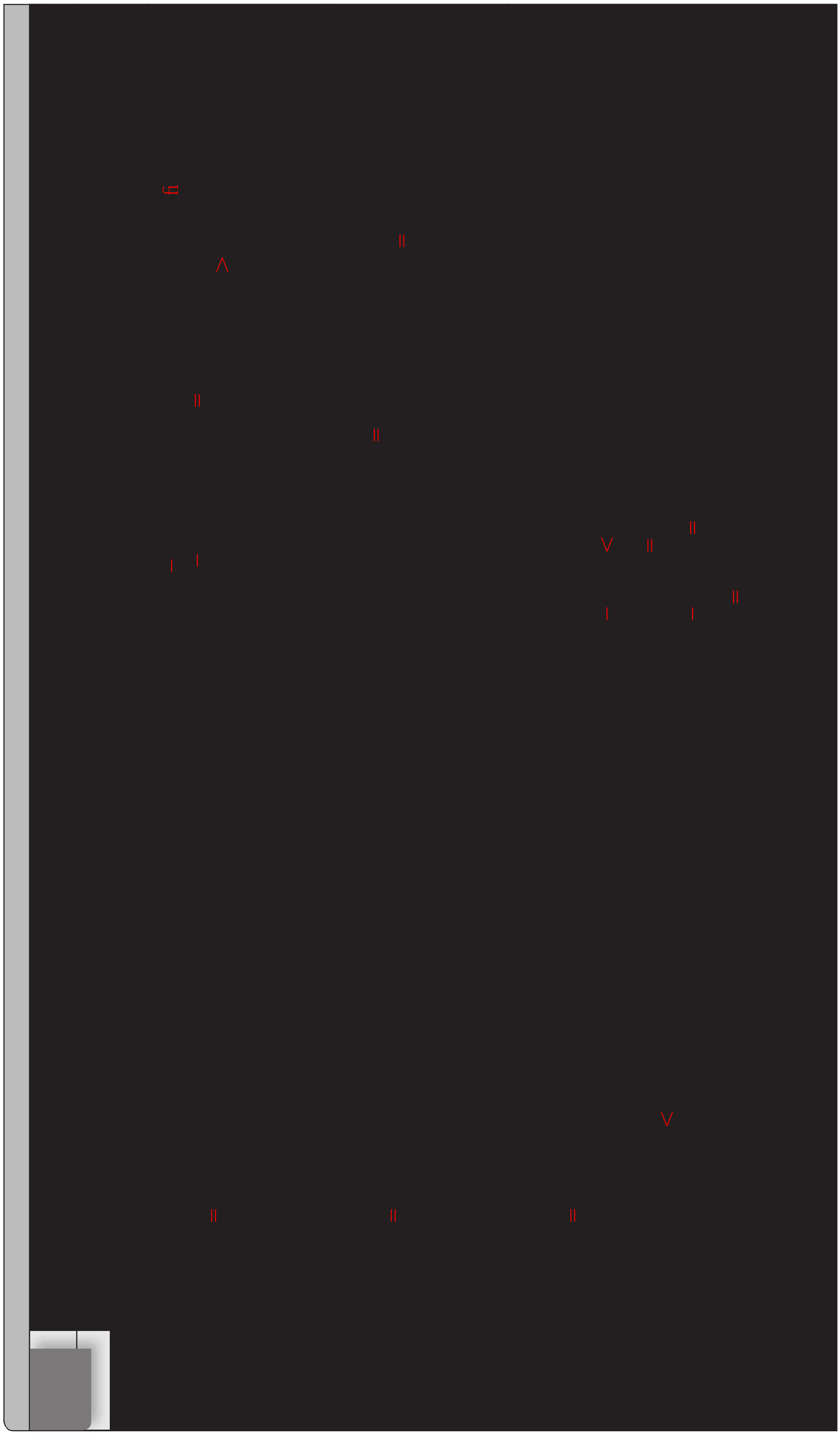
Further investigation into this topic has shown that the risk for kidney disease associated with the MYH9 gene is related to polymorphisms in another nearby location on chromosome 22q. The apolipoprotein 1 (ApoL1) gene encodes a serum factor that lyses Trypanosoma brucei rhodesiense. Case-control studies of African Americans with focal segmental glomerulosclerosis compared to African American controls reveal that the risk for renal disease was dependent on the presence of the ApoL1 alleles.[146] This has also been confirmed in African Americans patients with ESRD attributed to hypertensive nephrosclerosis.[146] This concept has important implications expanding from risk stratification of patients to expectations for managing hypertension in patients with nondiabetic kidney disease.

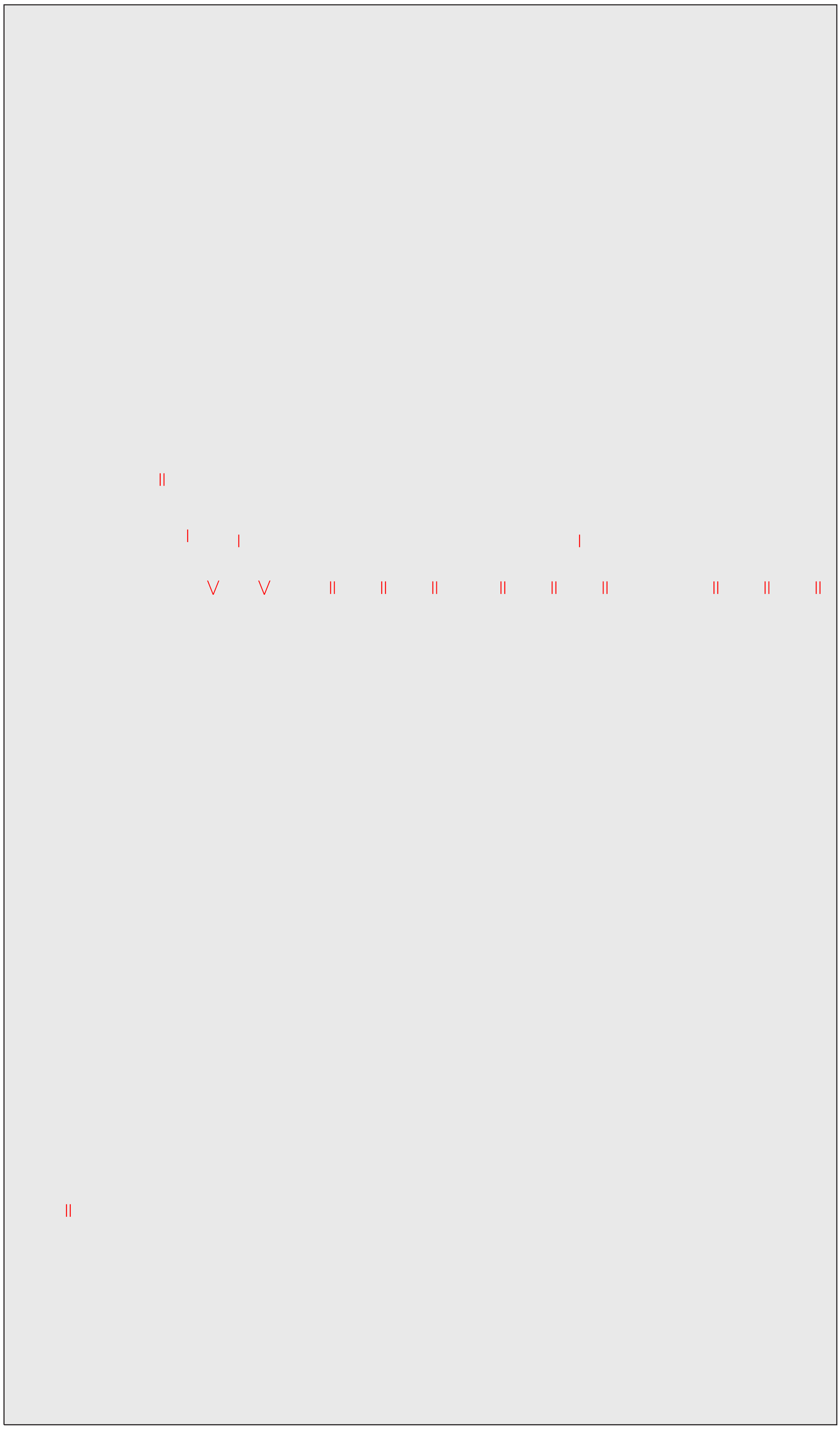
TREATMENT

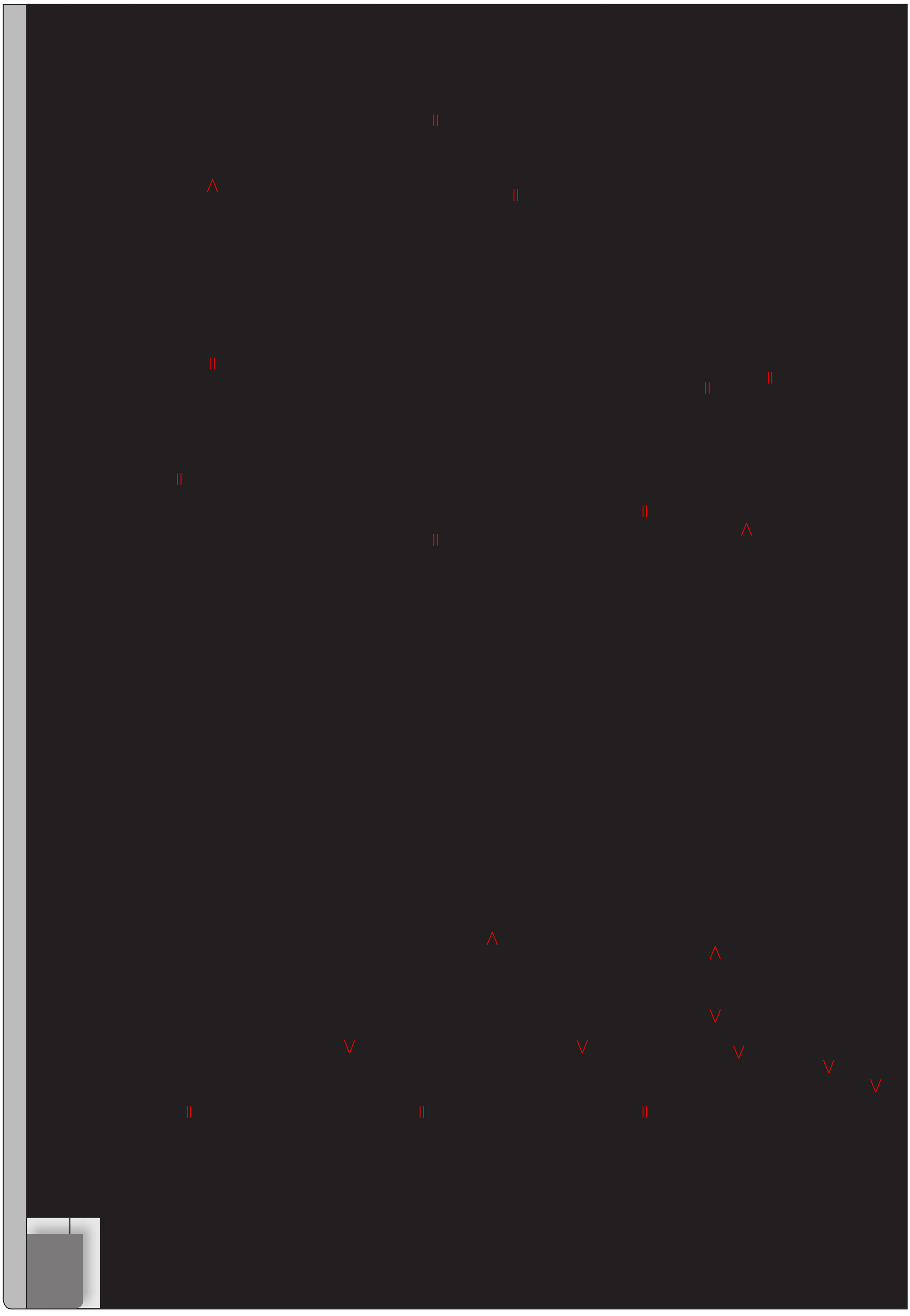
Pre–End-Stage Renal Disease

The management of hypertension in pre-ESRD CKD patients should be aimed at lowering the risk for progression to ESRD as well as reducing the risk for cardiovascular events and death. Current recommendations are to target a BP of 130/80 mm Hg in all CKD patients with consideration of a BP of 125/80 mm Hg in those with significant proteinuria.[147] These guidelines are based on the results and post hoc analyses of randomized clinical trials, which are summarized in Table 40.2. The Modification of Diet in Renal Disease (MDRD) studies randomized patients with GFR of 25 to 55 mL per minute (Study 1) and 13 to 24 mL per minute (Study 2) to a mean arterial pressure (MAP) <107 mm Hg or a MAP <92 mm Hg.[148] Although there was no difference between groups in the change in GFR with a mean follow-up of 2.2 years, a further analysis demonstrated that there was a significant reduction in GFR decline in those subjects with proteinuria randomized to the lower MAP goal.[149] The African-American Study of Kidney Disease and Hypertension (AASK) study randomized nondiabetic African American patients with a GFR of 20 to 65 mL per minute to either a MAP of 102 to 107 mm Hg or a MAP of <92 mm Hg. During the initial 3 months, there was a faster decline of GFR in the intensive BP group, but there was no difference between groups in the chronic or overall slope of GFR over time.[150] For patients with higher baseline proteinuria, there was a trend toward a slowed reduction in GFR with intensive BP control. Following the randomized study, all remaining subjects participated in a cohort study where the goal BP was set at 140/90 mm Hg. During the study, the goal BP was later changed to 130/80. For those with baseline proteinuria >0.22 g per day, there was a slower decline in GFR if they had been randomized to a MAP <92 mm Hg in the original study.[151] In contrast, the Ramipril Efficacy in Nephropathy 2 (REIN2) study randomized nondiabetic CKD patients with persistent proteinuria (1 to 3 g if the GFR <45 mL per minute, or >3 g if the GFR <70 mL per minute) to either standard BP control (diastolic BP <90 mm Hg) or intense BP control (<130/80 mm Hg). The study was halted at the first interim analysis because of similarities between BP groups in the outcomes of ESRD, proteinuria, and GFR decline despite the fact that systolic BP and diastolic BP were lower in the intensive BP group. These similarities persisted among the strata of baseline proteinuria.[152]

In summary, the following conclusions relevant to clinical practice emerge from these trials: (1) there is currently insufficient evidence from the cumulative trial data to demonstrate that, in patients with nondiabetic kidney disease, intensive BP goals (<130/80 mm Hg) slow the progression of CKD or reduce the incidence of ESRD and death, and (2) patients with proteinuria tend to have faster deterioration in renal function, but benefit the most from aggressive therapy. It is important to also acknowledge, based on studies in patients with diabetes, that renoprotection may not be synonymous with decreased overall cardiovascular risk reduction. The administration of combination therapy with perindopril and indipamide to participants with type 2 diabetes and varying levels of baseline renal disease (26% with microalbuminuria, 4% with macroalbuminuria, 19% with an estimated GFR <60 mL per minute) was associated with significantly reduced risk for the composite renal outcome of new onset microalbuminuria, new onset macroalbuminuria, new onset ESRD, or doubling of serum creatinine.[153] Achieving systolic BP <110 mm Hg offered the greatest renoprotection. Similarly, in post hoc analysis of the Irbesartan in Diabetic Nephropathy Trial (IDNT), the lowest quartiles of baseline and achieved systolic BP were associated with the lowest incidence of renal events.[154] However, the subgroup with systolic BP <120 mm Hg had a greater overall mortality than those with BP >120 mm Hg even after controlling for comorbidities. With systolic BP >120 mm Hg, cardiovascular mortality increased with each 10 mm Hg







increase, but also increased for each 10 mm Hg increment of diastolic BP lower than 85 mm Hg. Although these studies were inclusive of patients with diabetes and/or diabetic nephropathy and not completely generalizable to the whole CKD population, it is important to recognize potential limitations of overtreating BP in the context of outcomes other than renoprotection.

A large multicenter randomized clinical trial has been designed to address what the optimal BP target is for patients without diabetes mellitus in order to reduce the risk for cardiovascular events. The Systolic Blood Pressure Intervention Trial (SPRINT) will randomize almost 10,000 subjects to either an intensive BP goal (120 mm Hg) or a standard BP goal (140 mm Hg).[155] The inclusion criteria are age >55 years, hypertension, and the presence of a clinical or subclinical cardiovascular disease, excluding stroke. Inclusive in the definition is CKD with an estimated GFR of 25 to 59 mL per minute, but proteinuria >1 g will be one of the exclusion criteria. The primary outcome is the occurrence of the first major cardiovascular event, although a decline in renal function and the development of ESRD over a 6-year follow-up are some of the secondary end points. The large number of CKD patients expected to be enrolled in SPRINT will provide enough power to reach meaningful conclusions in this patient population. Given the exclusion criteria of diabetes and proteinuria >1 g per day, this study will be directly applicable to CKD patients considered at a lower risk for adverse events than some of the earlier mentioned studies.

Despite the existing debate for the ideal BP in a CKD patient, evidence shows that the majority of CKD patients fail to have BP adequately controlled.[5,10] Although most CKD patients will require more than one antihypertensive drug, all CKD patients should be prescribed a low sodium diet as the first step in managing BP. Dietary sodium intake will also affect the degree of proteinuria in CKD patients, such that increased dietary sodium intake abolishes the antiproteinuric effects of RAAS inhibition.[156] The current recommendations are to limit dietary sodium intake to less than 2.4 g per day.[147]

Inhibitors of the RAAS are the recommended first-line antihypertensive agents for most CKD patients, with the most commonly used agents being ACE inhibitors or ARBs. These agents should be titrated to the maximum recommended doses in order to achieve the greatest RAAS inhibition. Several randomized clinical trials have evaluated the efficacy of ACE inhibitors in CKD patients and, similar to the effects of implementing intensive BP lowering, show that the benefits are most appreciable in patients with higher baseline proteinuria. The African-American Study of Kidney Disease and Hypertension (AASK) and Ramipril Efficacy in Nephropathy (REIN) studies included randomization arms with different antihypertensive agents. In REIN, the ACE inhibitor ramipril decreased the risk of ESRD compared to placebo regardless of baseline proteinuria and slowed the decline of GFR in patients with nephrotic range proteinuria.[157,158] In AASK, subjects were randomized to the ACE inhibitor ramipril, the beta-blocker metoprolol, or the calcium channel blocker amlodipine. Ramipril caused a slower overall decline in GFR compared to metoprolol.[150] There was no difference in the overall decline in GFR between ramipril and amlodipine.[159] However, this was confounded by the acute increase in GFR with amlodipine because the chronic decline in GFR was slower with ramipril. Furthermore, for subjects with mild-to-moderate baseline proteinuria or renal impairment (GFR <40 mL per minute), there was a significantly slower reduction in GFR in the ramipril group. Ramipril also reduced the composite outcome of ESRD, the doubling of serum Cr, or death compared to metoprolol and amlodipine.[150,159]

The benefit of ACE inhibitors extends even to advanced nondiabetic cases of CKD. In a randomized clinical trial, patients with serum creatinine (Cr) between 3 and 5 mg per deciliter were randomized to either benazepril 10 mg twice daily or placebo, in addition to other antihypertensives.[160] Proteinuria >0.3 g per day was one of the inclusion criteria, and the mean baseline proteinuria was 1.6 and 1.7 g per day in the benazepril and placebo groups, respectively. The use of benazepril reduced the occurrence of the primary endpoint of the composite of doubling serum Cr, ESRD, or death. There was also a slower reduction in GFR and creatinine clearance over time, which was assessed as a secondary endpoint in the benazepril group. These findings occurred in the context of similar BP control between groups, and the average achieved systolic BP was <130 mm Hg in both groups.

The cumulative findings show that use of an ACE inhibitor offers benefits over other drugs even when a similar BP is achieved. In addition to these studies in nondiabetic kidney disease, there is robust evidence for BP control and RAAS inhibition in reducing renal endpoints in patients with diabetic kidney disease, specifically in the progression of overt nephropathy to composite clinical endpoints in type 1 and type 2 diabetes,[161–163] progression of microalbuminuria to macroalbuminuria in type 2 diabetes,[164] and the primary prevention of microalbuminuria in hypertensive patients with type 2 diabetes.[165,166] For CKD patients without diabetes, it should be emphasized that the benefits of RAAS inhibition are strongest in patients with the greatest risk for CKD progression. In fact, one meta-analysis confirmed that use of an ACE inhibitor was most beneficial in those with >500 mg per day of proteinuria.[167] The benefits of ACE inhibitors as first-line agents are less certain in those without proteinuria.

Additional agents are frequently required to control BP in CKD patients. Diuretic therapy addresses the impact of extracellular volume on BP and optimizes sodium balance. When GFR is significantly impaired, loop diuretics such as furosemide may be more effective than hydrochlorothiazide. It is important to dose loop diuretics at least twice daily and to consider increased doses in patients with significantly impaired GFR in order to optimize delivery of the drug to the site of action. Diuretics additionally offer the benefit of increasing renal potassium excretion in CKD patients who may be prone to hyperkalemia. Following the use of RAAS inhibitors and diuretics, the decision of which additional agents to

use will be dependent on other underlying comorbidities. Beta-blockers are recommended for patients with congestive heart failure, coronary artery disease, and arrhythmias.[147] Calcium channel blockers, vasodilators such as hydralazine and minoxodil, and clonidine may also be required if BP remains elevated. Although a post hoc analysis of the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial demonstrated that subjects with a GFR <45 mL per minute had decreased progression of CKD using the ACE inhibitor benazepril plus the calcium channel blocker amlodipine compared to benazepril plus hydrochlorothiazide,[168] it is unknown how the former combination compares to a regimen including an ACE inhibitor and a loop diuretic. A suggested approach to the selection of an antihypertensive regimen for CKD patients is provided in Figure 40.3.

Combination Renin-Angiotensin-Aldosterone System Inhibition

Because of the concerns of incomplete inhibition of the production or action of Ang II, combination therapy with both ACE inhibitors and ARB has been considered. This strategy has been shown to lower proteinuria in numerous studies of CKD patients, but it is uncertain how much of this effect is derived from additional BP lowering.[169] Because of the concerns of aldosterone escape and the non–ACE-related production of Ang II related to increased PRA in patients already receiving either an ACE inhibitor or ARB, add-on therapy using either a mineralocorticoid receptor blocker or a direct renin inhibitor has also been considered. None of these strategies is currently recommended for patients with CKD, but there is accumulating evidence of the effects of such regimens that is worth briefly discussing here.

Mineralocorticoid Receptor Blockers and Direct Renin Inhibitors. Most of the benefits of add-on therapy with a mineralocorticoid receptor antagonist (MRA) are related to a reduction in proteinuria as opposed to BP. In one clinical trial, patients with proteinuric CKD were randomized to ramipril plus dual placebo, ramipril plus spironolactone plus placebo, ramipril plus placebo plus irbesartan, or triple therapy with all three medications.[170] There were no differences in systolic BP during follow-up, but the groups receiving spironolactone as either dual or triple therapy had a clear benefit in proteinuria reduction compared to the group receiving only ramipril. Other studies involving MRA have been exclusively in patients with diabetic nephropathy and yielded similar results, suggestive of an antiproteinuric benefit from MRA,

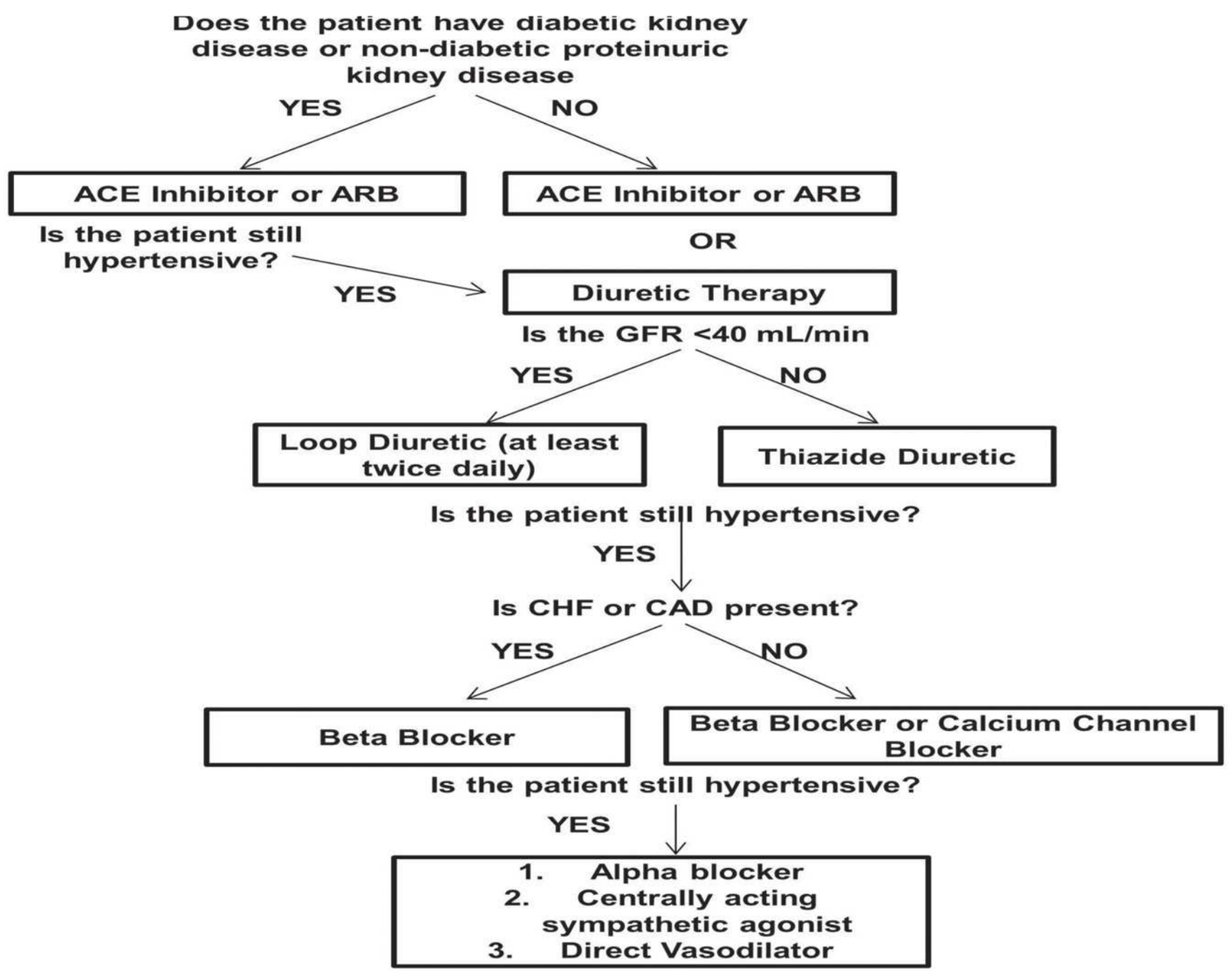


FIGURE 40.3 For chronic kidney disease (CKD) patients with diabetic kidney disease or nondiabetic proteinuric kidney disease, inhibition of the renin-angiotensin-aldosterone system (RAAS) system using either an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB) should be the first-line therapy. These drugs can also be used as first-line therapy in patients without proteinuria or diabetic kidney disease, but the evidence to support this decision is not as clear. Diuretics should be used to reduce extracellular volume. It is important to use a thiazide diuretic only if the glomerular filtration rate (GFR) is >40 mL per minute and to dose loop diuretics at least twice daily. For patients that remain hypertensive, those with coronary artery disease (CAD) or congestive heart failure (CHF) should receive an indicated beta-blocker, whereas for others, calcium channel blockers can be used. Finally, patients that are still hypertensive may be started on fourth- or fifth-line agents.

although BP differences have varied.[171–173] Clinical trials of the direct renin inhibitor aliskiren in CKD patients are mainly limited to diabetic nephropathy. Although the use of aliskiren as a single agent lowers BP and albuminuria,[174] the beneficial effects of combination therapy, including aliskiren compared to single agent RAAS inhibition, mainly stem from albuminuria reduction.[175,176]

End-Stage Renal Disease

The current recommendations for managing hypertension in ESRD patients is to target a pre-HD systolic BP <140 mm Hg and a post-HD systolic BP <130 mm Hg.[177] There is significant overlap in the etiology of hypertension in patients with ESRD and pre-ESRD CKD. There is increasing evidence of outcomes related to strategies aimed at targeting the underlying causes of hypertension in the HD population. Hemodialysis patients are even more vulnerable to the impact of volume overload than CKD patients because of the extremely limited ability to excrete sodium and water due to kidney failure. The mechanisms of increased vasoconstriction are also similar between ESRD and pre-ESRD CKD patients. Furthermore, the duration and timing of the weekly HD regimen may impact BP control in HD patients.

Identifying and achieving an HD patient's target dry weight are the initial steps that should be taken in managing BP. This process involves a careful clinical assessment of the patient's extracellular volume status, strict enforcement of dietary sodium restriction, and prudent application of ultrafiltration during the HD procedure. Current recommendations are to limit interdialytic sodium intake to 2 to 3 g per day in patients who appear volume overloaded.[177] In patients who remain hypertensive, there should be a consideration of a reduction in dry weight. A slow, progressive reduction in estimated dry weight over several weeks has been shown to reduce ambulatory BP in hypertensive HD patients.[48] Increasing the amount of time a patient spends on HD with either daily or nocturnal dialysis compared to thrice weekly sessions has also been shown to improve BP control. One RCT compared thrice weekly HD with a regimen consisting of six treatments per week.[178] The rates of death and a left ventricular (LV) mass were reduced after 12 months in the group randomized to more frequent HD. Pre-HD systolic BP and the number of antihypertensives required were also reduced in this group, although ambulatory BP was not measured. In an observational study, nocturnal HD has been associated with improved BP and LV mass.[179] One randomized study has demonstrated reductions in LV mass, pre-HD systolic BP, and the antihypertensive requirement in subjects converted to nocturnal HD.[180] Finally, another randomized study confirmed the improved BP with nocturnal HD, but failed to demonstrate improvement in the primary outcome of mortality and LV mass reduction.[181]

Despite the effects from dry weight reduction or more frequent HD treatments, pharmacologic treatment with antihypertensive agents is frequently required to further lower BP in HD patients. Overall, evidence from two meta-analyses supports the use of antihypertensive medications in HD patients to improve cardiovascular outcomes and mortality.[182,183] First-line recommended therapy for HD patients is the use of a RAAS-inhibiting drug.[177] In observational studies of HD patients, the use of ACE inhibitors was associated with reduced mortality.[184] However, this has not yet been confirmed in a randomized clinical trial. The Fosinopril in Dialysis (FOSIDIAL) trial randomized HD patients to either fosinopril or standard therapy and achieved a nonsignificant 8% reduction in the hazard ratio for mortality and cardiovascular death.[185] One small study that randomized hypertensive ESRD patients to one of several ARB (candesartan, losartan, valsartan) versus placebo demonstrated a reduction in both fatal and non-fatal cardiovascular events.[186] For patients that remain hypertensive after implementing a pharmacologic RAAS inhibition, other antihypertensive drugs should be prescribed based on existing comorbidities. The beta-blocker carvedilol has been shown to improve survival in patients with dilated cardiomyopathy,[187] and other beta-blockers may be used in the context of coronary artery disease. Additionally, the calcium channel blocker amlodipine has been shown to improve the composite outcome of mortality and nonfatal cardiovascular events in hypertensive HD patients already prescribed other agents, including ACE inhibitors and beta-blockers,[188] and should be considered as an add-on therapy. Fourth- and fifth-line agents, if necessary, include alpha-blockers, centrally acting sympathetic agonists (clonidine), and direct vasodilators. A suggested approach to the selection of an antihypertensive regimen for CKD patients is provided in Figure 40.4.

Despite the fact that peritoneal dialysis (PD) offers a more continuous treatment modality than intermittent HD for removing fluid and toxins, there is a high prevalence of hypertension in the PD population. In a multicenter Italian study of over 500 peritoneal dialysis patients, it was determined that the prevalence of hypertension was 88%.[189] Just as in HD patients, extracellular volume is an important determinant of BP, and both hypertensive status and the amount of sodium and fluid removal have been associated with increased mortality in PD patients.[190] Extracellular volume overload remains a primary concern in PD patients, and there is evidence using either an assessment of left atrial volume or bioimpedance analysis that PD patients are generally more volume overloaded than HD patients.[191,192] Further observational evidence, however, suggests that BP may not be as important of a predictor of the relative risk of death in the PD population.[193] Formal recommendations do not exist for the PD population as in HD patients, although attaining euvolemia in these patients remains an important goal.

CONCLUSION

Hypertension is a prevalent comorbidity associated with chronic kidney disease from the early stages to ESRD. Its existence in this population is related to multiple factors

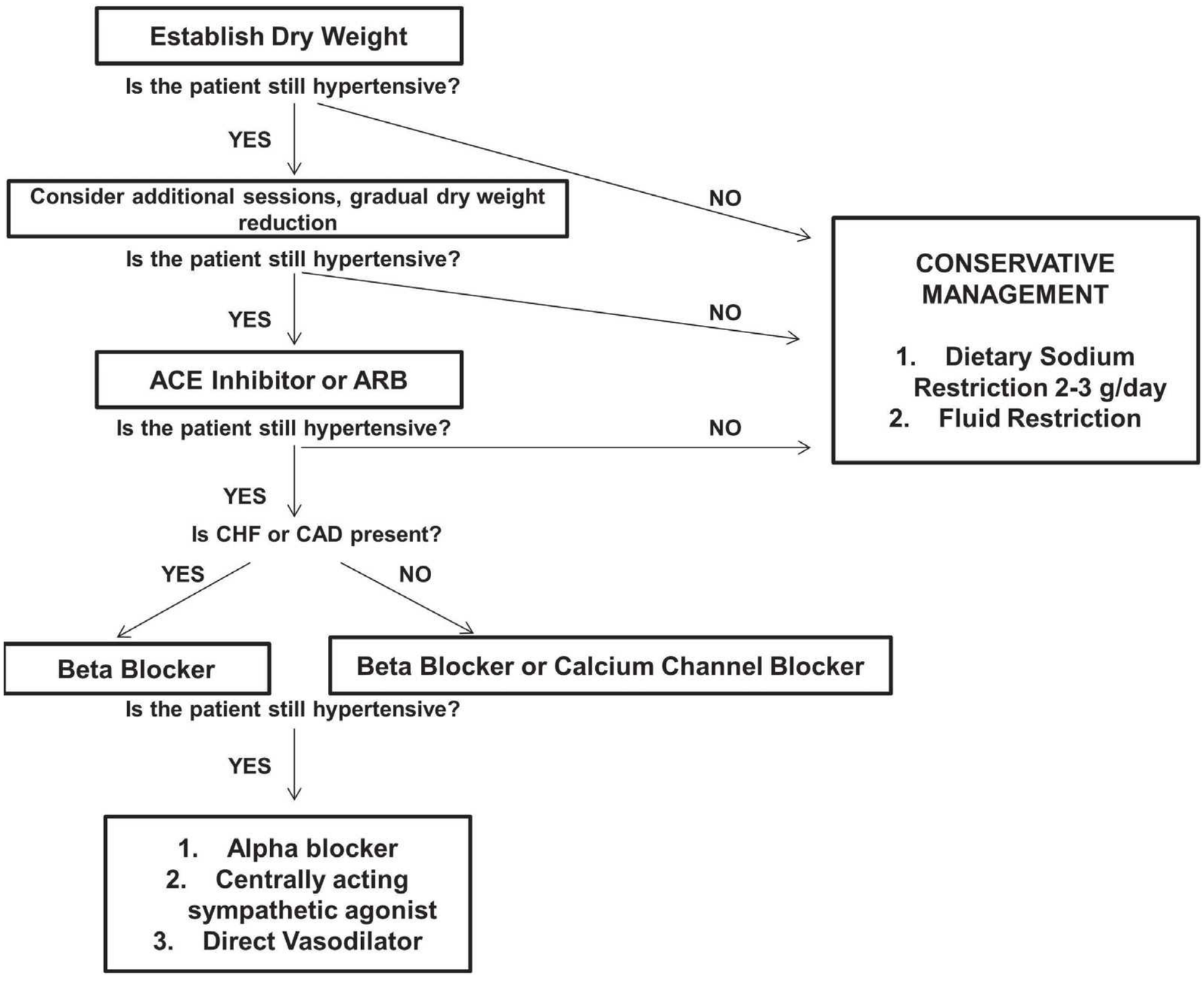


FIGURE 40.4 For hemodialysis (HD) patients, the first step in managing blood pressure (BP) should be to identify and achieve the accurate dry weight. Overtly volume overloaded patients may require additional HD treatments to achieve dry weight, and in others, a trial of gradual dry weight reduction may be possible. Inhibitors of the renin-angiotensin-aldosterone system (RAAS) (angiotensin-converting enzyme [ACE] inhibitors and angiotensin receptor blockers) are first-line pharmacologic therapy in HD patients. As in pre–end-stage renal disease (ESRD) chronic kidney disease (CKD) patients, additional therapy may be dictated by underlying comorbidities reserving indicated beta-blockers for patients with congestive heart failure (CHF) or coronary artery disease (CAD). Calcium channel blockers can be added next for patients that remain hypertensive because there is evidence that they reduce cardiovascular end points compared to placebo in HD patients. Finally, patients that are still hypertensive may be started on fourth- or fifth-line agents (alpha-blockers, centrally acting sympathetic agonists, direct vasodilators). All HD patients should limit their interdialytic weight gain through dietary salt and water restriction to prevent extracellular volume overload.

including extracellular volume overload and increased vasoconstriction. Uncontrolled hypertension is associated with the progression of CKD to ESRD and with cardiovascular morbidity and mortality. Home and ambulatory BP measurements are currently the gold standard for predicting outcomes and assessing the overall BP burden. The target BP for CKD patients that optimally reduces the risk for both renal and cardiovascular outcomes remains unknown, but subjects with increased proteinuria derive more benefit from aggressive BP lowering. The optimal BP for patients on HD also remains unknown, but appropriate estimation and achievement of dry weight are paramount in controlling BP for most of these patients. Pharmacologic antihypertensive agents will be required for most CKD and ESRD patients, and inhibitors of the RAAS appear to be the most beneficial in CKD patients with proteinuria, with accumulating evidence that there may also be an advantage to these drugs in ESRD patients.

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CHAPTER

41 Hypertension in End-Stage Renal Disease

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Hypertension among patients with end-stage renal disease (ESRD) is common. The diagnosis, pathophysiology, prognosis, and treatment of this condition are complex and subject of much controversy. The purpose of this review is to describe the epidemiology, pathophysiology, treatment, and control of hypertension among dialysis patients and point out controversies where they exist.

EPIDEMIOLOGY

The prevalence, treatment, and control of hypertension among people on hemodialysis have used varying definitions to diagnose hypertension. Most studies have been performed using blood pressure (BP) measurements obtained before and after dialysis and are discussed first. Studies using ambulatory BP recordings are discussed later.

Hemodialysis

Defined as a 1-week average predialysis systolic BP >150 mm Hg or diastolic BP >85 mm Hg or the use of antihypertensive medications in 2,535 clinically stable, adult hemodialysis patients participating in a multicenter trial, the prevalence of hypertension was found to be 86%.[1] Among hypertensive patients, 12% did not receive antihypertensive drugs, 58% were treated but not controlled, and only 30% were controlled. This is in line with other studies which have found the use of antihypertensive drugs to vary between 59% and 83%.[2–4] Furthermore, even among children on long-term hemodialysis similar findings have been reported.[5]

The determinants of hypertension in the general population (not on dialysis) are age, sex, and race. Among hemodialysis patients, sex and race are not found to be determinants of hypertension.[1] Thus, men and women, blacks and whites are equally at risk of having hypertension once they are on hemodialysis. In contrast to the general population, younger patients were more likely to be hypertensive. The independent determinants of hypertension are younger age, etiology of ESRD (either due to diabetes mellitus or hypertension), absence of obesity, and fewer years on dialysis. Thus, nondiabetic, obese, and older patients who have been on dialysis for several years are least likely to be hypertensive.

Treatment with antihypertensive drugs is independently associated with the following factors: younger age, diabetes mellitus, lack of obesity, and aspirin use. Control of hypertension is independently associated with the following: white race, absence of diabetes mellitus, longer years on hemodialysis, use of aspirin, and less antihypertensive drug use. Several studies have confirmed greater antihypertensive drug use to be associated with poorer control.[6,7] It appears that poor control of hypertension among hemodialysis patients is also associated with poor control of hypertension prior to reaching ESRD.[8]

The true prevalence of hypertension in people with ESRD on hemodialysis is difficult to ascertain. This is because among such patients, hypertension is difficult to diagnose. This uncertainty in making a firm diagnosis of hypertension is discussed later (see Diagnosis). Using 44-hour interdialytic ambulatory BP monitoring among 369 chronic hemodialysis patients, the prevalence, treatment, and control of hypertension has been ascertained.[7] The prevalence of hypertension, defined by either an interdialytic ambulatory BP of 135/85 mm Hg or more or the prescription of any antihypertensive agent, was 86%. Volume excess determined inferior vena cava diameter was an independent determinant of prevalent hypertension. Although hypertension was being treated with antihypertensive drugs in 89% of these patients, it was adequately controlled only in 38%. The independent determinants of poor control were the use of antihypertensive drugs and an expanded extracellular volume state. When antihypertensive medications were withdrawn nearly 80% of the participants became hypertensive. Poor control of hypertension was more likely when patients received multiple antihypertensive drugs. However, if patients had volume excess they were more likely to become hypertensive after withdrawing antihypertensive therapy.

The level of illness (comorbidities) among hemodialysis patients varies widely across the world. For example, the crude 1-year mortality rates show a wide range: 6.6% in Japan, 15.6% in Europe, and 21.7% in the United States.[9]

These differences in mortality are not fully accounted for by adjustment for observed comorbidities. Nonetheless, U.S. patients are the sickest. Similarly, there is substantial variation in the prevalence of hypertension among countries. These differences may be due to patient selection, demographic factors, and country-specific practices. As an example, in Tassin, France, patients are dialyzed using long hours of dialysis and administered a low sodium diet.[10–12] These patients have an astonishingly low prevalence of hypertension with <5% requiring antihypertensive therapy at 6 months after initiation of hemodialysis.[12] In contrast, as noted previously, the majority of patients in the United States require antihypertensive drugs.[1,7]

The epidemiology of hypertension has changed considerably since the introduction of hemodialysis. In the early years of dialysis, low sodium dialysate was prescribed, dietary sodium was restricted, and great attention paid to dry weight.[13] The prevalence of hypertension was then reported to be 10% to 15%.[13] The prevalence estimates of 85% to 90% currently reported may be due to several factors such as increased prevalence of diabetes mellitus but also because of change in dialysis practice over the years. For example, the introduction of higher sodium dialysate and less attention to dietary sodium and dry weight may have contributed to the epidemic of poorly controlled hypertension.

Peritoneal Dialysis

The epidemiology of hypertension in patients on long-term peritoneal dialysis (PD) has been less well studied.

Some studies suggest that hypertension control in patients on PD is superior compared to those on hemodialysis.[14,15] For example, among 1,202 patients participating in the 1995 Peritoneal Dialysis Core Indicators Study, the average blood pressure among peritoneal dialysis patients was 139/80 mm Hg.[16] This is in contrast to the predialysis BP of 152/82 mm Hg among 1,238 participants in the HEMO study.[3] Only 29% of PD patients had systolic blood pressure that exceeded 150 mm Hg and only 18% had a diastolic blood pressure greater than 90 mm Hg.[16] Putatively, these observations are explained by removal of vasopressors and sodium pump inhibitors by PD.[17] Nonetheless, high quality head-to-head studies are sparse and the epidemiology of hypertension may be similar to that seen among hemodialysis patients.[18]

Among 414 Italian PD patients, 24-hour ambulatory BP recordings were obtained.[19] The prevalence of hypertension was 88% based on office BP ≥140/90 mm Hg, and 69% based on BP load >30%. BP load is defined as number of BP recordings above a certain threshold. In another study, comparison of 22 hemodialysis patients with 24 patients on PD with 44-hour ambulatory BP monitoring showed no differences in daytime and nighttime BP.[20]

As seen among hemodialysis patients,[21] ambulatory BP among PD patients was related to left ventricular mass.[22,23] This was not the case for office BP.[22] The arterial distensibility of the common carotid artery was significantly related to systolic BP and concentric left ventricular hypertrophy (LVH).[22] On the other hand, overhydration, as assessed by tracer dilution, was common and was related to diastolic BP elevation and eccentric LVH.[22] Volume overload in PD patients may be affected by the peritoneal transport characteristics.[24] High transporters tend to have a higher BP; ultrafiltration may restore their BP to more normotensive levels.[24] In a small study, patients on continuous cyclic PD were reported to have a greater left ventricular mass compared to those on continuous ambulatory PD.[23] This was thought to be due to greater volume overload.[23]

Dipping Phenomenon in Dialysis

Among dialysis patients the physiologic decline in BP from day to night during sleep, known as nocturnal dipping, is nearly universally blunted.[25–29] This blunted nocturnal dipping, or nondipping, has been associated with left ventricular hypertrophy and mortality in some reports[30–33] but not all.[34,35] Although nondipping is said to be a manifestation of volume excess,[26] improving dry weight in a randomized trial did not restore dipping despite improving interdialytic ambulatory BP.[36] Whether nondipping is causally related to LVH and mortality remains unclear.

PATHOPHYSIOLOGY

Both cardiac output and systemic vascular resistance are directly related to BP. An increase in cardiac output without a concomitant reduction in systemic vascular resistance will therefore elevate BP. Conversely, if systemic vascular resistance is increased without a parallel reduction in cardiac output blood pressure would also increase. The pathophysiology of hypertension among dialysis patients is further analyzed using this hemodynamic construct.[37]

Increased Cardiac Output Due to Increased Sodium Load and Extracellular Fluid Volume Expansion

The kidneys are the primary regulators of sodium balance. In the absence of kidney function, sodium regulation is impaired. Sodium balance then has to be maintained by its removal by dialysis. Whereas it is not difficult to remove the interdialytic weight by ultrafiltration, subtle loss of lean body mass can lead to volume overload that is difficult to recognize. Typical clinical signs such as pedal edema are often not useful in detecting the presence of volume excess.[38] Volume excess evokes increase in cardiac output which over time provokes increase in systemic vascular resistance.[37,39] Increase in systemic vascular resistance manifests clinically as hypertension. The rise in blood pressure provokes natriuresis which normalizes BP. However, hemodialysis patients have markedly impaired ability to excrete sodium and therefore to reduce BP. Unless excess extracellular fluid volume is removed with ultrafiltration, a rise in vascular resistance sustains hypertension in these individuals. Thus, assessment

and treatment of volume excess is an important and modifiable target of therapy in patients with ESRD. Whereas both dialysate and dietary sodium restriction are important for achieving euvolemic state, unless dry weight is achieved, sodium restriction by itself is unlikely to produce euvolemia.[40]

Intercurrent illnesses such as catheter-related bacteremia, pneumonia, and diabetic foot infection may cause declines in BP and result in difficulties with ultrafiltration. If these illnesses are substantially severe and cause loss of lean body mass, then recovery from these inflammatory illnesses may be associated with volume overload if loss of lean body mass is not recognized. Thus, as a practical corollary, dry weight should be reassessed especially after a recent intercurrent illness even when BP may not be elevated.

In more than 90% of patients, mean arterial pressure with ultrafiltration dialysis falls. This decline in BP has been somewhat misleadingly called "volume-dependent hypertension."[41,42] In contrast to the fall in BP within hours, volume-dependent hypertension is characterized by a decline in BP in response to sodium and volume restriction that takes several weeks or months to manifest. For example, in patients with essential hypertension who are prescribed thiazide diuretics, an initial fall in extracellular fluid (ECF) volume is followed by a slower decline is systemic vascular resistance that finally evokes a reduction in BP.[43] Similarly, in patients with stage 3 and 4 chronic kidney disease (CKD) who are prescribed loop diuretics there is an initial fall in ECF volume associated with a rise in plasma renin activity; subsequently reduction in BP is seen.[44] In dialysis patients reduction in body weight with ultrafiltration, long-duration dialysis coupled with dietary sodium restriction leads to improvement in BP over several weeks.[36] In contrast to the intradialytic reduction in BP, it is the long-term changes in BP evoked by a reduction in ECF volume that are examples of volume-dependent hypertension.

Some investigators have described a delayed reduction of BP after dry weight is lowered. This is called the lag phenomenon. This phenomenon was described using observations on predialysis or postdialysis BP who had dry weight lowered when they were new to dialysis (incident patients).[45] The existence of the lag phenomenon in this population has been questioned by the findings of a trial among prevalent hemodialysis patients.[36] In this randomized trial, a prompt improvement in interdialytic ambulatory BP within 4 weeks was noted; no further reduction in BP at 8 weeks was found. Therefore, these findings question the existence of a lag phenomenon. Several other reports when patients are dialyzed more frequently have also found prompt improvements in BP.[46,47] In one such report, prompt reduction in BP was seen within 1 month, with no reduction in BP over 1 year.[47] It is also important to recognize fluid weight loss may need to reach a certain threshold before BP improves among hemodialysis patients. In other words, BP may not decline until a threshold of euvolemia is achieved. Once achieved, BP decline may be rapid and precipitous. Accordingly, among incident patients where dry weight is challenged gradually a lag phenomenon may appear to exist when in fact this may be simply a threshold effect of volume on BP.

Patients with ESRD may have salt craving and may therefore consume excess salt. Compared to 11 healthy volunteers, among 29 patients with CKD who underwent a taste test with sodium impregnated test strips dietary sodium intake was proportional to the taste threshold for sodium.[48] The taste threshold for sodium was blunted in patients with CKD; it was particularly blunted in patients on oral diuretics. Furthermore, zinc deficiency was associated with this latent taste dysfunction. These findings suggest that latent gustatory dysfunction and zinc deficiency may underlie excess sodium intake among patients with CKD. Although this study was limited to patients with earlier stages of CKD, similar mechanisms may mediate gustatory dysfunction among those with ESRD.

Emerging data suggest that sodium may have a more complex role in the pathogenesis of hypertension. For example, high-salt diet in rats provokes interstitial hypertonic sodium accumulation in skin—this evokes proliferation of the lymph capillary network. The mechanisms underlying these effects on lymphatics involve activation of tonicity-responsive enhancer binding protein (TonEBP) in macrophages that are present in the interstitial compartment of the skin. Activation of TonEBP causes vascular endothelial growth factor (VEGF)-C secretion by macrophages. This in turn increases the density of the lymph capillary network. Interrupting VEGF-C signaling augments interstitial hypertonic volume retention and elevates BP in response to a high-salt diet.[49] These data provide support to the notion that, besides external sodium balance, the redistribution of sodium between the skin and circulation provides extrarenal regulation of body fluid volume and BP control. The relevance of this finding to sodium balance in humans is not yet clear.

Increased Systemic Vascular Resistance

In has been long recognized that increased systemic vascular resistance may play an important role in maintaining hypertension among patients undergoing nephrectomy and hemodialysis.[50] However, there is no consistent hemodynamic pattern that distinguishes hypertensive from normotensive dialysis patients.[51] Mediators that may influence systemic vascular resistance are discussed in the following sections.

Renin-Angiotensin System

It has also been long recognized that among uremic patients with hypertension, although many patients may have plasma renin activity in the normal range, plasma renin activity may be inappropriately increased in relationship to exchangeable sodium.[52] That the renin-angiotensin system (RAS) is activated even in hemodialysis patients is illustrated by the following: (1) renin increases with ultrafiltration dialysis suggesting that kidneys with nearly no excretory function can still sense volume[53]; (2) blood pressure improves with

an infusion of saralasin, an angiotensin II antagonist[54]; and (3) patients treated with angiotensin-converting enzyme (ACE) inhibitors have a dose-dependent increase in plasma renin activity[55] and an improvement in blood pressure.[55,56]

Sympathetic Nervous System

Besides serving as an excretory organ for salt and toxin removal, the kidneys, even when dysfunctional as in ESRD, somewhat surprisingly, also serve as an afferent sensor for the activation of the sympathetic nervous system (SNS). Strong evidence has emerged that implicates enhanced sympathetic activity as a cause of hypertension in patients with ESRD.[57,58]

Initial studies provided indirect evidence of increased sympathetic nerve activity. For example, investigators reported diminished vascular response to norepinephrine in animals with chronic renal failure.[59] This was explained by a chronic increase in sympathetic nerve activity that downregulated adrenergic receptors. However, later studies provided more direct evidence of elevated sympathetic tone in patients with ESRD.[57] This was made possible by direct measurement of efferent sympathetic nerve activity.[60] That latter technique involves microneurography in which a fine tungsten electrode is placed in the sympathetic nerves that run along the peroneal nerve. Using this technique, sympathetic activity is noted to be markedly increased in those patients on chronic hemodialysis who still have their native kidneys. In contrast, patients with bilateral nephrectomy have reduced sympathetic activity, lower calf vascular resistance, and lower mean arterial pressure.[57] Thus, the kidney, although devoid of excretory function, serves as an afferent organ to signal the midbrain region to increase sympathetic activity. The central mechanisms of increased sympathetic activity may involve endogenous nitric oxide; endogenous nitric oxide may inhibit sympathetic activity in several brain nuclei involved in the neurogenic control of BP.[61] Dopaminergic neurons may be responsible. For example, experiments in hypertensive hemodialysis patients show that administration of the dopamine-releasing drug bromocriptine decreased plasma norepinephrine and other markers of sympathetic outflow, and lowered mean arterial pressure.[62] Baroreceptor desensitization has also long been recognized in hypertensive patients with ESRD and may contribute to elevated BP.[63]

Recent discovery of an enzyme that catabolizes catecholamines may be important in the pathogenesis of hypertension among dialysis patients. This enzyme called renalase is an amine oxidase which is secreted into the blood by the kidney.[64] Renalase is secreted as a proenzyme and degrades circulating catecholamines. This enzyme is activated by an increase in BP or brief surges in plasma catecholamines. Infusion of recombinant renalase lowers BP by reducing peripheral vascular resistance as well as cardiac output. Markedly reduced levels of circulating renalase are found among hemodialysis patients; renalase levels are inversely related with glomerular filtration rate (GFR). Renalase knockout in the mouse is associated with hypertension and these mice are sensitive to cardiac ischemia.

Nitric Oxide and Its Circulating Inhibitors

Endothelial derived nitric oxide plays a critical role in the maintenance and regulation of vascular tone and modulates key processes mediating vascular disease including leukocyte adhesion, platelet aggregation, and vascular smooth muscle proliferation.[65] Endothelial nitric oxide synthase enzymatically produces nitric oxide from the substrate L-arginine. L-arginine supplementation can partially reverse renal failure-associated endothelial dysfunction[66] and improve BP.[67] A circulating inhibitor of nitric oxide synthase, asymmetrical dimethyl arginine (ADMA), competes with L-arginine for nitric oxide synthase. In humans with salt-sensitive hypertension, administration of a high-salt diet increases plasma ADMA and BP.[68] Circulating ADMA is increased in subjects with CKD[69] and ESRD[70] and may contribute to endothelial dysfunction and increased BP. In patients with ESRD, ADMA is correlated with increased left ventricular (LV) thickness and reduced ejection fraction, consistent with its ability to increase systemic vascular resistance.[71] Furthermore, ADMA is highly correlated with norepinephrine in dialysis patients.[70] Both are strongly linked to mortality in cohort studies.[70] As discussed previously, nitric oxide may be important for the control of nuclei that control sympathetic activity.[61] This suggests a strong relationship between impaired NO availability and sympathetic activation in these patients.

In normal healthy subjects, approximately 300 μmol ADMA is generated per day, but only 50 μmol per day is excreted by the kidneys; the remaining is degraded enzymatically by dimethylarginine dimethylaminohydrolase (DDAH).[72] Pharmacologic inhibition of DDAH (with a small molecule, 4124W) causes accumulation of ADMA and generalized vasoconstriction. As may be expected, overexpression of DDAH in genetically engineered mice reduces ADMA, improves NO bioavailability, and reduces systolic BP. Oxidative stress impairs DDAH activity by oxidizing a sulfhydryl moiety critical to enzymatic activity; this leads to accumulation of ADMA and promotes endothelial dysfunction. Inflammation, increased homocysteine levels, reduced antioxidant defenses, and increased free radicals in ESRD may therefore provide an explanation for the relationship between oxidative stress, endothelial dysfunction, and the generation of hypertension. Cohort studies show an association between increased ADMA and cardiovascular events or death in hemodialysis patients.[70] However, lowering homocysteine does not prevent cardiovascular events.

Erythropoietin

Hypertension is a common but frequently overlooked adverse effect of erythropoietin therapy.[73–78] Hypertension can be missed because of variability in BP from predialysis to postdialysis and the lack of home or ambulatory BP measurements.[2] Studies that failed to detect increases in BP with erythropoietin therapy may have managed hypertension more aggressively through the prescription of antihypertensive drugs or closer attention to dry weight.[79,80]

Erythropoietin therapy was an independent predictor of hypertension diagnosed by ambulatory BP monitoring.[7] Some studies show association of erythropoietin use with nondipping.[81] Increase in BP with erythropoietin occurs more commonly in those people with preexisting hypertension[77,82] or those with a family history of hypertension.[82]

Although the exact mechanism by which erythropoietin increases BP is not known, it may be multifactorial such as increased vascular reactivity to norepinephrine,[83] increased generation of endothelin-1,[84–86] and vasoconstrictor prostaglandins.[84] In line with the generation of vasoconstrictor prostaglandins, use of antiplatelet therapy has been postulated to prevent the development of hypertension among patients treated with erythropoietin.[87] The critical importance of nitric oxide in erythropoietin-induced hypertension has been studied in a mouse model.[88] Transgenic mice overexpressing human erythropoietin were generated. Despite hematocrit levels of 80%, adult transgenic mice did not develop hypertension or thromboembolism because of a compensatory increase in endothelial nitric oxide (NO) synthase levels, NO-mediated endothelium-dependent relaxation, and circulating and vascular tissue NO levels. Administration of the NO synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) led to vasoconstriction, an increase in vascular resistance, hypertension, and death of transgenic mice; the wild-type siblings developed hypertension but did not show increased mortality. L-NAME-treated polyglobulic mice revealed acute left ventricular dilatation and vascular engorgement associated with pulmonary congestion and hemorrhage. Thus, endothelial NO appears to be critical in maintaining normotension, preventing cardiovascular dysfunction, and survival in vivo when erythropoietin is used.

Erythropoietin levels were correlated with systemic vascular resistance and ambulatory BP among untreated patients with essential hypertension.[89] In carefully conducted studies to determine the mechanism of increase in BP with erythropoietin, it was observed that the systemic vascular resistance was uniformly increased with the administration of erythropoietin.[90] Furthermore, a good relationship was seen between exchangeable sodium and increase in BP. Somewhat surprisingly, aldosterone was also increased with this therapy. Accordingly, close attention should be paid to volume status and sodium balance while using this drug.[90] All patients prescribed this therapy should have careful BP monitoring.

Uncertainty exists regarding the determinants of hypertension among patients treated with erythropoietin. For example, some studies show that although increase in hemoglobin is dose-dependent, an increase in BP is not.[73,91] On the other hand, during the first 5 weeks of administration of erythropoietin, the change in hemoglobin concentration was directly related to increase in diastolic BP ($r = 0.42$, $P < .001$).[75] Interestingly, in animal experiments hypertension does not track with increase in hemoglobin.[92] If erythropoietin is administered to anemic animals with chronic renal failure, but hemoglobin is kept stable by feeding an iron deficient diet, hypertension still occurs. In blood vessels harvested from these animals treated with erythropoietin, vasodilatory responses to NO donors were impaired, but response to several vasoconstrictors was normal. Among patients on long-term hemodialysis, treatment with iron to increase hemoglobin was not associated with parallel increases in BP.[93]

Others

A variety of other substances have been described which can have vasoconstrictive properties. These are discussed in detail elsewhere in the book, but for the sake of completeness, they are summarized briefly here. For example, compounds that block the sodium pump, such as digoxin-like immunoreactive substance and ouabain-like compound, can lead to an increase in intracellular calcium that may elicit vascular small muscle contraction.[17] Plasma endothelin-1 levels are noted to be increased among hypertensive dialysis patients compared to normotensive dialysis controls.[94]

Parathyroid hormone (PTH) can increase intracellular calcium and aggravate hypertension[95] and parathyroidectomy may improve BP.[96] Not all investigations support this notion. For example, elevated PTH levels can reduce the pressor response to norepinephrine in animals with chronic renal failure[97] and parathyroidectomy may not correct hypertension.[98]

Loss of medullary prostaglandins and other renal-derived vasodilators may also be responsible for hypertension in this population. Plasma concentrations of the vasoconstrictive peptide endothelin are elevated in patients on hemodialysis[99,100] and much more so than in those patients requiring no dialysis.[100]

Use of illicit drugs such as cocaine or prescription drugs such as decongestants containing pseudoephedrine may also contribute to increased BP. Nonadherence with dialysis treatments may make hypertension difficult to control.[101] This may be related to both excess accumulation volume and toxins.

Unlike the general population in which obesity is associated with hypertension, absence of obesity is consistently associated with less prevalence of hypertension and better control. The mechanism of this relationship is not clear. It is possible that even subtle volume excess among lean patients leads to hypertension. Those with obesity may be able to buffer the excess volume without manifesting hypertension.[102]

Sleep apnea is very common in dialysis patients and is often associated with volume overload. Hypopneic spells during the night lead to nocturnal hypoxemia and provoke intense sympathetic arousal and an increase in nocturnal BP.[103]

Vascular Changes as a Basis of Systolic Hypertension

Systolic BP increases with age.[104] In contrast, there is an increase in diastolic BP until about age 55 years and then a fall. Accordingly, pulse pressure widens with age. Structural and functional changes in the arterial circulation that occur

with aging are accelerated with hypertension[105] and CKD.[106] Vascular aging is associated with increase in arterial stiffness. Arterial stiffness is strongly and directly related to systolic BP[107] and mortality in ESRD.[107] Arterial stiffness is also related to ventricular stiffness making intradialytic hypotension and interdialytic hypertension two faces of the same disease.[108] Diabetes mellitus, age, and smoking are some of the factors that also accelerate vascular aging. These are common among those with CKD. Factors peculiar to uremia that contribute to accelerated vascular stiffening include hyperparathyroidism, increased calcium × phosphorus product, increased circulating endothelin concentration,[109] sympathetic activation,[57] and vascular inflammation; increases in intravascular volume and inappropriately high angiotensin II further augment vascular stiffness.[110] Accordingly, it is not surprising that systolic hypertension is common in those with ESRD.

DIAGNOSIS

The diagnosis of hypertension among patients on hemodialysis is challenging and misdiagnosis may lead to overtreatment or undertreatment of hypertension.[111–114] Diagnosing hypertension is difficult because of several reasons.[115] BP in these patients is often measured without attention to technique.[116] BP declines during hemodialysis with ultrafiltration. This decline in BP can be variable and in part is related to the magnitude and intensity of ultrafiltration.[117] For example, those patients who have a large volume removed over a short period of time may have a large decline in BP. These patients may also gain the removed volume back over the interdialytic interval and have a large increase in BP.[118] Predialysis BP may therefore be hypertensive and postdialysis BP hypotensive. It therefore becomes unclear which BP to use to diagnose hypertension[119] and substantial errors can occur both in the detection of hypertension and assessing its severity.[120,121] Both predialysis and postdialysis BP measurements are highly variable from one visit to the next. The variability within patients (between visits) is so much that it approaches the variability in BP between patients.[122] Additionally, hemodialysis patients have significant seasonal variability in BP; BP is highest during winters and lowest during summers.[123] This may be related to temperature-induced vasodilatation. Although significant relationships exist between both predialysis and postdialysis BP and interdialytic ambulatory BP,[124] a meta-analysis has shown that predialysis and postdialysis BP measurements agree poorly with interdialytic ambulatory BP.[125] Accordingly, among hemodialysis patients, large errors are possible when using predialysis or postdialysis BP to judge the magnitude of elevation in interdialytic ambulatory BP.

The current National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines recommend that prehemodialysis and posthemodialysis BP should be <140/90 and <130/80 mm Hg, respectively.[126] Use of predialysis or postdialysis BP measurements to make management decisions in the interdialytic period can be problematic. For example, in a survey in the United Kingdom, centers that achieved better postdialysis BP targets had more intradialytic hypotension.[127] What is clear is that interdialytic weight gain increases predialysis BP[3,128–131] and provokes the use of antihypertensive therapy.[128,129] However, interdialytic weight gain does not correlate with interdialytic ambulatory BP.[132,133] Therefore, whether achieving these pre- or postdialysis BP targets would cause clinical harm (or benefit) remains unknown.

Using all BP measured during a midweek dialysis treatment may serve as a more useful tool to estimate interdialytic ambulatory BP.[134] Although the mean intradialytic BP serves as a useful tool to assess hypertension, the calculation of median intradialytic BP is computationally less intense. It may therefore be used as a bedside tool to predict interdialytic ambulatory BP. Midweek median intradialytic BP of 140/90 mm Hg or more has sensitivity and specificity that exceeds predialysis or postdialysis measurements and can serve as a rapid and convenient tool to assess hypertension in long-term hemodialysis patients.[134] However, this is the method of last resort because better methods are available to evaluate hypertension in hemodialysis patients. Why these distinctions are important is discussed later.

Home BP monitoring is a practical way to diagnose and manage hypertension in all patients with kidney disease.[135,136] Home BP monitoring is recommended by both the American Heart Association and the European Society of Hypertension for diagnosing and managing hypertension.[137,138] Home BP monitoring is especially valuable in diagnosing and managing hypertension for those on hemodialysis for the following reasons.[139] Home BP correlates more closely with ambulatory BP compared to predialysis or postdialysis BP recordings.[140] Home BP can track changes in BP evoked by reduction in dry weight.[141] Home BP, compared to predialysis or postdialysis BP recordings, is much more reproducible from one week to the next.[141] Home BP is superior to measurements made in the dialysis unit, even when the dialysis unit measurements are made using recommended techniques, in predicting the presence of target organ damage (echocardiographic LVH)[21,142] or long-term outcomes such as cardiovascular events [143] or mortality.[143–146] The association of BP and outcomes is discussed further in the section on Prognosis. A recent trial randomized stable hemodialysis patients to home BP-guided therapy or predialysis BP-guided therapy.[147] The primary endpoint was to assess change in interdialytic ambulatory BP at 6 months and change in echocardiographic LVH. There was no change in ambulatory BP at 6 months in the predialysis BP-guided therapy group. A significant fall was noted at 6 months in ambulatory BP. Between group differences were significant. Given the small number of patients and variability in timing of echocardiographic left ventricular mass measurements, no between groups differences in the latter measurement were seen. Another trial randomized 17 hemodialysis patients to usual care and 17 to home BP monitoring. Significant

improvement in average weekly systolic BP was seen in the home BP group only.[148] These data support the use of home BP measurement to manage patients on hemodialysis.

Among hemodialysis patients, the timing and frequency of home BP monitoring is of particular importance. Home BP increases on average at a rate of 4 mm Hg every 10 hours elapsed after dialysis.[149] Therefore, measurement soon after dialysis or just before dialysis will underestimate or overestimate the burden of hypertension. Therefore it is important to measure BP at various intervals following dialysis. Simply obtaining BP 20 minutes postdialysis may not yield the most representative interdialytic BP.[150] We recommend that measurements be made twice daily (on waking up in the morning and just before going to sleep) following a midweek dialysis for 4 days.[151] These measurements allow an adequate number for diagnosing and managing hypertension. For the long-term follow-up, monthly measurement should suffice in most patients. More frequent measurements may be needed in those who are clinically unstable.

Ambulatory BP monitoring, among hemodialysis patients, is held to be the gold standard for diagnosing hypertension.[119,152–154] Compared to peridialytic BP recordings, it correlates better with LVH,[21] and all-cause mortality.[155] Using a validated monitor,[156] we recommend measuring BP over the entire interdialytic interval (44 hours). We recommend recording BP every 20 minutes from 6 AM to 10 PM and every 30 minutes from 10 PM to 6 AM.[157] As in the case of home BP, interdialytic systolic BP increases but at a lower rate of 2.5 mm Hg every 10 hours.[158,159] Because, compared to home BP, typically a much greater number of measurements during the interdialytic interval are available, patterns of BP can be evaluated. Figure 41.1 illustrates the pattern of BP and heart rate over an interdialytic interval.

Evaluating the pattern of ambulatory BP offers insights into the volume state and arterial stiffness. A cohort of long-term hemodialysis patients underwent evaluation for arterial stiffness by aortic pulse wave velocity and interdialytic ambulatory BP monitoring.[159] In a cross-sectional analysis from 11,833 blood pressure measurements from 125 long-term hemodialysis patients, it was found that aortic pulse wave velocity and interdialytic weight gain had a substantial impact on interdialytic ambulatory BP level, trends, and rhythms (Fig. 41.2). Arterial stiffness was associated with an overall increase in the level (intercept) of systolic, diastolic, and pulse pressure. Interdialytic weight gain, on the other hand, was associated with interdialytic increase (linear trend) in BP. The circadian amplitude was blunted by increments in either arterial stiffness or interdialytic weight gain. These results suggest that arterial BP patterns may be of prognostic importance.

If there was a causal relationship between dry weight and patterns of BP it would follow that challenging dry weight will alter the pattern of BP. If dietary patterns were not clamped, but dry weight is challenged it is expected that

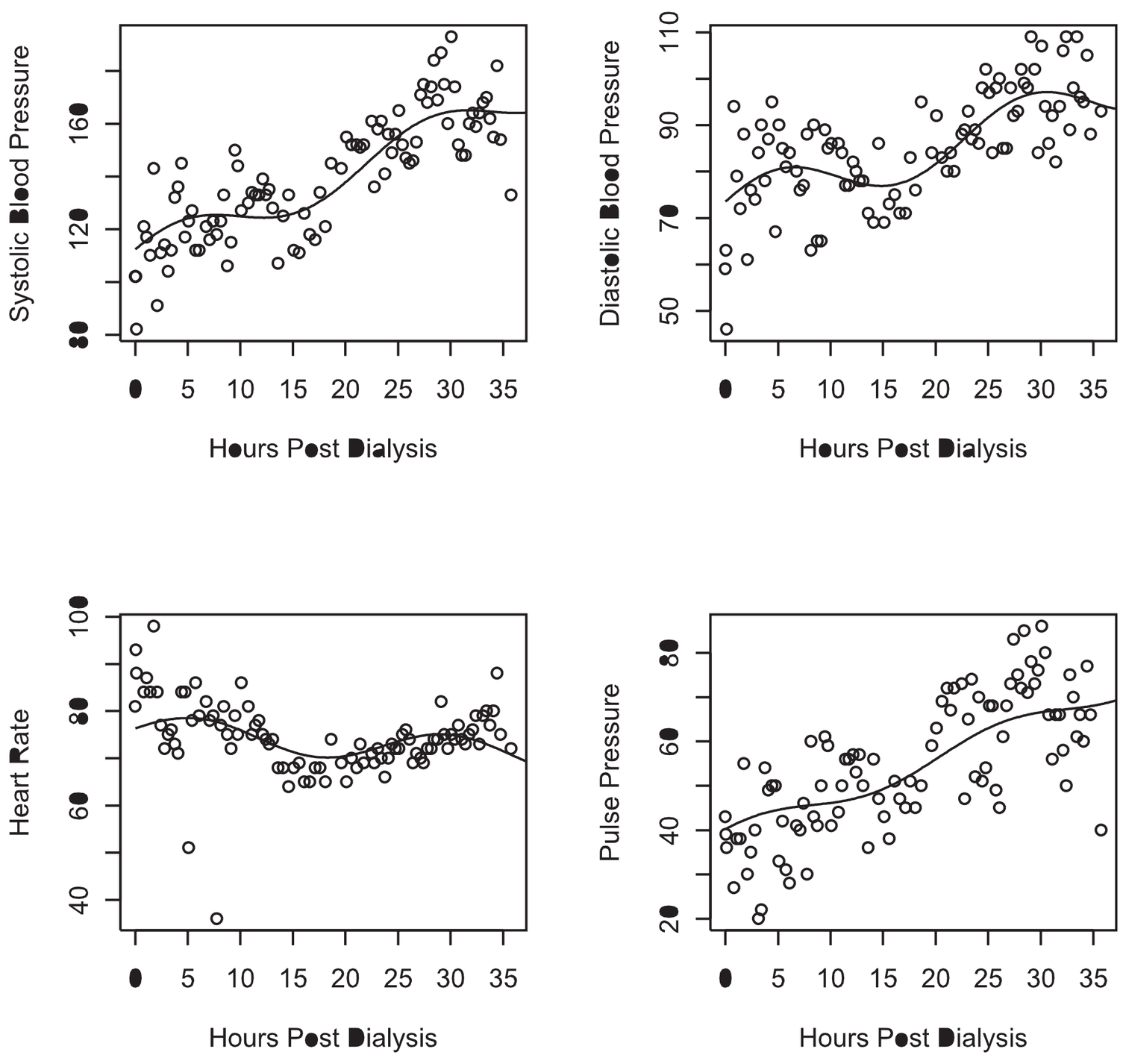

FIGURE 41.1 Modeled trended cosinor blood pressure and pulse rate in a hemodialysis patient. Notice the linear trend in systolic, diastolic, and pulse pressure but lack thereof in heart rate. (Reprinted from Kelley K, Light RP, Agarwal R. Trended cosinor change model for analyzing hemodynamic rhythm patterns in hemodialysis patients. *Hypertension*. 2007;50:143.)

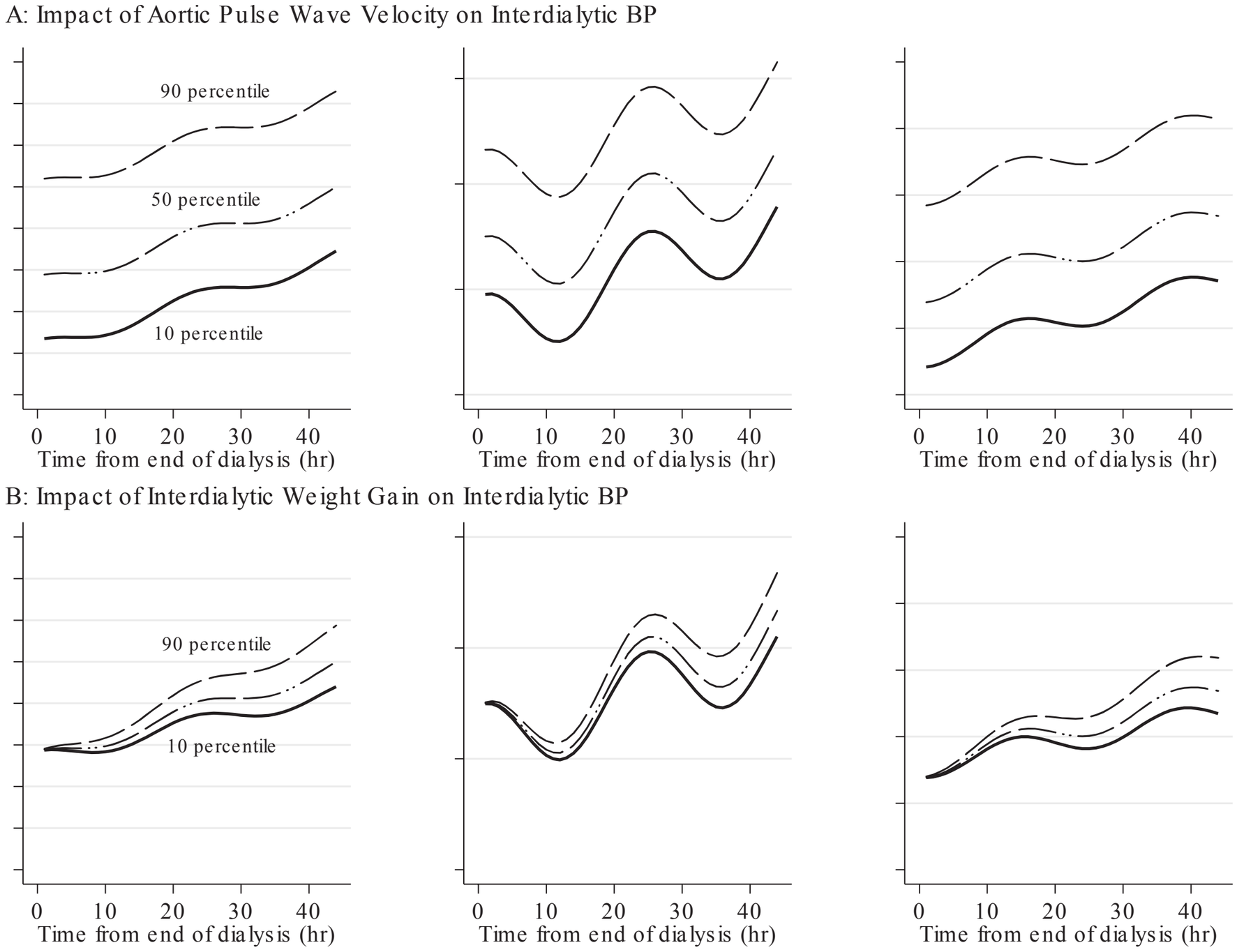

FIGURE 41.2 Impact of aortic pulse wave velocity and interdialytic weight gain on ambulatory blood pressure between two consecutive dialysis treatments. The top three graphs show the impact of increasing pulse wave velocity at 10th percentile (4.1 m/s, *solid line*, bottom), 50th percentile (6.00 m/s, *dashed and dotted line*, middle), and 90th percentile (10.6 m/s, *dashed line*, top) on blood pressures with interdialytic weight gain plotted at the median (2.4 kg). Only the intercept blood pressure changes. The bottom three graphs show the impact of increasing interdialytic weight gain at 10th percentile (0.9 kg, *solid line*, bottom), 50th percentile (2.4 kg, *dashed and dotted line*, middle), and 90th percentile (4.6 kg, *dashed line,* top) on blood pressures with aortic pulse wave velocity plotted at the mean (6.0 m/s). The slope of the line is influenced by interdialytic increase in blood pressure. (Reprinted from Agarwal R, Light RP. Arterial stiffness and interdialytic weight gain influence ambulatory blood pressure patterns in hemodialysis patients. *Am J Physiol Renal Physiol.* 2007;294:F303–F308.)

interdialytic ambulatory systolic BP will be lowered but interdialytic weight gain will increase. Compared to a control group, patients who had dry weight challenged experienced steepening of the interdialytic systolic BP slopes and lowering of intercept systolic BP (Fig. 41.3). Thus flat interdialytic BP slopes and high overall systolic BP may reflect volume overload.[118]

In approximately 10% to 15% of the patients, instead of decreasing, BP paradoxically increases during dialysis.[160] These patients have intradialytic hypertension. Intradialytic hypertension is defined in different ways. These definitions include the following: (1) a discrete change in BP from pre- to postdialysis in a certain proportion (typically >50%) of dialysis sessions; (2) regression of all intradialytic BP with slope >0; and (3) change from pre- to postdialysis of >0 mm Hg. Intradialytic hypertension is associated with greater short-term (6 month) mortality in hemodialysis patients.[161] In another cohort, increasing systolic BP by more than 10 mm Hg during hemodialysis occurred in about 10% of incident patients, and although increasing systolic BP during hemodialysis was associated with decreased 2-year survival, these findings were limited to patients with predialysis systolic BP less than 120 mm Hg.[162] Although the exact mechanism of this relationship is unclear,[163,164] a study shows that intradialytic hypertension in hemodialysis patients using definition 2 is associated with both volume excess and interdialytic hypertension.[165] Another study, using definition 1, confirmed the association between intradialytic hypertension and interdialytic hypertension.[166]

At least two studies suggest that lowering dry weight may improve interdialytic hypertension. Cirit et al. studied seven hypertensive patients on hemodialysis with marked cardiac dilatation who experienced paradoxical hypertension during dialysis.[167] After probing dry weight, both BP and postdialysis weight was reduced; BP reduction was 46/22 mm Hg and postdialysis weight was reduced by 6.7 kg. They concluded that BP may paradoxically rise with ultrafiltration when patients are volume overloaded. In

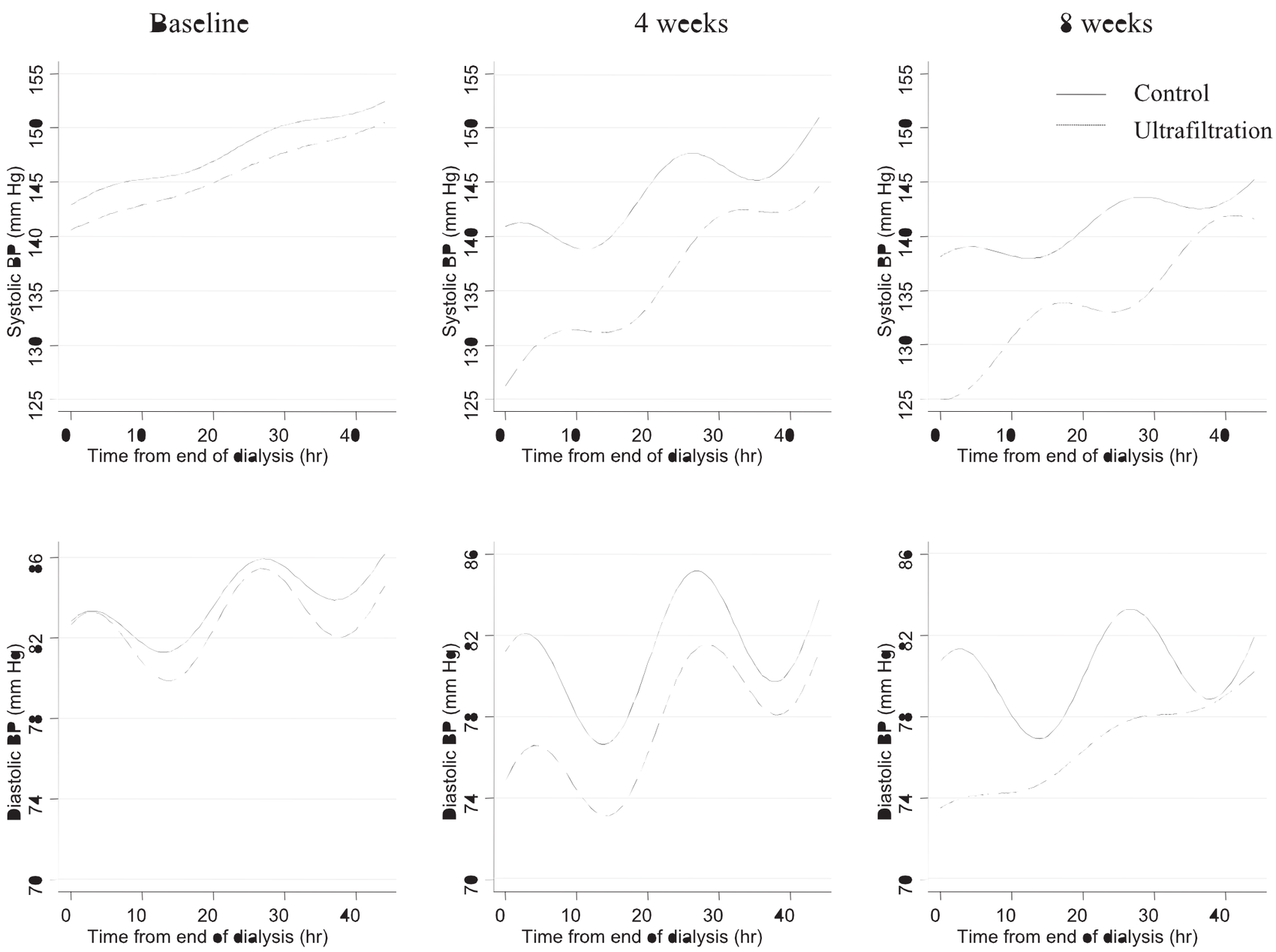

FIGURE 41.3 Patterns of systolic and diastolic blood pressure obtained using ambulatory blood pressure monitoring over 44 hours during an interdialytic period and analyzed using the trended cosinor change model. The *solid line* is the control group and *dotted line* the ultrafiltration (UF) group. The left panel represents recordings at baseline, the center panel measurements at 4 weeks, and the right panel 8 weeks following randomization. UF caused a reduction in intercept systolic and diastolic blood pressure but steepened the slope of change over time at 4 weeks and 8 weeks. The amplitude of variation increased in the control group compared to UF group at 4 weeks and 8 weeks in the case of diastolic but not systolic blood pressure. (Reprinted from Agarwal R. Volume-associated ambulatory blood pressure patterns in hemodialysis patients. *Hypertension*. 2009;54:241.)

another randomized trial (DRIP trial, discussed later), dry weight was reduced progressively.[36] Those patients who had intradialytic hypertension who had additional ultrafiltration and therefore reduction in dry weight had improvement in both intradialytic and interdialytic hypertension.[165] This suggests that an appropriate therapy for intradialytic hypertension would be to further lower dry weight.

A meeting report of the Kidney Disease Improving Global Outcomes (KDIGO) controversy conference concluded the following: "Although a worthy goal, neither measurement of ambulatory blood pressure monitoring nor self-measured home BP may be feasible for most patients throughout the world, leaving pre-hemodialysis and post-hemodialysis BP measurements to be used, but with caution and with the knowledge that these are inferior."[168] This discussion makes it clear that the opinions of the author differ from that of the work group. Home BP measurement is a practical way to measure and manage hypertension among hemodialysis patients. The targets of therapy using home BP monitoring will need to be defined in future trials. Guidelines of the American Heart Association define hypertension in the general population as home BP of at least 135/85 mm Hg; lowering BP in the interdialytic period to at least 140/90 mm Hg appears to be a reasonable goal.

NONPHARMACOLOGIC TREATMENT

Once an accurate diagnosis is made (see section on how to make an accurate diagnosis), the therapy of hypertension among hemodialysis patient rests on nonpharmacologic management. Although scarcely studied, one small study lasting 6 months showed a beneficial effect of exercise on BP and medication requirements.[169] Exercise consisted of using a stationary bicycle during dialysis.[169] Besides this promising strategy, the nonpharmacologic management of hypertension is based on four principles: dietary sodium restriction, individualizing dialysate sodium, the management of dry weight, and providing an adequate dialysis. These principles are discussed in the following sections.

Dietary Sodium Restriction

Dietary sodium restriction limits interdialytic weight gain and improves the feasibility of achieving dry weight.[170,171] Instead of restricting dietary sodium, patients on hemodialysis

are sometimes prescribed fluid-restricted diets. With the exception of treating hyponatremia, there is no scientific basis for prescribing a fluid-restricted diet in these patients.[172]

Recent guidelines suggest that the elderly and those with CKD are most likely to derive the greatest benefits of dietary sodium restriction.[173] These guidelines recommend restricting sodium intake even more than those advocated earlier (2 g per day). Dietary sodium restriction to no more than 1.5 g sodium (or about 65 mmol) per day is now recommended.

Although no randomized trials have been performed among patients with ESRD, observational studies among long-term hemodialysis patients suggest that restricting dietary sodium and achieving dry weight can improve LVH.[174]

Although dietary sodium intake remains a common cause of excess volume accumulation, an often overlooked source of sodium loading among hemodialysis patients is through the prescription of dialysate sodium.

Individualizing Dialysate Sodium

Short dialysis treatment sessions provokes hemodynamic instability; hypertonic dialysate was introduced into practice to provide better hemodynamic stability. However, this practice came at a cost. It has been recognized for a long time now that substantial increments in interdialytic weight gain and thirst can be provoked with the prescription of hypertonic dialysate.[175] Reducing interdialytic weight gain and thirst are now being recognized as important treatment targets.[176] The prescription of high Na dialysate triggers increased fluid volume removal, hemodynamic instability, and prescription of even a higher dialysate sodium.[177] In some patients, worsening of BP control may ensue.[178] The vicious cycle can be interrupted by individualizing dialysate sodium concentration[179] which may improve BP control.[180] In a pilot study of 16 patients, dialysate sodium at end dialysis was decreased in four phases from 137.8 mmol to 135.6 mmol.[181] As a result of this maneuver, the net sodium loss increased nearly 100 mmol from 383 to 480 mmol; this also reduced interdialytic weight gain and BP.[181] Thus, facilitating diffusive sodium losses in addition to convective loss can increase net sodium removal and therefore BP. Sodium ramping which is prescribed to offer intradialytic hemodynamic stability is associated with fewer hypotensive episodes on dialysis but greater interdialytic fatigue and thirst, greater interdialytic weight gain, and hypertension.[182] Interdialytic 24-hour ambulatory BP increased when time-averaged concentration of Na was extremely elevated at 147 mEq/dL.[183] Therefore, one sodium prescription may not fit all patients.

In a nonrandomized trial improvement in nocturnal mean arterial pressure was found among patients who were prescribed a low dialysate sodium.[40] However, if the low dialysate sodium was not accompanied by reduction in dry weight, BP did not change. Simply prescribing low dialysate sodium without altering dry weight may not improve BP control. On the other hand, BP increments provoked by higher sodium dialysate can be adequately controlled by adjustment of dry weight.[184]

Management of Dry Weight

The management of dry weight poses several challenges. First and foremost, there is not even a universally agreed upon definition of dry weight.

The concept of dry weight was proposed in the early years of dialysis.[185] In 1967, Thomson and colleagues defined dry weight as reduction of BP to hypotensive levels during ultrafiltration and unassociated with other obvious causes.[185] Then, in 1980, dry weight was defined by Henderson as the weight obtained at the conclusion of a regular dialysis treatment below which the patient more often than not will become symptomatic and go into shock. In 1996, dry weight was defined by Charra and colleagues as the body weight at the end of dialysis at which the patient can remain normotensive until the next dialysis despite the retention of saline and ideally without the use of antihypertensive medications.[186] In 2008, Raimann et al. proposed a definition of dry weight defined by continuous calf bioimpedance analysis during dialysis. They defined dry weight as a flattening of the baseline/instantaneous impedance ratio curve for at least 20 minutes in the presence of ongoing ultrafiltration. Finally in 2009, Sinha and Agarwal proposed a definition that combined subjective and objective measurements.[187] According to this definition, dry weight is defined as the lowest tolerated postdialysis weight achieved via gradual change in postdialysis weight at which there are minimal signs or symptoms of either hypovolemia or hypervolemia.

Assessment of Dry Weight

The physical examination is notoriously unreliable in excluding volume overload. For example, pedal edema does not correlate with dry weight very well. In a case control study, Agarwal et al. found that inferior vena cava diameter, blood volume monitoring, plasma volume markers, and inflammation markers were not determinants of edema.[38] In hemodialysis patients, pedal edema correlated with cardiovascular risk factors such as age, obesity, and left ventricular mass but not volume markers. For the most part, the assessment and achievement of dry weight is an iterative process that often provokes uncomfortable intradialytic symptoms such as hypotension, dizziness, cramps, nausea, and vomiting. These symptoms often lead to interventions such as cessation of ultrafiltration, administration of saline, the premature cessation of dialysis, or placing the patient in the head-down (Trendelenburg) position. Interestingly, placing the patient in the head-down position does little to protect the BP[188]; whereas raising the leg passively without lowering the head can be effective in raising ventricular filling pressure.[189] Often physicians will respond to these distressing symptoms by raising dry weight, which may result in the necessity of adding more antihypertensive medication. Paradoxically, this may make

subsequent achievement of dry weight even more difficult. However, there are strategies to gently reduce target weight by setting the ultrafiltration goal slightly above the interdialytic weight gain (by ~0.2 to 0.3 kg in an adult) optimally by prolonging the dialysis time to allow for slower ultrafiltration. Then dry weight can often be successfully achieved without troublesome symptoms (as discussed further later in this chapter).

Technologies to Assess Dry Weight

Although several technologies claim to assess dry weight none can be regarded as a reference standard. Some of these technologies are discussed further.

Relative plasma volume (RPV) monitoring utilizes photooptical technology to noninvasively measure absolute hematocrit through a transparent chamber affixed to the arterial end of the dialyzer. The assumption underlying this technology is that in the absence of blood loss and uniform mixing of blood, the rise in hematocrit is proportional to the amount of fluid withdrawn during the dialysis procedure. Accordingly, percent blood volume change during the dialysis procedure can be calculated in real time. RPV slope then is a function of ultrafiltration rate and the plasma refill rate. Patients who are "wet" have large interstitial fluid volumes and therefore a high plasma refill rate—their RPV slope will be flat. Patients with a low plasma refill rate will have steeper slopes and are more likely to be at their "dry weight" (Fig. 41.4). Prospective, randomized trials suggest that relative blood volume-controlled dialysis can improve the predialysis BP, reduce the frequency of intradialytic hypotension, as well as reduce the cardiothoracic ratio.[190]

In the Dry-weight Reduction In Hypertensive Hemodialysis Patients (DRIP) trial (discussed later in the chapter), RPV monitoring was performed in all patients at the beginning and end of the study.[191] RPV slopes were defined as flat when they were less than the median (1.33% per hour) at the baseline visit. The study found that flat RPV slopes may indicate a volume-overloaded state. This is because of the following four reasons: (1) probing dry weight in these patients leads to steeper slopes; (2) those with flatter slopes at baseline have greater weight loss; (3) both baseline RPV slopes and the intensity of weight loss are important for subsequent change in RPV slopes; and, most importantly, (4) RPV slopes predict the subsequent reduction in interdialytic ambulatory systolic BP—those with the flattest slopes have the greatest decline in BP on probing dry weight. Thus, RPV slope monitoring may be useful to assess dry weight among hypertensive hemodialysis patients. RPV monitoring, combined with clinical assessment of intradialytic hypovolemia, and postdialytic fatigue, can help assess patient dry weight and optimize volume status while reducing dialysis-associated morbidity.[192] Several studies in hypertensive pediatric hemodialysis patients have successfully utilized RPV monitoring to improve BP control.[193,194]

Certain echocardiographic parameters have been reported to assess volume among hemodialysis patients. Cheriex et al. in a small study of 18 hemodialysis patients first reported the usefulness of inferior vena cava (IVC)

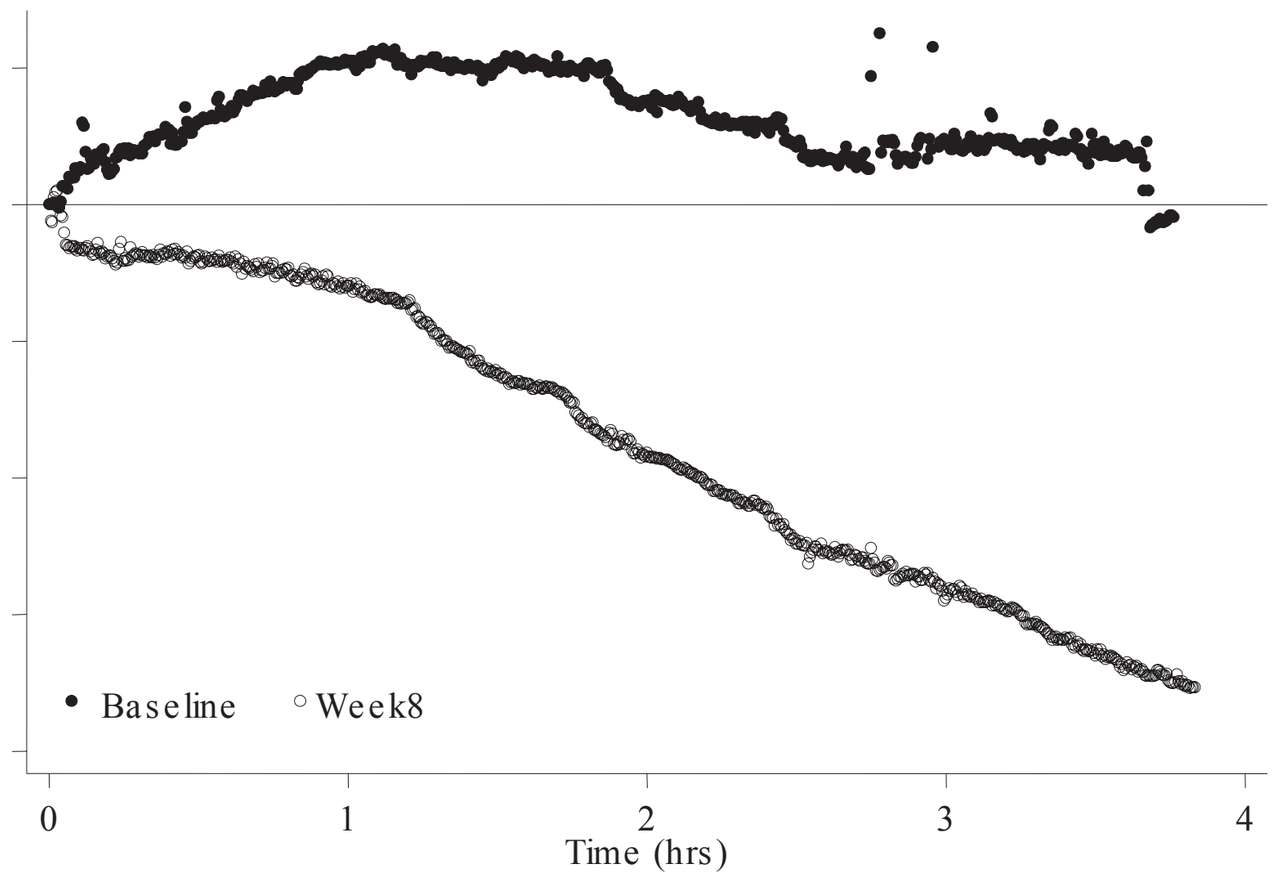

FIGURE 41.4 A 42-year-old black man with end-stage renal disease on chronic hemodialysis for 8 years treated with four antihypertensive medications consented to participate in the DRIP trial after he was noted to be hypertensive. Interdialytic ambulatory blood pressure monitoring revealed blood pressure of 149/89 mm Hg. At baseline, relative plasma volume monitoring demonstrated no change in relative plasma volume. Dry weight was probed in the subsequent 8 weeks. He lost 2.0 kg of postdialysis weight from 62.0 kg to 60.0 kg. At 8 weeks relative plasma volume monitoring revealed 3.15% reduction in relative plasma volume per hour. Interdialytic ambulatory blood pressure improved to 125/77 mm Hg. Relative plasma volume monitoring may be a useful tool to assess dry weight. (Reprinted from Agarwal R, Weir M. Dry-weight: A concept revisited in an effort to avoid medication-directed approaches for blood pressure control in hemodialysis patients. *Clin J Am Soc Neph*. 2010;5:1255.)

diameter and its collapse with inspiration as a marker of volume.[195] A good relationship was found both between IVC diameter and right atrial pressure and between collapse index and right atrial pressure; the right atrial pressure was measured invasively. However, the collapse index was found not to correlate with changes in blood volume. These results were confirmed by Agarwal et al. in the context of a larger randomized trial.[196] The end point was not right atrial pressure but the relationship of reduction in IVC diameter and interdialytic ambulatory BP.

Not all studies have given such encouraging results. For example, Katzarski et al. suggest that IVC diameter measured at the end of dialysis or shortly thereafter gives a misleading indication of volume overload in these patients.[197] Among children on chronic hemodialysis, IVC diameter also did not vary significantly after changing dry weight.[198] Another problem with the use of IVC diameter is that there is no uniform definition of the threshold used to classify patients into hypovolemic, euvolemic, or hypervolemic groups. For example, depending on the criterion used, before dialysis, hypovolemia was found in an astounding 39% to 47% of the patients.[199] An additional 21% to 25% were euvolemic before dialysis despite being above dry weight. Thus, further evaluation is needed before this test can be become mainstream in determining dry weight.

Left atrial diameter is a part of routine echocardiographic evaluation and is volume responsive.[196] Thus, this measurement can be easily used among patients who do not have other reasons to have left atrial enlargement such as mitral regurgitation. Furthermore, left atrial volume has been reported to be a correlate of fatal and nonfatal cardiovascular events among hemodialysis patients.[200] Hepatic vein Doppler evaluation can also assess right atrial pressure but has not been found to be of value to determine dry weight. One reason for this may be the technical difficulty associated with its measurement especially in the postdialysis state where the hepatic veins may be so collapsed that they are hard to visualize.

Another technique to assess dry weight is through body impedance analysis.[201–203] Wabel et al. measured body composition through body impedance analysis and predialysis systolic blood pressure among 500 patients from eight dialysis centers in Europe.[204] The joint consideration of volume state and BP may provide a useful tool to classify patients in terms of volume sensitive and volume resistant hypertension. One third of the patients had normal BP and normal fluid status by the definitions used by the authors. Hypertension and volume expansion was found in 15%, hypertension without volume expansion in 13%, and normotension with volume expansion in 10%. The remaining patients had reasonably controlled BP and volume state. Volume expansion in follow-up of a cohort of 269 patients found expanded extracellular fluid volume to be associated with increased mortality.[205]

Mass spectrometers, through determination of distribution phase of orally administered deuterated water, can provide an accurate estimate of total body water. Absolute measurements of total body water may become more feasible with the use of portable mass spectrometers. Chan et al. reported on the use of a flowing afterglow mass spectrometer following ingestion of heavy water immediately after dialysis among 12 hemodialysis patients.[206] Measurements of total body water immediately after hemodialysis and immediately preceding the following dialysis showed excellent agreement between the two measurements after accounting for insensible losses and urine output. The coefficient of variation in total body water between the two measurements was 2.6%. This proof of principle study demonstrated that absolute total body water can be determined among hemodialysis patients. Further work is required before this study can be used for day-to-day decision making about volume management.

Other strategies to assess dry weight such as through measurement of natriuretic peptides[207] are still being evaluated for the management of hypertension in dialysis patients.

In summary, there is no established gold-standard test or marker of dry weight in dialysis patients. The technologies available can assist the detection of volume excess, but clinical judgment provides the ultimate guidance on what the most appropriate postdialysis weight of a patient should be.

Benefits of Probing Dry Weight

Probing dry weight may be defined as a gradual change in postdialysis weight at which there are minimal signs or symptoms of either hypovolemia or hypervolemia. Probing is the current gold standard by which dry weight is defined. Briefly, dry weight is the lowest tolerated postdialysis weight achieved via gradual change in postdialysis weight at which there are minimal signs or symptoms of either hypovolemia or hypervolemia.

To test the hypothesis that hypertension among hemodialysis patients who do not manifest overt signs of volume overload is mediated by excess volume, dry weight was probed without changing the dialysis time in a randomized controlled trial of hypertensive hemodialysis patients.[36] Notably, in this study, patients with obvious volume overload were excluded. Interdialytic ambulatory BP monitoring was performed at baseline, 4 weeks, and 8 weeks in 50 patients randomized to a control group and 100 patients randomized to the ultrafiltration group. In the intervention group, ambulatory BP was reduced within 4 weeks by 11/6 mm Hg.[36] This level of BP reduction was achieved despite stable concurrent use of 2.7 antihypertensive drugs. The magnitude of reduction in BP is therefore much larger than what would be expected by adding an additional antihypertensive agent. Because the control group had a placebo effect, a correction for this effect is needed. Even after the correction for the placebo effect, ambulatory BP reduction was significant at 7/3 mm Hg. This antihypertensive effect was sustained for 8 weeks of observation. Despite provoking occasional uncomfortable intradialytic symptoms, the quality of life was not impaired. Even in this randomized trial, the presence or absence of edema, which is often taken to be a reliable sign of volume overload, had no predictive value in separating

the responders from nonresponders. Furthermore, 10% of the patients in the control group developed accelerated hypertension defined as BP ≥175/105 mm Hg by interdialytic ambulatory monitoring. This study provides strong support to the hypothesis that among hemodialysis patients, probing dry weight is an effective strategy for reducing BP.

Observational studies also support the practice of probing dry weight. In 1969, Vertes et al. reported that 35 of 40 patients became normotensive by achieving dry weight.[13] In a report from Turkey, Kayikcioglu et al. compared the benefit of nonpharmacologic to pharmacologic therapy for control of left ventricular mass among hemodialysis patients.[208] In a case-control study patients who had been treated at one center with salt restriction and dry weight reduction were compared to patients at another center where antihypertensive-based therapy was the primary method for management of hypertension. The center using dry weight and salt restriction as a primary strategy had the following benefits: lower antihypertensive drug use (7% vs. 42%); lower interdialytic weight gain; lower left ventricular mass; better diastolic and systolic left ventricular function; and fewer episodes of intradialytic hypotension. These observations are important and of clinical relevance; they suggest that probing for dry weight as opposed to adding more antihypertensive drugs perhaps diminishes the risk for cardiac remodeling and mitigates LVH and preserves systolic and diastolic left ventricular function. Although a case-control study cannot assert causation, the results of this study support the use of nonpharmacologic therapies in the management of patients with ESRD.

Dry Weight and Outcomes

Studies among hemodialysis patients in both adults and children suggest that managing intradialytic RPV may reduce the number of hospital admissions due to fluid overload,[192,209] improve BP control, and decrease hypotension-associated dialysis symptoms.[210] It is possible that the latter benefit is, in part, related to diminished use of antihypertensive medication. Accordingly, monthly monitoring of RPV and home BP may offer an attractive way to assess the adequacy of volume control among hemodialysis patients.

To study the effect of volume status on mortality, Wizeman et al. followed 269 prevalent hemodialysis patients for several years.[211] They measured hydration state using a body composition analyzer. If there was greater than 15% excess of extracellular water (2.5 L volume excess), they classified such patients as volume overloaded; 25% of the patients had excess extracellular fluid (ECF) volume. In a multivariate adjusted analysis, they found that excess volume was associated with high mortality. Compared to those without excess ECF volume, the hazard ratio of mortality with excess fluid volume was 2.1 (P = .003). Although the study did not examine reduction in ECF volume on subsequent outcomes, such studies need to be performed in the future.

Inrig et al. compared the change in pulse pressure during dialysis as a risk factor for hospitalization and mortality among prevalent hemodialysis patients participating in a randomized controlled trial.[212] They found that patients who had the least change in pulse pressure from before to after dialysis had clinical characteristics indicating volume overload. Among these patients, lowering of the pulse pressure from before to after dialysis was associated with lower hospitalization and mortality outcomes. Because pulse pressure is largely driven by systolic BP, it is likely that lowering of pulse pressure with dialysis reflects more volume loss, a lower ECF volume state, and may provide better cardiovascular outcomes, perhaps through less pressure/volume stress on the heart. Further research is needed to establish a cause and effect relationship.

Potential Hazards of Probing Dry Weight

There are potential hazards related to probing dry weight, none of which have been adequately examined. These include the following: (1) increased risk of clotted angioaccess; (2) increased rate of attrition in residual renal function; and (3) complications related to interdialytic hypotension. Intradialytic hypotension, besides requiring more nursing interventions, can be complicated by cerebral hypoperfusion, seizures, myocardial dysfunction, and mesenteric ischemia. Furthermore, it has been associated with mortality.[213] The relative risks and benefits of probing dry weight need to be examined in long-term randomized trials.

Providing Adequate Dialysis

The European Best Practice Guidelines recommend that dialysis should be delivered at least three times a week and the total duration should be at least 12 hours per week, unless substantial residual renal function is present.[214] An increase in treatment time and or frequency should be considered in patients who experience hemodynamic instability or remain hypertensive despite maximal possible fluid removal.

In the United States, a recent study reported that the average duration of dialysis among 32,065 participants in the ESRD Clinical Performance Measures Project was 217 minutes.[215] The interquartile range was 195 to 240 minutes. This means that one fourth of the patients were receiving <3 hours and 15 minutes of dialysis and only one fourth of the patients were receiving >4 hours of dialysis.

Although the adequacy of dialysis is still debated, it is clear that patients who shorten treatment have hypertension that is more difficult to control.[101] Patients that are dialyzed 8 hours three times a week have excellent BP control, minimal requirement for antihypertensive drugs, and excellent long-term survival.[11,133] In a randomized cross-over trial of 38 patients, the effects of 4 hours dialysis to 5 hours dialysis were evaluated.[216] Hemodynamic stability and hypotensive episodes were fewer with longer dialysis, especially among older patients (>65 years of age). However, these data are difficult to generalize since treatment was evaluated only over 2 weeks and those requiring >4 L ultrafiltration were excluded. Longer or more frequent dialysis sessions, in general, are associated with less hemodynamic instability, better

achievement of target postdialysis weight, better control of blood pressure, and the reduced need for antihypertensive drugs. These are discussed further in the next section.

Frequent Dialysis and Its Effect on Blood Pressure

Observational studies suggest that conversion of patients from three times a week conventional dialysis to nocturnal dialysis may improve BP and left ventricular mass.[217] In a cumulative analysis of 72 patients from nine centers it was noted that predialysis systolic and diastolic BP falls within 1 month of dialysis by 13/7 mm Hg from 163/94 mm Hg.[47] This reduction was accompanied by 1% decline in postdialysis weight. Although BP did not change after 1 month, the number of antihypertensive agents declined significantly. At baseline, 54% were not taking antihypertensive drugs whereas at 12 months after switching to daily dialysis, 75% were not taking antihypertensive agents.

Several observations have suggested improvements in BP and left ventricular mass among patients undergoing more frequent dialysis. For example, Chan reported an improvement in both systolic and diastolic BP, reduction in antihypertensive drugs and doses, and reduction in left ventricular mass in patients undergoing nocturnal dialysis.[217] This group has also reported an improvement in pharyngeal size among nocturnally dialyzed patients.[218] This may improve sleep apnea and, consequently, ambulatory BP. Another mechanism of BP reduction is suggested to be an increase in arterial compliance and consequently improvement in baroreflex sensitivity.[219] Others may be better volume and toxin removal.[220] In another small study, both systolic and diastolic BP improved with six times per week compared to thrice weekly dialysis, whether they were recorded predialysis or postdialysis improved. The number of medications and predialysis and postdialysis weight remained unchanged. Intradialytic symptoms commonly attributed to volume shifts also improved. A randomized two-period crossover study compared the effect of short daily hemodialysis versus standard hemodialysis on BP and left ventricular mass index among 12 hypertensive patients with ESRD.[221] At the end of 6 months of standard hemodialysis and 6 months of daily dialysis, 24-hour ambulatory BP monitoring, echocardiography, and bioimpedance were performed. Interdialytic ambulatory BP declined by 20/6 mm Hg with daily dialysis. The decrease in BP was accompanied by the withdrawal of antihypertensive therapy in seven of eight patients during daily dialysis. Left ventricular mass index decreased by 28.6 g per m^2 from 148.7 g per m^2. Extracellular water content decreased 5.1% from 52.7% and correlated with 24-hour improvements in left ventricular mass index and systolic BP.

A randomized controlled trial assigned 52 hemodialysis patients to either frequent dialysis, six nights per week, or conventional three times a week treatment. In the frequent dialysis group, improvement was seen in cardiac magnetic resonance imaged left ventricular mass and reduction in the need for BP medications.[222] The Frequent Hemodialysis Network (FHN) study randomized hemodialysis patients to either conventional three times weekly dialysis or more frequent in-center dialysis; the primary endpoint was an improvement in both LVH and physical health composite. The primary endpoint was met but perhaps the most notable finding was an improvement in systolic BP, reduction in antihypertensive drug use, and improvement in left ventricular mass.[223] These findings suggest better achievement of dry weight in these patients.[224] Increasing the treatment duration may improve hemodynamic stability of dialysis and make the procedure more tolerable but is not a requirement for improvement in left ventricular mass. Shortening the procedure to tailor dialysis to a minimum Kt/V may provoke intradialytic symptoms, postdialysis fatigue, and nonadherence to therapy—thus, this is not recommended.[225] Normotension can be achieved independently of the duration of dialysis if the control of volume is adequate.[226] In fact, left ventricular mass index was also improved to a comparable degree in the DRIP trial participants where the duration of dialysis was not altered but the dry weight was aggressively challenged.[227]

PHARMACOLOGIC TREATMENT

All classes of antihypertensive drugs, except diuretics, are useful for managing hypertension in hemodialysis patients.[228] Diuretics are generally ineffective at very low GFR. There is no role for loop diuretics even when given in high doses, as high as 250 mg intravenously of furosemide, among anuric hemodialysis patients.[229] Measurements using tissue Doppler echo images found that central cardiac hemodynamics were unaltered when anuric hemodialysis patients were given even such high doses of loop diuretics. Given the ototoxicity associated with high doses of loop diuretics, their use, especially in high doses, is not recommended.

Both angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are useful for lowering BP[55,230–232] and some recommend their use in all patients.[233] But not all agree with this recommendation.[234] Consideration of pharmacokinetics is important when prescribing these drugs.[235] For example, lisinopril is renally removed and can be administered just three times a week after dialysis.[55] Ramipril may also accumulate with repeated dosing.[236] The pharmacokinetics of losartan have been carefully examined and remain unaltered in hemodialysis patients.[237] Unique among antihypertensive drugs, the use of ACE inhibitors or ARBs can reduce angiotensin II which can worsen anemia.[238] Whether ACE inhibitors attenuate response to erythropoietin among dialysis patients is debated.[239–241] Also unique among antihypertensive drug classes, the use of ACE inhibitors can trigger anaphylactoid reactions when used concomitantly with AN69 membranes; this adverse reaction appears less frequent with ARBs.[242] A multicenter, open label, 6-month study was performed in 406 patients to test the tolerability and efficacy of losartan in patients on hemodialysis.[242] Fifteen patients discontinued the study

due to adverse reactions related to losartan, and in seven of them the adverse reaction was hypotension. In two patients a possible anaphylactoid reaction was reported after dialysis with an AN69 membrane, necessitating premature termination of dialysis and losartan in one patient. In contrast, nine patients with a history of previous anaphylactoid reaction with an ACE inhibitor and AN69 did not show this complication with losartan and AN69. Thus, losartan appears to have a lower incidence of anaphylactoid reactions than those detected with ACE inhibitors and AN69. Taste disturbances may occur with captopril.[230] Although one small cohort study found reduction in mortality with the use of ACE inhibitors,[243] a larger cohort did not reveal any improvement in survival.[244] In a randomized controlled trial, fosinopril, an ACE inhibitor, was not associated with reduced mortality.[245] Another small study failed to show improvement in myocardial mass or diastolic function with ACE inhibitor therapy.[246]

Beta-blockers can lower BP among hemodialysis patients.[247,248] Atenolol is completely removed by the kidney and is not metabolized in the body. Accordingly, some have suggested that this is a contraindication for using atenolol in patients with CKD. However, we have taken the reverse approach and used it liberally among hemodialysis patients. Even when administered three times a week after dialysis it effectively lowers BP and retains its effect over the entire interdialytic interval.[249,250] Several other β-blockers such as propranolol[251–254] and carvedilol[255,256] have been successfully used. Compared to other classes of drugs, the use of β-blockers has been associated with 16% improvement in all-cause mortality among patients with ESRD.[244]

Calcium channel blockers are commonly prescribed to patients with ESRD. They can effectively lower BP even in the volume expanded state.[257,258] Their use has been associated with improved aortic pulse wave velocity[258] and reduced mortality[259] but other studies dispute mortality claims.[260] For example, no one class of antihypertensive drugs was associated with improved survival in one study.[260] Furthermore, use of both amlodipine and valsartan has been associated with reduced oxidative stress.[261] The pharmacokinetics of amlodipine are unaltered among dialysis patients and the drug is not dialyzable.[262] Therefore, the dose of amlodipine does not need to be modified among dialysis patients.

Vasodilators such as minoxidil and hydralazine are sometimes used as drugs of last resort to control BP. These drugs are powerful vasodilators and provoke reflex tachycardia, the control of which may require the administration of β-blockers.[263] The duration of action of hydralazine is short and its use may result in large variation (spikes and valleys) in BP control. In contrast, the duration of minoxidil is long and likely results in smoother BP control.[264] Minoxidil use can produce vexing side effects such as pedal edema and hirsutism. A serious adverse drug reaction associated with minoxidil is pericardial effusion and is not rare.[265] Hydralazine use can result in a lupuslike illness.

Centrally acting agents such as clonidine can effectively lower BP by reducing autonomic activation.[266,267] Clonidine clearance is GFR dependent[268,269] but the drug is not removed much by dialysis.[269] Because clonidine can be delivered through a transdermal delivery system that lasts 1 week, its use may be associated with lesser nonadherence.[270] However, dry mouth, dizziness, and somnolence are troublesome side effects of this drug. In contrast, guanfacine, another centrally acting agent, is associated with less somnolence and dry mouth.

Alpha-blockers, in our experience, are associated with orthostatic hypotension and intradialytic hypotension events. Among 22 hypertensive hemodialysis patients treated with the α-blocker prazosin, 11 patients described transient dizziness in the first month of therapy and one patient had recurrent syncope requiring termination of therapy.[271] Accordingly, α-blockers should be used cautiously among hemodialysis patients.

There is a renewed interest in spironolactone as a therapy to improve hypertension and left ventricular hypertrophy. Small trials suggest improvement in predialysis BP without the occurrence of hyperkalemia.[272]

In observational studies, the use of antihypertensive drugs has been associated with improvement in all-cause mortality.[273–275] An interventional study used antihypertensive drugs in addition to both the correction of anemia and management of dry weight to evaluate multiple outcomes.[276] It reported that the multitargeted intervention was associated with reduced LVH, reduced cardiovascular mortality, and reduced all-cause mortality. Substantial variation in the use of antihypertensive drug class between countries has been noted.[277] Lopes et al. analyzed the variable use of antihypertensive drug classes among hemodialysis patients and subsequent mortality.[277] Most often, use of antihypertensive drugs was associated with improved outcomes. For example, facilities that treated 10% more patients with ARBs had on average 7% lower all-cause mortality independent of patient characteristics and prescription patterns of other antihypertensive medications. At the patient level, benefits were seen with the prescription of β-blockers (RR = 0.88, $P < .0001$), peripheral α-blockers (RR = 0.89, P = .02), and long-acting dihydropyridine calcium channel blockers (RR = 0.92, P = .003). On the other hand, the use of short-acting dihydropyridine calcium channel blockers was associated with all-cause mortality (RR = 1.67, $P < .0001$). Cardiovascular mortality at the patient level was reduced with the use of β-blockers, ARB, and α-blockers but increased with short-acting dihydropyridine calcium channel blockers. At the facility level, improvement in cardiovascular mortality was seen only with angiotensin receptor blockers; mortality was increased with the use of central antagonists and long-acting dihydropyridine calcium channel blockers. Because these data are observational in nature, causality cannot be implied; randomized trials are needed to test the safety and efficacy of antihypertensive agent use among hypertensive hemodialysis patients.

Regrettably, the reduction in all-cause mortality with antihypertensive drug therapy has not been realized with

adequately powered randomized controlled trials. This may be due to multiple reasons including the low numbers of patients. Nonetheless, meta-analyses of these trials show improvement in the cardiovascular event rate.[278,279] These benefits are especially seen among those who have hypertension.[279]

Nephrectomy for Resistant Hypertension

Nephrectomy has been reported as therapy for resistant hypertension as early as 1967. Of the 40 patients reported, 35 had BP controlled with dry weight reduction; the remaining five required nephrectomy.[13] A case series of 10 patients who underwent bilateral nephrectomy for drug-resistant hypertension reportedly had no antihypertensive drug requirement after the procedure and had a good quality of life.[280]

PROGNOSIS

Among hemodialysis patients, the relationship of BP with outcomes is a subject of much controversy.[274,281–286] Some studies confirm the association of high BP with strokes,[287,288] cerebral atrophy,[289] cardiovascular events,[290] complex cardiac arrhythmias,[291] the development of congestive heart failure,[284] and all-cause mortality[292] as is noted in the general population. Other studies suggest that the relationship of BP and outcomes is the reverse of that seen in the general population. These studies show that low BP measured either predialysis or postdialysis is associated with increased mortality.[273,274,293,294] This association of low BP and mortality is magnified further when BP is considered as a time-dependent covariate.[273] High BP measured either predialysis or postdialysis is either not associated or minimally associated with increased mortality. This phenomenon has been labeled as reverse epidemiology of hypertension. This has raised concerns regarding lowering of BP among hypertensive hemodialysis patients.[295,296] Other studies, however, have demonstrated a direct relationship between BP and mortality.[10,11,144,292] Consideration of the patient characteristics, dialysis practices, and BP measurement techniques are useful when evaluating these outcomes and addressing these controversies.

Patient characteristics differ which is reflected in long-term outcomes that also differ markedly around the world. The lowest crude mortality rate of about 50/1,000 patient years has been reported from centers in Tassin, France, and Okinawa, Japan.[10,11,297,298] Intermediate mortality rates of about 100/1,000 patient years are reported from some centers in Europe and South America.[8,283,299] The highest mortality rates of about 160 to 180/1,000 patient years are those reported from the United States.[275]

In the population of patients who have a lower crude mortality rate and those who have been followed for several years, hypertension is a risk factor for poor outcomes.[282,292,300] In contrast, in the population of patients who have a higher crude mortality rate and those who have been followed for a year or less low BP is a risk factor for poor outcomes and high BP is either protective or has no ill consequence.[276] Some insights can be derived from examining the cohorts with intermediate risks. One such cohort demonstrated that deaths within 2 years (early deaths) were related to deaths due to cancer and withdrawal from dialysis whereas deaths after 2 years (late deaths) were related to cardiovascular and noncardiovascular causes.[283]

Consideration of the level of illness and considering the vintage of the patient are also instructive to ascertain the value of hypertension as a risk factor among hemodialysis patients. Examining the outcomes of 2,770 patients on peritoneal dialysis provides such insights.[18] These patients were studied between 1997 and 2004 and had been on peritoneal dialysis for at least 180 days in England and Wales. In fully adjusted analyses, greater systolic, diastolic, mean arterial, and pulse pressure were associated with decreased mortality among patients who had been on dialysis for less than 1 year. However, greater systolic pressure and pulse pressure (but not mean arterial pressure and diastolic BP) were associated with increased mortality among patients who had been on peritoneal dialysis for 6 or more years. A subgroup of patients was placed on the transplant wait list within 6 months of starting renal replacement therapy and was presumably healthier. Among these patients, greater systolic, diastolic, mean arterial, or pulse pressure were not associated with decreased mortality in the first year. Similarly, among 16,959 dialysis patients in the United States, low systolic BP (<120 mm Hg) was associated with increased mortality in years 1 and 2.[301] However, high systolic BP (150 mm Hg or more) was associated with increased mortality among patients who survived at least 3 years.[301]

Regional differences in mortality are unlikely to be due to patient specific characteristics alone. For example, in France the center in Tassin reports a mortality rate of 45/1,000 patient years. In contrast, Degoulet et al., also from France, have reported a mortality rate of 96/1,000 patient years.[299] Differences in outcomes may be due to center-specific practices. For example, patients reported by Charra et al. in Tassin are dialyzed long hours with low sodium dialysate and given low sodium bread from the dialysis unit. The vast majority of these patients become normotensive without needing antihypertensive drugs. In these patients, the conventional epidemiology of hypertension holds. In fact, normotension has been advocated as a criterion for adequacy of dialysis. This is not unreasonable given that sodium is a ubiquitous uremic toxin and its excess is critically important for the pathogenesis of hypertension among hemodialysis patients.

Difference in techniques used for BP measurement is also quite likely to contribute to variation in the observed relationship between BP and outcomes. For example, Amar et al. were the first to discover the strong relationship between ambulatory BP and mortality.[144] These authors reported that nocturnal systolic BP was directly related to mortality. We have used ambulatory BP to detect its

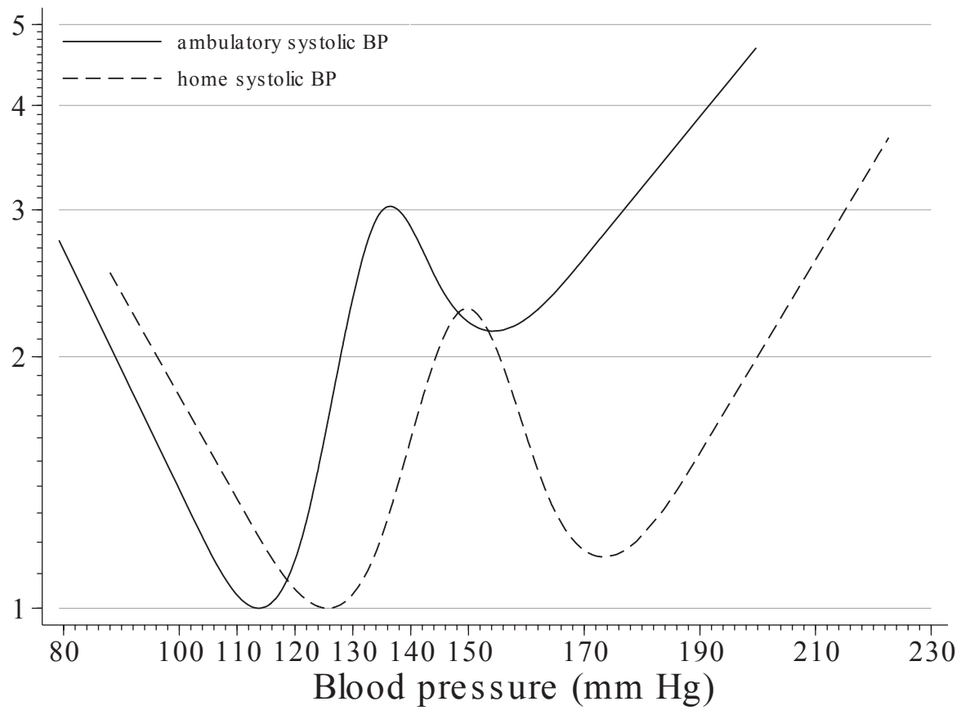

FIGURE 41.5 Nonlinear relationship of systolic blood pressure obtained outside the dialysis unit and subsequent mortality over up to 6 years. The best outcome was seen when ambulatory systolic blood pressure was between 110 and 120 mm Hg and home systolic blood pressure was between 120 and 130 mm Hg. The splines are calculated at the average age of this cohort which was 55 years. (Reprinted from Agarwal R. Blood pressure and mortality among hemodialysis patients. *Hypertension*. 2010;55:762.)

relationship with mortality. In a cohort of approximately 150 patients we found a direct and statistically significant relationship of both home (self-measured) and ambulatory (automatically measured) BP with mortality.[145] No such relationship was detectable using pre- and postdialysis BP recordings. In a larger cohort followed for a longer time we found a W-shaped relationship between both ambulatory BP and home BP with all-cause mortality (Fig. 41.5).[302] At extremes of BP, mortality was noted to be high. Compared to ambulatory BP, the optimal BP ranges for home BP were about 10 mm Hg higher.

CONCLUSION

Hypertension is common, difficult to diagnose, and poorly controlled among patients with ESRD. Although there is controversy surrounding its diagnosis and treatment, evidence suggests that home BP monitoring may help make a more accurate diagnosis of hypertension. Goal home BP of <140/90 mm Hg is 5 mm Hg more than the goal suggested by the American Heart Association to be a reasonable target for dialysis patients. The primary strategies to control BP are dietary and dialysate sodium reduction, an adequate prescription of dialysis, and achievement of dry weight. Pharmacologic therapy should be second line. Use of antihypertensive drugs may improve cardiovascular outcomes. Frequent dialysis is effective in improving BP control and increasing treatment times among hypertensive patients may be a reasonable way to both afford hemodynamic stability and improve BP control.

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CHAPTER

42 Renal Artery Stenosis, Renovascular Hypertension, and Ischemic Nephropathy

Stephen C. Textor

The identification and management of renovascular disease presents a clinical challenge that is directly relevant to nephrologists, and also one that relates to many other medical specialties, including cardiovascular specialists, internal medicine physicians, vascular surgeons, and interventional clinicians. How best to manage renovascular disease remains controversial, in part because of the near simultaneous development of far more effective diagnostic tools, medical therapy, and revascularization techniques over the past decade that has ever been available before. It behooves clinicians caring for patients with renal disease to have a solid understanding of these concepts as part of their clinical responsibility to prevent an irreversible loss of kidney function and adverse effects of arterial hypertension.

Few conditions are more rewarding to treat than new onset severe hypertension and/or progressive renal insufficiency that reverses after the successful restoration of renal blood flow. Occlusive lesions of the main renal arteries can now be detected readily with any of a variety of imaging tools. Determining when to pursue renovascular lesions in clinical hypertension, renal insufficiency, or circulatory congestion; establishing their pathophysiologic role; and whether the hazards associated with revascularization are warranted are pressing concerns regularly faced by nephrologists. Although recent trial data fail to provide compelling evidence in favor of endovascular stenting for all patients with atherosclerotic disease, the validity of prospective clinical trials in this disorder has been fiercely challenged.[1,2] Experienced clinicians understand that renal revascularization in these disorders sometimes should be undertaken both to improve hypertension and to salvage renal function.

Recognition that reduced renal perfusion activates pressor systems that raise systemic blood pressure remains one of the seminal observations and most widely studied mechanisms in cardiovascular physiology. Reversal of renovascular hypertension can provide major benefits to patients with accelerating hypertension (e.g., allowing effective blood pressure control and reduction of long-term drug therapy). Selecting patients and determining optimal timing for vascular intervention at a reasonable risk is rarely simple, however.

These issues are complicated further by the rapid expansion of endovascular procedures over the past two decades. Although restoring lumen patency in partially occluded vessels intuitively may seem beneficial, recent trials indicate that revascularization procedures carry both substantial costs and some risks, whereas the clinical benefits remain ambiguous.[3,4] This is a remarkable turn of events, insofar as renovascular hypertension traditionally has been considered a prototype for "curable" secondary hypertension. Most diseases of the renal arteries are progressive, and the clinical manifestations develop gradually over time, either because the vascular compromise worsens or because adaptive mechanisms to offset hemodynamic effects become overwhelmed. Because advances in medical therapy have allowed more effective antihypertensive drug treatment than ever before, more patients are appearing clinically at later stages in their disorder with a manifest loss of kidney function and/or circulatory disorders.[5] It may be argued that such patients face more severe consequences of renovascular compromise and may have less of a likelihood of benefit from restoring the renal circulation. Hence, it behooves nephrologists to recognize the importance of a close follow-up of vascular disease in the kidney, as with many other vascular conditions. Before moving forward with renal revascularization, both affected patients and physicians should consider carefully the potential benefits and risks. Understanding the pathophysiologic basis for the clinical syndromes associated with renal artery stenosis is an important first step in this process. This chapter will review the background and basis for much of the clinical information related to these disorders.

The basic clinical syndromes to be discussed are outlined in Figure 42.1. Many renal artery stenoses produce little hemodynamic effect and represent "incidental" disease. Such lesions sometimes may be found in asymptomatic, normotensive individuals. Because many renal arterial lesions are detected in patients with preexisting hypertension, the role of renovascular disease itself may be obscured. Renovascular hypertension denotes the syndrome of rising arterial pressures specifically caused by impaired renal perfusion that leads to the activation of pressure pathways. When the severity and duration of reduced blood flow threatens the viability of kidney tissue, many authors

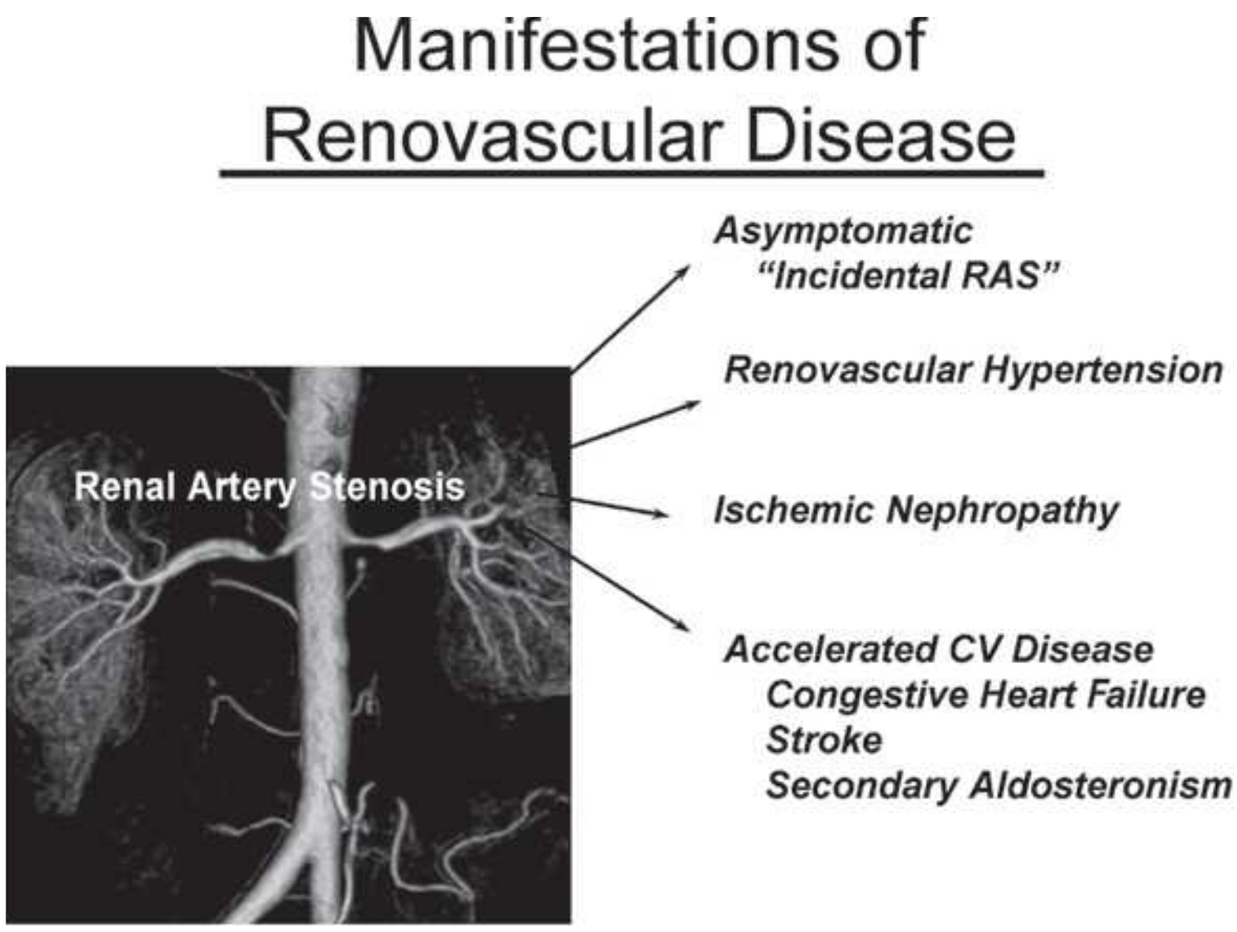


FIGURE 42.1 Clinical manifestations of renal artery stenosis range across a broad spectrum. Many, perhaps most, represent incidental lesions with minimal hemodynamic effects. Some reach levels wherein the activation of pressor mechanisms produces a rise in blood pressure, identified as renovascular hypertension, and at some point, threaten kidney function sufficiently to warrant the term ischemic nephropathy (see text). Particularly when the entire renal mass is affected, impaired kidney function and solute excretion from renovascular disease can accelerate cardiovascular morbidity, sometimes identified as one of the cardiorenal syndromes, with worsening congestive heart failure. The task facing the clinician is to identify where along this spectrum an individual patient lies. *CV,* cardiovascular; *RAS,* renal artery stenosis.

have designated this condition as ischemic nephropathy, which some believe is a major cause for some patients reaching end-stage renal disease (ESRD).[6,7] More recently, attention has been focused on the role of renovascular disease in impairing cardiac function, both by reducing the systemic excretion of sodium and volume and by producing abrupt rises in arterial afterload that magnify cardiac dysfunction. The primary task of the clinician is to elucidate the role of renal arterial stenosis in a given patient and to direct therapy accordingly.

HISTORICAL PERSPECTIVE

Observations in the 1800s regarding blood pressure measurements revealed important connections between fluid volume, arterial pressure, and vascular resistance. How these observations ultimately led to the elucidation of the renin-angiotensin-aldosterone system has been reviewed.[8] In 1898, Tigerstedt and Bergman established that extracts of the kidney had pressor effects in the whole animal, and these authors are credited with the identification of renin. The identification of each component of the renin-angiotensin system represents a remarkable series of research ventures spanning a half century and several investigators in many countries. Goldblatt and others provided seminal experiments with the development of an animal model in which reduced renal perfusion produced hypertension, published between 1932 and 1934. Numerous investigators thereafter identified the peptide nature of angiotensin, the role of renin substrate or angiotensinogen, the role of nephrectomy in sensitizing the animal to the pressor effects of angiotensin, and the sequential phases of renovascular hypertension. Hence, the renin-angiotensin system owes its initial discovery and nomenclature primarily to early studies related to the regulation of blood pressure by the kidney. Only recently have the multiple additional effects of angiotensin become evident regarding vascular remodeling, the modulation of inflammatory pathways, and interactions with fibrogenic mechanisms. Understanding that reduced renal blood flow produces sustained elevations in arterial pressure led to a broad study of the mechanisms underlying many forms of hypertension. Experimental models of two-kidney and one-kidney renal clips (two-kidney and one-kidney Goldblatt hypertension) represent some of the most extensively studies models of blood pressure and cardiovascular regulation.

Extension of these studies into clinical medicine followed soon thereafter. A time line highlighting these developments is illustrated in Figure 42.2.[9] Some patients presented with malignant forms of hypertension during the late 1930s and 1940s, so designated due to remarkably poor survival if the patient's blood pressure could not be lowered successfully. Few antihypertensive agents were known until the 1950s, and intervention consisted mainly of extremely low sodium intake diets and/or lumbar sympathectomy.

Recognition that some forms of severe hypertension were secondary to occlusive renovascular disease led surgeons to undertake unilateral nephrectomies for small kidneys in 1937.[10] The fact that some of these were indeed pressor kidneys and blood pressure fell to normal levels provided proof of concept and led to more widespread use of nephrectomies. Unfortunately, achieving a cure of hypertension after the nephrectomy was rare, and Homer Smith reviewed the poor results overall in a 1956 paper discouraging this practice.[10]

The 1960s marked the introduction of methods of vascular surgery to restore renal blood flow. These procedures carried substantial morbidity associated with aortic surgery, but offered an opportunity to improve the renal circulation and to potentially reverse renovascular hypertension. One result of this development was a series of studies aiming to characterize the functional role of each vascular lesion in producing hypertension, thereby allowing a prediction of the outcomes of vascular surgery.[10] A large, cooperative study of renovascular hypertension[81] included major vascular centers and reported on the results of more than 500 surgical procedures. These results provided limited support for vascular repair,

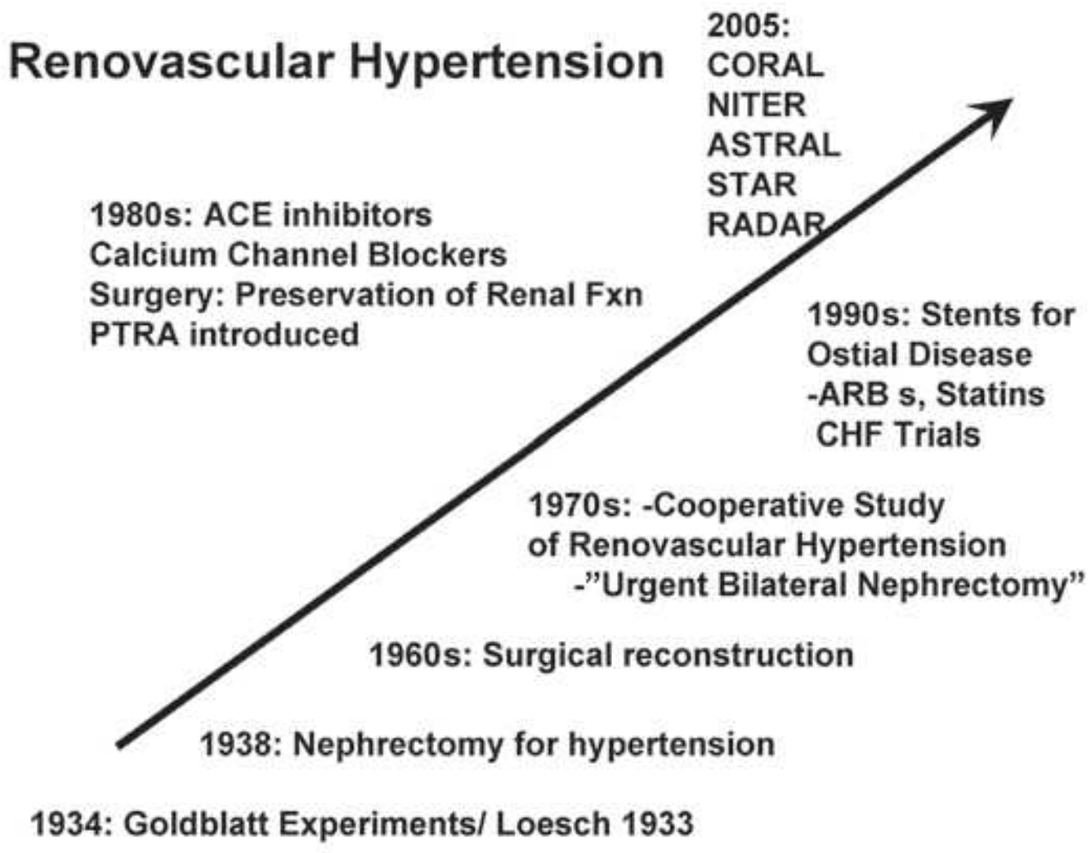


FIGURE 42.2 A time line with major events in the understanding of renovascular hypertension. Goldblatt and Loesch are identified as the original investigators in the 1930s who linked reduced kidney perfusion to the development of sustained hypertension. Surgical revascularization only became technically feasible in the 1960s, with the emergence of effective medical therapy and endovascular procedures in the 1980s and 1990s. The application of effective medical therapy, including statins, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin-receptor blockers (ARBs), have led to clinical equipoise that is the basis for prospective, randomized trials comparing optimal medical treatments with or without revascularization, beginning around 2005. *PTRA*, percutaneous transluminal renal angioplasty; *CORAL*, cardiovascular outcomes for renal atherosclerotic lesions; *NITER*, nephrotherapy ischemic therapy; *ASTRAL*, angioplasty and stent for renal artery lesions; *STAR*, stent placement in patients with atherosclerotic renal artery stenosis and impaired renal function; *RADAR*, randomized, multicenter, prospective study comparing best medical treatment versus best medical trearment plus stenting in patients with hemodynamically relevant atherosclerotic renal artery stenosis; *CHF*, congestive heart failure.

but identified relatively high associated morbidities and mortalities, particularly in patients with atherosclerotic disease.

In the 1980s and 1990s, further developments led to both improved medications and the introduction of endovascular procedures, including percutaneous angioplasty and stents. These both broadened the options for treating patients with vascular disease and raised new issues regarding timing and overall goals of intervention. Recent developments highlighted the need for intensive cardiovascular risk factor reduction and more stringent standards of blood pressure control. Antihypertensive medications have improved dramatically, both with regard to efficacy and tolerability. As emphasized in the following, the broad application of angiotensin-converting enzyme inhibitors and angiotensin-receptor antagonists for reasons other than hypertension alone changed the clinical presentation of disorders associated with renal artery stenosis.

Uncontrollable hypertension is now less commonly the reason to intervene in renovascular disease. Often, the main objective is the long-term preservation of renal function. In recent years, endovascular techniques make possible renal revascularization with relatively low morbidity in many patients previously considered unacceptable surgical candidates. The challenge for clinicians is how and when to apply these tools most effectively in the management of individual patients.[11]

DEFINITIONS AND CLASSIFICATION

The syndrome of renovascular hypertension (RVH) refers to hypertension primarily mediated by the reduction of renal artery perfusion pressure. The prevalence of renovascular hypertension is not known with precision, but available data suggest that it occurs in 0.5% to 5.0% of the general hypertensive population.[12] RVH can develop with a variety of arterial lesions, including arterial dissection, extrinsic compression, embolic infarction, or thrombosis (Table 42.1).

42.1 Lesions Producing Renovascular Hypertension and Ischemic Nephropathy

Causes of Renal Artery Stenosis
Atherosclerotic renal artery disease
Fibromuscular dysplasias
Medial fibroplasia
Perimedial fibroplasia
Intimal fibroplasia
Medial hyperplasia
Endovascular aortic stent graft crossing the renal artery
Acute arterial embolism/thrombosis (e.g., antiphospholipid syndrome)
Arterial trauma
Aortic dissection
Neurofibromatosis
Arterial aneurysm
Arteriovenous malformation/fistulae
Cholesterol emboli
Systemic necrotizing vasculitis
Polyarteritis nodosa

Atherosclerotic renal artery disease is the most common cause of renal artery stenosis, accounting for about 80% of renal arterial lesions. Fibrous renal artery diseases, as a group, account for less than 20% of renal arterial lesions. Reports of resistant hypertension suggest that when renovascular disease is a factor, more than 84% is atherosclerotic renal artery disease and 16% is fibromuscular renal artery disease.[13] Fibrous renal artery disease has been reported in 2% to 6% of potential renal transplant donors (usually normotensive individuals).[14,15] The vast majority of incidental renal artery lesions in angiographic series are atherosclerotic.

In addition to atherosclerotic and fibrous renal artery disease, a number of less common clinical entities can produce renovascular hypertension. These include acute arterial thrombosis or embolism, cholesterol emboli, aortic dissection, renal arterial trauma, arterial aneurysm, arteriovenous malformation of the renal artery, neurofibromatosis, polyarteritis nodosa, and Takayasu arteritis. Recent expansion of the use of endovascular aortic stent grafts, sometimes with structural impingement of the main renal arteries, is a new addition to the list of iatrogenic causes of renovascular hypertension.[4,16,17] Renal artery thrombosis occurring as a complication of umbilical artery catheterization has been recognized as causing renovascular hypertension in infants.[18] Transplant renal artery atherosclerosis, intimal hyperplasia, or vascular kinking may contribute to renal transplant renovascular hypertension (see Chapter 82).

Table 42.2 presents a classification of atherosclerotic and fibrous renal artery diseases with a description of their morphology and histology. These types of renal artery occlusive diseases represent a heterogeneous group of diseases, occurring in different age groups and behaving differently with regard to their individual natural history. An appreciation of these differences may be important for therapeutic decision making in patients with renal artery occlusive disease.

Fibromuscular Dysplasias

Fibromuscular dysplasia (FMD) lesions produce distortions of the luminal diameter of large- and medium-sized arteries due to nonatherosclerotic arteriopathies. The lesions usually involve the mid to distal vessel beyond the first 1 to 2 cm from the aorta (Fig. 42.3). The prevalence of clinically apparent renovascular FMD is estimated at 4 out of 1,000, with lower prevalence of cerebrovascular involvement (1 out of 1,000). Observations from screening angiographies in normotensive potential kidney donors indicate that some degree of FMDs can be observed in 3% to 6% of otherwise healthy, normotensive individuals.[15] Clinically apparent FMDs are most common among young females between the ages of 15 and 50 years. It may be familial in 10% of cases and tends to involve both renal arteries. It has been associated with subclinical carotid fibromuscular disease in first-degree relatives, which, in some cases, is consistent with an autosomal dominant inheritance. Renal arteries are involved with FMDs in 65% to 70% of cases, whereas 25% to 30% involve cerebral vessels. Both sites are involved in approximately 15% of patients. FMDs may develop in association with hereditary disorders of the connective tissue, such as Ehlers-Danlos and Marfan syndromes.

The lesions of FMD develop as disruptions of vascular wall components with abnormal deposition of collagen

42.2 Histologic Classifications of Fibromuscular Dysplasia and Angiographic Appearance

Type	Frequency	Histology	Angiographic Appearance
Medial		Alternating ridges of collagen/ loss of elastic membrane	String of beads Medial: bead diameter is larger than lumen diameter Perimedial: bead diameter is smaller than lumen diameter Medial hyperplasia: smooth stenosis without beads
Medial fibroplasia	85%–100% most common		
Perimedial fibroplasia	Rarer (10%–15%)	True smooth muscle hyperplasia: no fibrosis	
Medial hyperplasia	Rarest		
Intimal	<10%	Circumferential deposition of collagen in intima: fragmented or duplicated internal elastic lamina	Concentric smooth stenosis: long, smooth vessel narrowing
Adventitial	<1%	Dense collagen replaces fibrous tissue in adventitia and surrounding tissue	Smooth stenosis or diffuse attenuation of vessel lumen

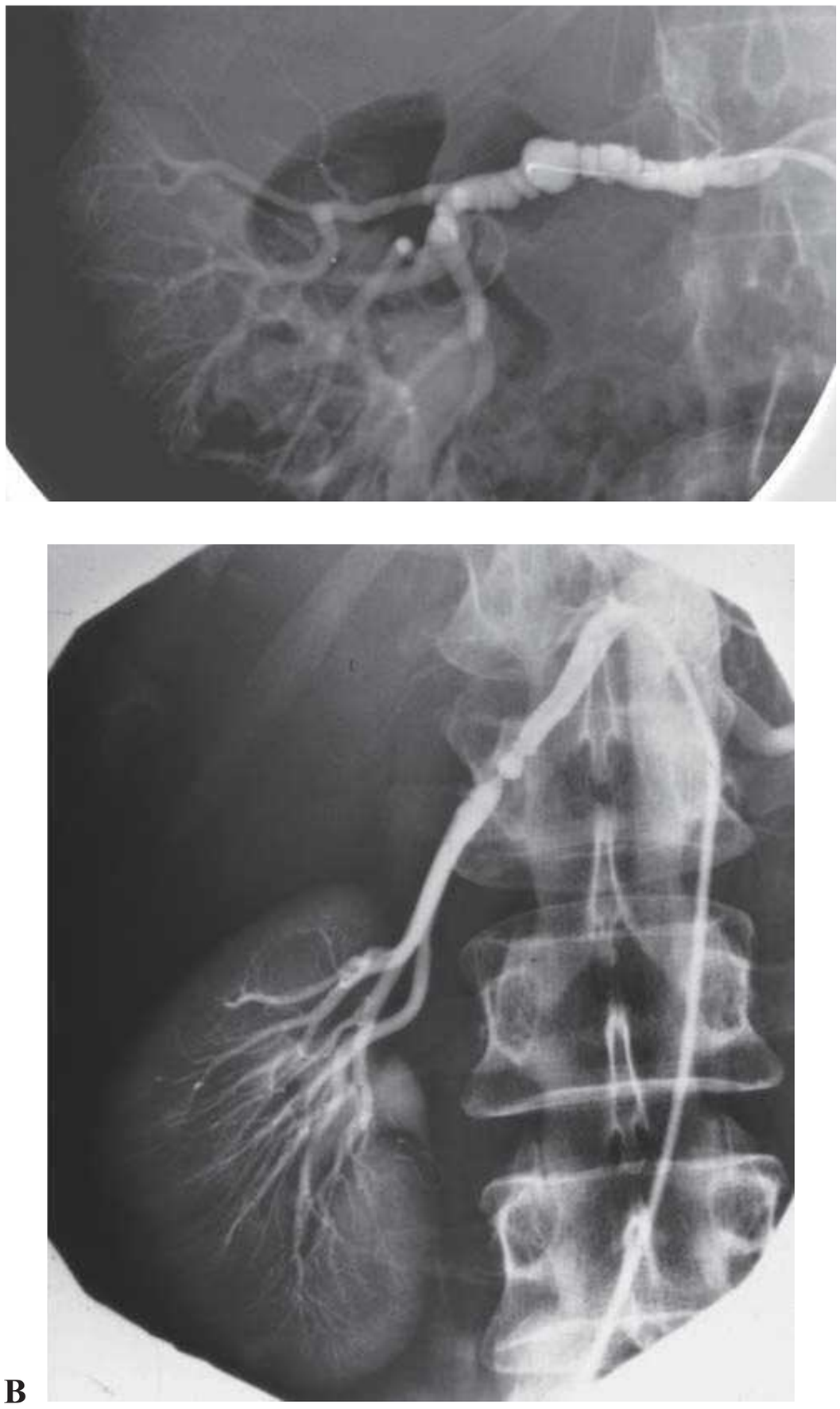


FIGURE 42.3 Examples of fibromuscular disease of the renal artery. **A:** An image of the string of beads appearance that is typical of medial fibroplasia with aneurysmal bulging beyond the artery. **B:** An image of a tight, focal lesion that is typical of intimal hyperplasia in a young male.

in bands, sometimes with disruption of the vascular elastic membrane. The molecular basis for these disruptions is unknown, although several candidate genes have been proposed. The primary subtypes of FMDs are designated as medial fibroplasia, intimal fibroplasia, and periadventitial fibroplasia, defined initially on the basis of histologic analysis of surgically resected vessels. Medial fibroplasia is the most common phenotype, and often manifests as a string-of-beads appearance with alternating stenoses with apparent luminal dilations, as summarized in Table 42.2. The other phenotypes appear as focal or elongated vascular occlusions. Medial fibroplasia affects the distal half of the main renal artery, frequently extends into the major branches, is often bilateral, and angiographically gives the appearance of multiple aneurysms wherein the diameters of the aneurysms are wider than the apparently unaffected portion of the main renal artery (Fig. 42.3A). Most cases of medial fibroplasia are diagnosed in women between the ages of 30 and 50 years. Although medial fibroplasia progresses to higher degrees of stenosis in about one-third of patients, complete arterial occlusion and/or ischemic atrophy of the kidney ipsilateral to the renal artery stenosis are rare. The stenotic lesions in medial fibroplasia are secondary to thickened fibromuscular ridges replacing the normal structure of the intima and the media of the artery. These thickened ridges alternate with thinned areas that may not have an internal elastic membrane, thereby becoming aneurysmal.

Perimedial fibroplasia (subadventitial fibroplasia) accounts for approximately 15% of fibrous renal artery lesions. This lesion also occurs predominantly in women, typically between the ages of 15 and 30 years. Angiographically, it is often characterized by a small string-of-beads appearance, with the beads being of similar or of smaller diameter compared to the diameter of the apparently unaffected portion of the renal artery. This lesion typically affects the distal half of the main renal artery, is frequently bilateral and highly stenotic, and may progress to total arterial occlusion. Collateral blood vessels and renal atrophy on the involved side are commonly observed.[19,20]

Medial hyperplasia and intimal fibroplasia account for only 5% to 10% of fibrous renal artery lesions. Intimal fibroplasia occurs primarily in children and teenagers and angiographically appears as a localized, highly stenotic, and smooth lesion with poststenotic dilation. It may occur in the proximal portion of the renal artery and when it does, it can resemble atheroma. Intimal fibroplasia is progressive and is occasionally associated with dissection or renal infarction and renal atrophy (Fig. 42.4C). Medial hyperplasia, also rare, is found predominantly in teenagers, and angiographically also appears as a smooth, linear stenosis, sometimes appearing as though a ligature were tied around the renal artery. There is considerable difficulty in distinguishing between intimal fibroplasia and medial hyperplasia by angiography alone, and these two types of fibrous artery disease are sometimes grouped together in the literature.[21]

Renal artery aneurysms can develop in up to 50% of these lesions, which sometimes produce local dissection and/or occlusion (Fig. 42.4C). Arteriovenous fistulae and thrombosis can also occur.

Rates of progression differ between histologic subtypes and are poorly understood. Many lesions remain below hemodynamic thresholds and have minimal clinical importance. Others progress to produce hypertension and occasional thrombosis, particularly in women, although this happens far more commonly with atherosclerotic lesions. Smoking appears to be the major risk factor for progressive disease in young women.

Takayasu arteritis is a variant form of systemic vasculitis that affects large arteries such as the aorta and its main branches, including the renal arteries. It mainly affects young women. It can cause discrete stenosis of the aorta

FIGURE 42.4 **(A)** and **(B)** Illustrate the ability of percutaneous transluminal renal angioplasty (PTRA) to open otherwise tight fibrous bands and successfully reverse renovascular hypertension in a young athlete. **C:** Fibromuscular disease complicated by a renal artery aneurysm that produced a segmental infarction and renovascular hypertension not amenable to renal revascularization.

and its main branches, including the common carotid, and the subclavian and the renal arteries.[22] Renal artery involvement is not uncommon in Asian and African patients and is a major cause of renovascular hypertension in these countries.[23] Koide[24] reported renovascular hypertension in 278 (18.8%) of 1,475 patients in a Japanese nationwide survey of Takayasu arteritis. Takayasu arteritis is a common cause of renovascular hypertension in Southeast Asia, including India and China, and accounts for between 20% and 60% of RVH cases.[23,25]

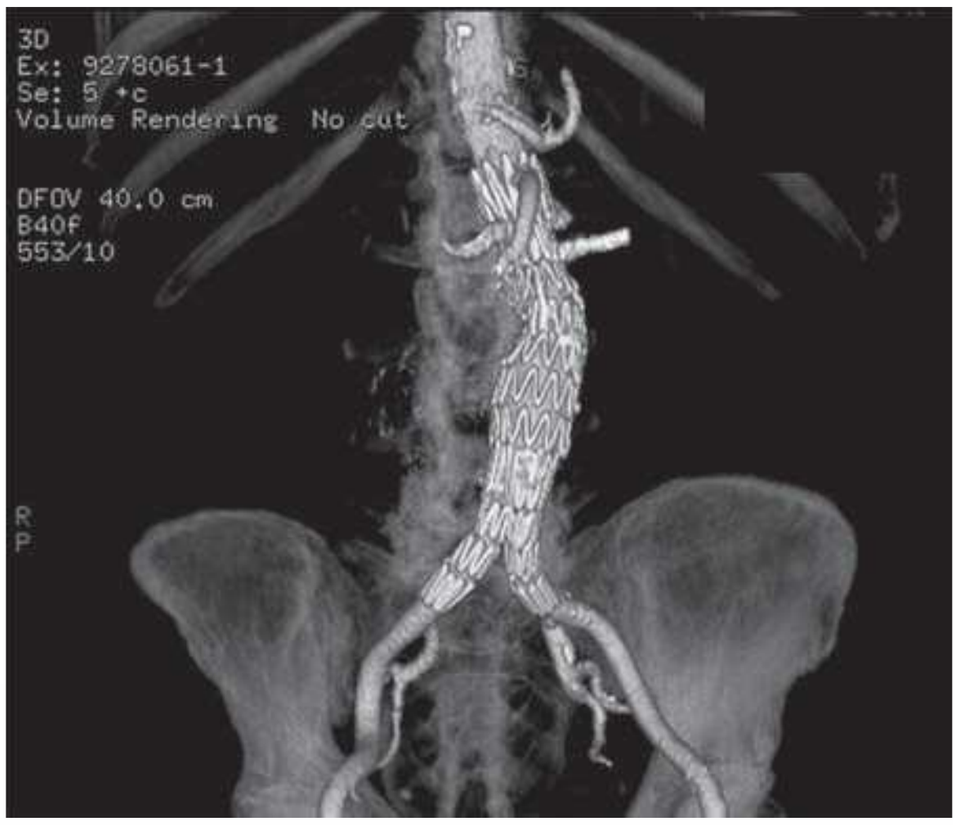
A recent expansion in the use of endovascular procedures for aortic aneurysm repair (EVAR) has produced a new clinical source of renal artery occlusion (Fig. 42.5A). Many of these stent grafts are placed in close proximity to the origins of the renal arteries. Occlusion of the renal arteries is observed in up to 6% of these procedures, sometimes related to migration or inadvertent coverage of the vessels.[4,26] Some grafts are designed for deliberate extension of uncovered struts above the renal arteries for anatomic reasons. Remarkably, the frequency of renal artery compromise is modest, and current endovascular procedures can restore luminal patency if promptly recognized.[27] Identifying and treating vascular occlusion due to aortic stent grafts is important, because failure to achieve patency is associated with a clinically important loss of kidney function in up to 20% of subjects.[28]

Acute and chronic renal artery occlusion secondary to renal artery emboli, aortic or renal artery dissection, neurofibromatosis, arterial trauma, arteriovenous malformations, and polyarteritis nodosa are discussed elsewhere in this book.

Atherosclerotic Renal Artery Stenosis

Atherosclerotic renal artery stenosis (ARAS) most often develops in older patients (> 50 years of age) and is associated with systemic atherosclerosis. ARAS is the most common

FIGURE 42.5 Examples of atherosclerotic renal artery stenosis. **A:** An aortic aneurysm repair using an endovascular stent graft with an extension to cover portions of the renal arteries, which represents a new form of treatment-associated renovascular compromise. (See Color Plate.) **B:** High-grade bilateral renal arterial stenoses from ostial lesions and **(C)** a total renal occlusion to the right kidney and high-grade stenosis to a solitary functioning kidney. Such lesions are becoming more common because effective antihypertensive drug therapy may be delaying the identification of ARAS until later stages with deteriorating kidney function and blood pressure control.



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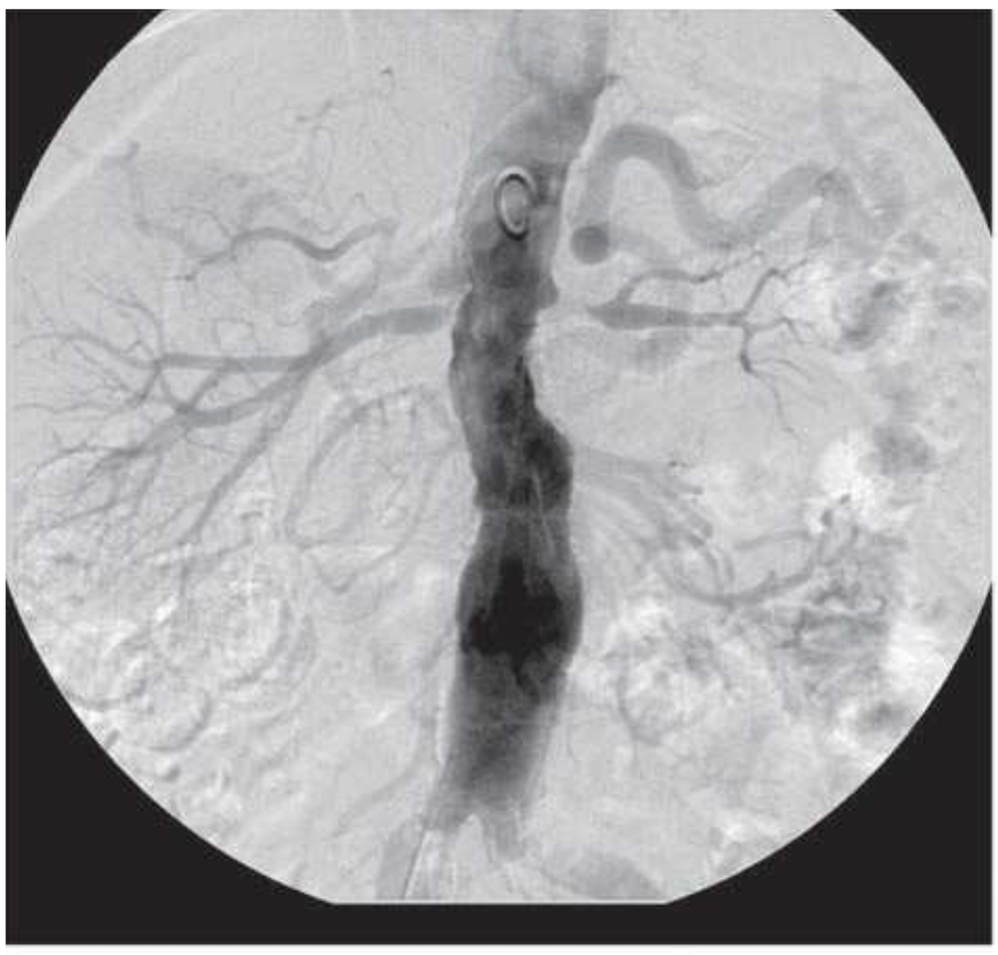

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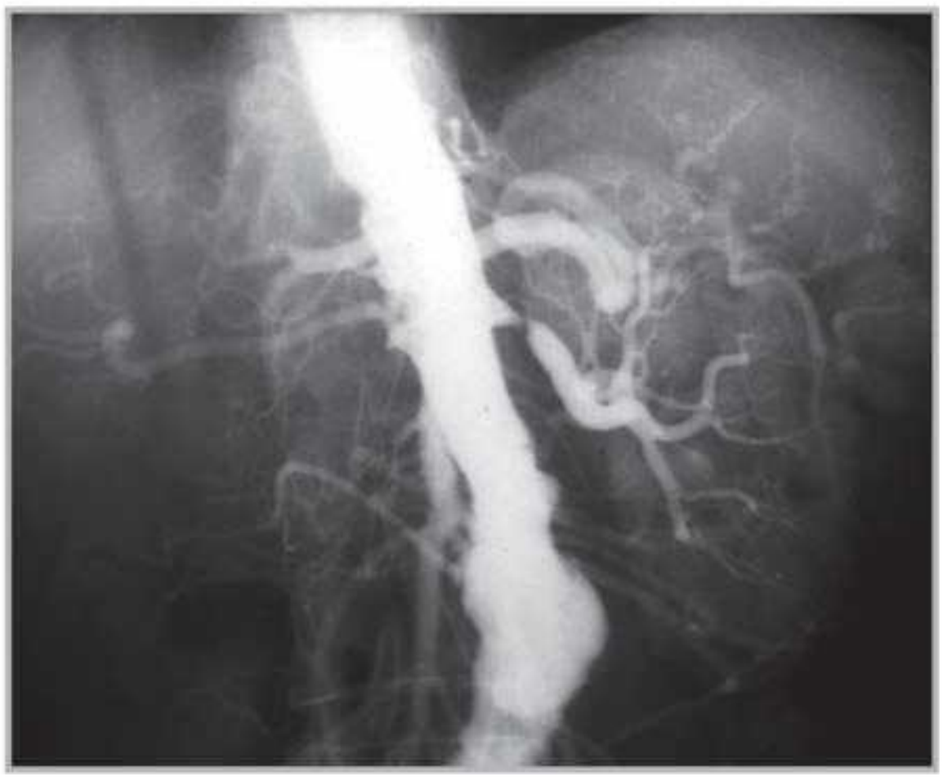


C

cause of RVH and can contribute to a loss of renal function leading to ESRD (Fig. 42.5B,C). Atherosclerotic plaque often arises in the first 1 to 2 cm of the renal artery or may extend directly from the aorta into the renal ostium. Aortic and renal vascular calcification is often present. Some 75% to 80% of patients with renal artery atherosclerosis and renovascular hypertension have ostial atherosclerotic lesions, and 25% to 30% of lesions are in the nonostial location. ARAS is a manifestation of systemic atherosclerotic disease and is associated with coronary, cerebrovascular, peripheral vascular, and aortic disease.[29,30] The prevalence of ARAS appears to be increasing. This probably reflects the fact that more people are living long enough for atherosclerotic vascular disease in the visceral abdominal vessels to reach critical levels, thus aggravating hypertension when the kidney is affected. In a recent systematic analysis of patients undergoing an angiography of the peripheral or coronary circulations, ARAS was found in 11% to 42% of cases.[30] Predictors of ARAS include a history of hypertension, the presence of renal functional impairment, coexisting vascular or coronary artery disease, the presence of abdominal bruits, and a history of smoking. Renal artery lesions are bilateral in 20% to 40% of such patients. Estimates of the prevalence of ARAS depend on the population screened. One population-based study of a cohort of 870 patients older than 65 years screened with renal artery duplex sonography found a 6.8% prevalence of ARAS, defined as greater than 60% stenosis. No differences in prevalence were detected between African Americans and Caucasians,[31] although reported rates for surgically corrected renovascular hypertension are lower for African Americans.[32] Men are affected more often than women, but this gender difference declines with advancing age. Recent series indicate a distinct shift toward women in series referred for revascularization.[33,34] Several studies suggest that atherosclerotic renal vascular disease is associated with adverse coronary events[33,35] and increased mortality. Autopsy series report an overall prevalence of 4% to 20%, with progressively higher rates for those older than 60 years (25% to 30%) and 75 years (40% to 60%). These studies suggest that ARAS leading to renal artery stenosis is the single most common cause of secondary hypertension in patients older than 50 years.[36] It also commonly leads to systolic hypertension with wide pulse pressures. Furthermore, renal artery stenosis has been reported to contribute

to the decline in renal function in 15% to 22% of patients reaching ESRD.[37,38]

Adaptation and Progressive Vascular Occlusion in Atherosclerotic Renal Artery Stenosis

With improved antihypertensive drug therapy and the recognized potential for advanced ARAS to lead to reduced kidney function, clinicians need to consider the potential for progressive renovascular occlusion during medical therapy. This has been a controversial subject. Initial retrospective studies of serial angiograms suggested that progressive occlusion could develop in 40% to 50% of subjects[37] with 15% progressing to total occlusion over periods between 3 to 5 years.[39] A series of studies in the 1990s with ARAS followed prospectively with high-resolution Doppler ultrasound indicated that measurable hemodynamic progression occurred in nearly 50% of subjects over 5 years,[40] although the rates of clinical progression defined by changes in kidney size (24%), loss of GFR, and/or progression to total occlusion were much lower.[41] The 3-year cumulative incidence of renal artery disease progression for 295 renal arteries initially classified as normal, less than 60% stenosis, and greater than or equal to 60% stenosis, was 18%, 28%, and 49%, respectively. In this prospective series, the early progression to total occlusion occurred only in nine arteries (3%), all of which had a baseline reduction in lumen diameter greater than 60% (Fig. 42.6). The cumulative incidence of progression to total occlusion in patients with baseline stenosis of 60% or more was 4% at 1 year, 4% at 2 years, and 7% at 3 years. Factors associated with the risk of renal artery disease progression during the time of monitoring included systolic blood pressure (BP) greater than or equal to 160 mm Hg, diabetes mellitus, and high-grade stenosis (more than 60%) in either the ipsilateral or contralateral renal artery.[37] One of the prospective treatment trials from Europe suggested that 16% of subjects treated without revascularization developed total occlusion, based on renography.[39] Recent prospective treatment trials indicate far lower rates of disease progression. BP control and statin therapy in these trials have been more effective and more widely applied than before, with crossover rates from medical therapy to renal revascularization below 10% over reporting periods between 3 and 5 years.[42] It should be emphasized that the epidemiologic and clinical hazard of progressive stenotic disease is closely related to the level of initial stenosis. For lesions that are more than 75% occluded, further progression is not only far more likely to occur, but the consequence regarding tissue ischemia and loss of functioning tissue is more severe (see Pathophysiology, which follows).

PATHOPHYSIOLOGY OF CLINICAL SYNDROMES WITH RENOVASCULAR DISEASE

Renovascular Hypertension

Renovascular occlusive disease from any cause that reaches a "critical" level can activate pressor mechanisms that tend to raise systemic arterial pressure and restore renal artery

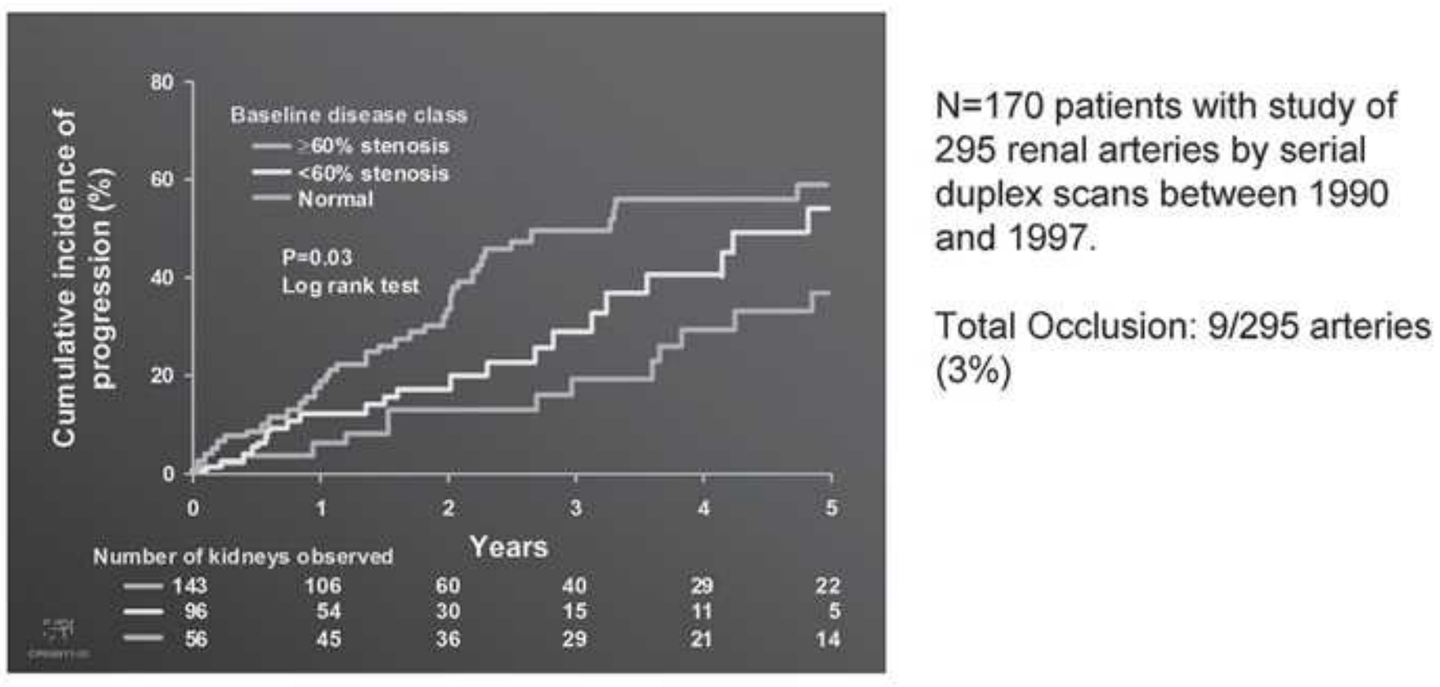


FIGURE 42.6 The progression of atherosclerotic renal artery stenosis (ARAS) of varying severity during a serial follow-up by Doppler ultrasound. There were 295 arteries that were followed sequentially at 6-month intervals for 5 years. Measurable hemodynamic progression (defined as an increase in peak systolic velocity of at least 100 cm per second) was identified in 31% by 3 years, and more than 50% of the group with more than 60% at baseline. Importantly, clinical progression was uncommon (defined by a change in serum creatinine, loss of kidney size, or total occlusion). Predictors of progression included systolic blood pressure, age, and diabetes. (Adapted from Caps MT, Perissinotto C, Zierler RE, et al. Prospective study of atherosclerotic disease progression in the renal artery. *Circulation*. 1998;98:2866–2872.) (See Color Plate.)

perfusion pressures. Luminal occlusion of less than 60% (cross-sectional area) rarely produces any measurable gradient for either pressure or flow. Hence, a fall in renal perfusion pressure sufficient to initiate RVH occurs only when luminal occlusion is relatively severe, usually in the 70% to 80% cross-sectional occlusion range (Fig. 42.7). When critical stenosis develops and reduces renal perfusion pressure, multiple mechanisms are activated in the kidney to restore renal blood flow. Foremost among these pathways is the release of renin from the juxtaglomerular apparatus, leading to the activation of the renin-angiotensin-aldosterone system (RAAS). Release of plasma renin occurs only after poststenotic pressures fall by at least 10% to 20% compared with aortic pressures.[43] This is mediated in part by the stimulation of neuronal nitric oxide synthase and cyclooxygenase 2 in the macula densa. Blockade of the RAAS at the time an experimental renal artery lesion is created prevents the development of hypertension. Animals genetically modified to lack the angiotensin (Ang 1) receptor fail to develop two-kidney one-clip hypertension.[44] Experiments using kidney transplantation from AT1 receptor knockout mice indicate that both systemic and renal angiotensin receptors participate in additive fashion to blood pressure regulation.[45]

In the presence of an intact RAAS, systemic arterial pressures increase until renal perfusion is restored. Studies in both experimental models and humans indicate that additional mechanisms add to long-term elevation of blood pressure in the presence of renal artery stenosis, including activation of the sympathetic nervous system, impairment of nitric oxide generation, and release of endothelin as well as hypertensive microvascular injury to the nonstenotic kidney.

Although there are differences among species (rat, dog, rabbit) in experimental renovascular hypertension, two basic models of Goldblatt hypertension are recognized: the two-kidney, one-clip (2K-1C) model (in which one renal artery is constricted and the contralateral renal artery and kidney are left intact) and the one-kidney, one-clip (1K-1C) model (in which one renal artery is constricted and the contralateral kidney is removed). These two experimental models of renovascular hypertension are diagrammed in Fig. 42.8A,B. Mechanisms responsible for sustained RVH differ according to whether one or both kidneys are affected. Both of these models depend initially on impaired renal perfusion and activation of the RAAS with sodium retention. However, the presence of a normal contralateral kidney allows pressure natriuresis to occur, by which the elevated perfusion pressure produces sodium excretion in the nonstenotic kidney. Because the nonstenotic kidney eliminates excess sodium and volume, the level of perfusion pressure to the stenotic side remains reduced, leading to the ongoing activation of the renal artery underperfusion and RAAS stimulation. This sequence of events producing angiotensin II (Ang II)–dependent hypertension and secondary aldosterone excess with hypokalemia is summarized in Fig. 42.8A.

By contrast, 1K-1C hypertension represents a model in which the entire renal mass is exposed to reduced pressures

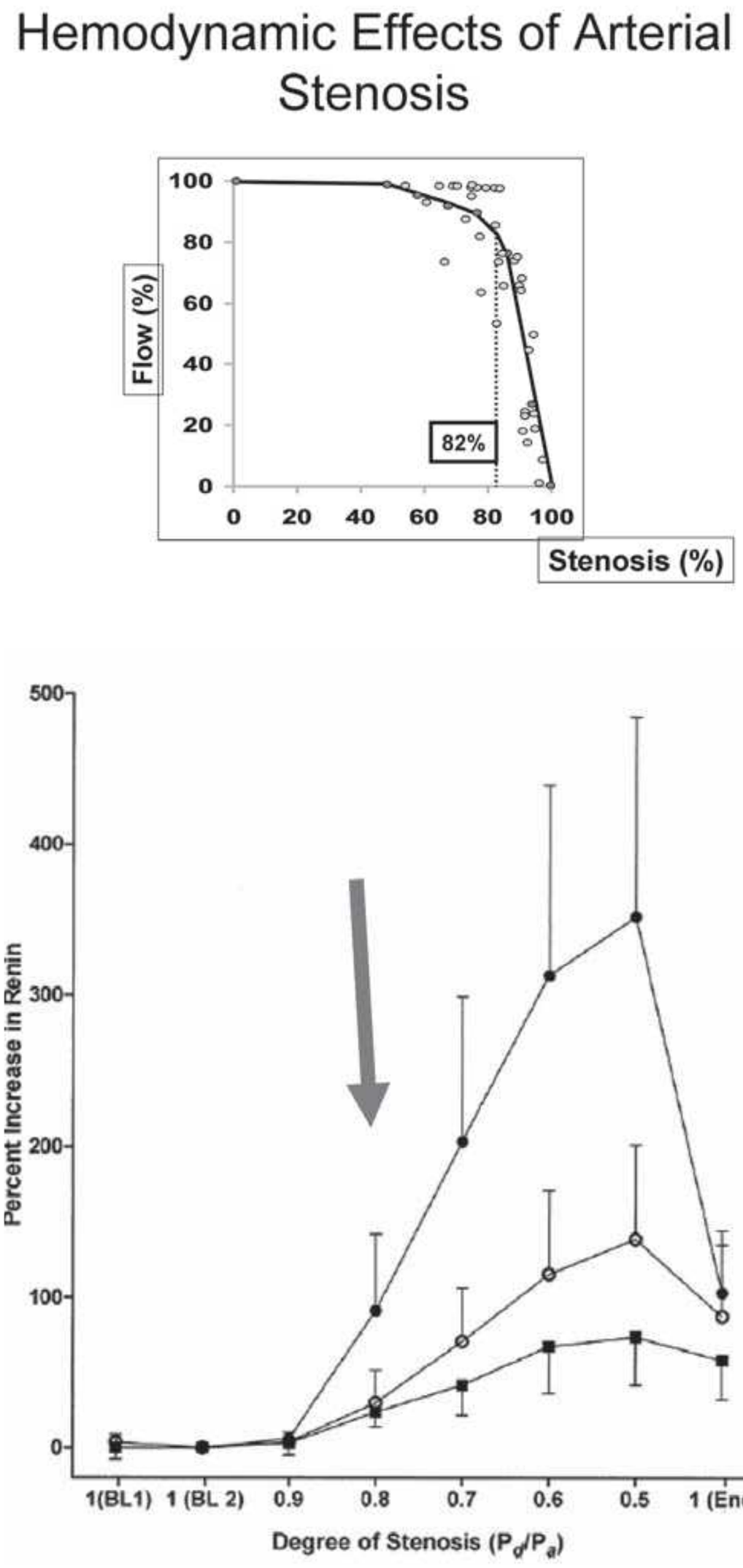


FIGURE 42.7 **A:** Arterial pressure depicted as a function of degree of luminal occlusion. These data highlight the fact that no change in postlesion pressure or flow can be identified until cross-sectional occlusion is severe, usually more than 70% to 80%. These experimental data in dogs are consistent with measurements in human subjects **(B),** wherein renal vein renin release was not detected during balloon occlusion until translesional gradients between the aorta and renal artery of at least 10% to 20% were produced. MAP, mean arterial pressure. (Data from May et al.[229] and De Bruyne et al.[43])

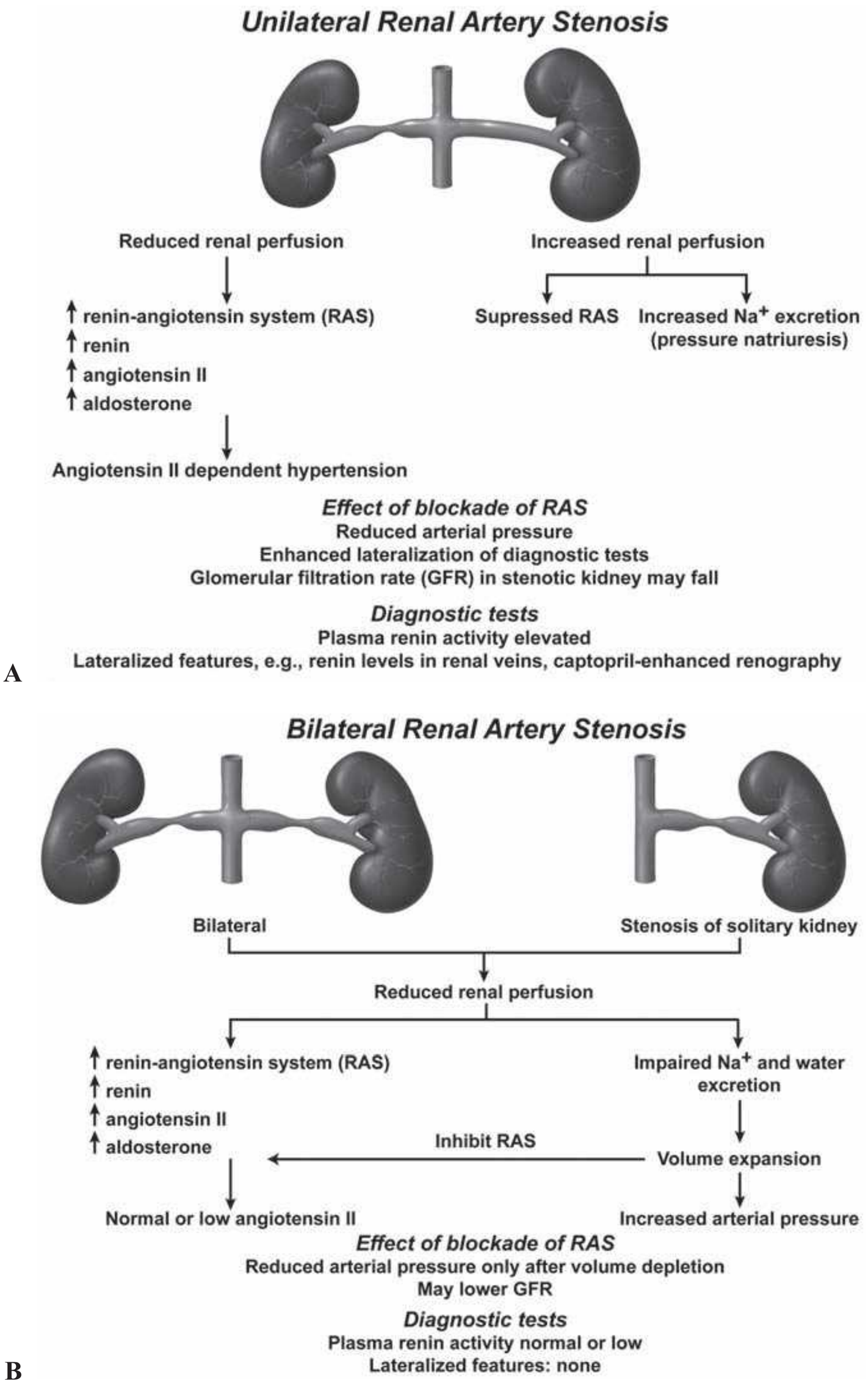


FIGURE 42.8 A: A schematic illustration of hormonal and hemodynamic effects of unilateral renal artery stenosis to produce renovascular hypertension. Unilateral disease is identified as 1-clip-2-kidney Goldblatt hypertension and manifests with the activation of the renin-angiotensin system so long as the contralateral kidney continues to undergo pressure natriuresis (see text). (Adapted from Safian and Textor[91].) Panel **(B)** illustrates the sequence of events with 1-clip-1-kidney Goldblatt hypertension, wherein no normal contralateral kidney is available to excrete sodium and volume in response to rising arterial pressures. In this model, the initial activation of the renin-angiotensin system is temporary due to volume expansion.

beyond a stenosis. There is no normal or nonstenotic kidney to counteract increased systemic pressures. As a result, sodium is retained and the blood volume is expanded, which eventually feeds back to inhibit the renin-angiotensin system (RAS) (Fig. 42.8B). Therefore, 1K-1C hypertension is typically not angiotensin dependent unless the removal of volume is achieved that reduces renal perfusion pressure and again activates the RAAS.

Most renovascular disease in humans is asymmetric and is thought most often to resemble 2K-1C renovascular models. The mechanistic differences between these forms of RVH have clinical implications regarding diagnosis and treatment. Many diagnostic studies classically used to evaluate the functional significance of renal artery lesions depend on comparisons of the different physiologic response of the two kidneys, which may not be evident if both kidneys are affected or if only one kid ney is present. Furthermore, diagnostic tests that depend on differences in responses to alterations in sodium status (such as the measurement of renal vein renin levels after sodium depletion or individual

kidney sodium reabsorption) may be problematic, because high levels of Ang II and aldosterone stimulate sodium reabsorption in both the stenotic and nonstenotic kidney.

The Phases of Renovascular Hypertension

Many of the primary physiologic drivers, such as activation of the RAAS, underlying renovascular hypertension manifest differently at various points in time. These changing manifestations complicate defining primary pathogenic mechanisms and establishing whether renovascular lesions are actually causal in hypertension. Rarely is it known exactly when critical levels of stenosis are attained. In experimental models, these observations have been summarized by separating two-kidney renovascular hypertension into sequential phases.[46] In phase 1, renal ischemia and activation of the RAS are central and the BP elevation is renin dependent. The acute administration of Ang II antagonists or angiotensin-converting enzyme (ACE) inhibitors, removal of the renal artery stenosis (i.e., removal of the clip), or removal of the stenotic kidney normalizes the BP. In the absence of these maneuvers, a transition phase (phase 2) subsequently develops, bridging the acute (phase 1) and chronic (phase 3) phases of experimental renovascular hypertension. This transition phase variably lasts from a few days to several weeks depending on the experimental model and animal species. During this transition phase, plasma renin levels gradually fall, but the BP remains elevated. Salt and water retention are observed as a consequence of the effects of hypoperfusion of the stenotic kidney, augmented proximal renal tubular reabsorption of sodium and water, and secondary aldosteronism.[47,48] In addition, the high levels of Ang II stimulate thirst, further contributing to an expansion of the extracellular fluid volume. The expanded extracellular fluid volume results in suppression of peripheral plasma renin activity (PRA). During this transition phase, the hypertension is still responsive to removal of the unilateral renal artery stenosis, to Ang II blockade, or to unilateral nephrectomy, although these maneuvers do not normalize the BP as promptly and consistently as in the acute phase. These changes may depend on the recruitment of additional mechanisms of Ang II action, including the generation of reactive oxygen species,[49,50] the quenching of nitric oxide,[51] and the generation of endothelium-derived substances such as endothelin[52,53] and thromboxanes.[54] Some of these mechanisms depend on slowly developing effects of Ang II at levels that do not reverse rapidly with direct Ang II blockade.[55] Additional mechanisms recruited over time that sustain BP elevation and induce tissue injury are summarized in Table 42.3.

After several days or weeks, a chronic phase (phase 3) evolves, wherein the removal of the stenosis by unclipping the renal artery in the experimental animal or nephrectomy of the stenotic kidney fails to reduce the BP to baseline levels. The mechanism maintaining elevated arterial pressure—that is, the failure of "unclipping" to lower the BP in this chronic phase of 2K-1C hypertension—is multifactorial but is thought to reflect widespread arteriolar damage in the contralateral kidney consequent to elevated systemic pressure and the initial high levels of Ang II. The BP remains elevated even though the PRA has returned to a normal level. The pressure natriuresis of the contralateral kidney blunts the extracellular fluid volume expansion initially generated by the stenotic kidney (Fig. 42.8A), but because the contralateral kidney suffers vascular damage from prolonged exposure to the increased BP, its excretory function diminishes and

42.3 Interactive Mechanisms Underlying Hypertension and Kidney Injury in Atherosclerotic Renal Artery Stenosis

Tissue Underperfusion	Recurrent Local Ischemia
Activation of the renin-angiotensin system	ATP depletion
Altered endothelial function: (endothelin, NO, prostaglandins)	Tubulointerstitial injury
Sympathoadrenergic activation	Microvascular damage
■ Increased reactive oxygen species	Immune activation
■ Cytokine release/inflammation (NF-κB, TNF, TGF-β, PAI-1, IL-1)	Vascular remodeling
■ Impaired tubular transport functions	Interstitial fibrosis
Apoptosis/necrosis	Activation of the renin-angiotensin aldosterone system
	Sympatho-adrenergic activation
	Endothelin
	Disturbances of "oxidative stress" Oxidized-LDL

NO, nitric oxide; NF-κB, nuclear factor kappa B; TNF, tumor necrosis factor; TGF-β, transforming growth factor-beta; PAI-1,plasminogen activating factor inhibitor; IL-1, interleukin 1; ATP, adenosine triphospate; LDL, low-density lipoprotein. From Textor SC. Atherosclerotic renal artery stenosis: overtreated, but underrated? J Am Soc Nephrol. 2008;19:656–659, with permission.

extracellular fluid volume expansion persists. In phase 3 of 2K-1C hypertension, acute blockade of the RAS fails to lower the BP. Sodium depletion may ameliorate the hypertension, but does not normalize it.

Exactly how and whether this sequence of events applies directly to humans is not known. All of these features obscure the diagnosis of true renovascular hypertension and limit predictability of the BP response to revascularization. Not surprisingly, a documented history of a brief duration of hypertension suggests a more favorable response to revascularization procedures.[56]

There are additional important clinical correlates of this process. First, many of the diagnostic studies that depend on the lateralization of effects have only modest predictive value when results are negative. As a general rule, these tests are most useful when results are positive, meaning that high-grade lateralization of renin release, differences in renal function, and changes in the glomerular filtration rate (GFR) after the administration of an ACE inhibitor most accurately predict improvement after revascularization when results are markedly positive. A negative test result, however, may also be associated with a beneficial outcome. Second, coexistent intrarenal disease, such as arteriolosclerosis with glomerulosclerosis, is usually associated with persistent hypertension despite the correction of renal artery stenosis, particularly for patients with ARAS.[57] In such patients, a long duration of hypertension favors the development of arteriosclerotic lesions and renal injury in the contralateral kidney. Thus, older age and a long duration of hypertension for more than 3 to 5 years predict a poorer clinical BP outcome after renal revascularization. Most older patients with ARAS also have impaired renal function that itself predisposes one to long-term hypertension.[11]

The Pathogenesis of Ischemic Nephropathy

The activation of pressor mechanisms producing RVH often occurs with a minor loss of renal size or function, particularly with fibromuscular disease.[58–60] Conversely, improved BP control after revascularization sometimes may be achieved without an appreciable improvement in kidney function. However, the more common clinical scenario with ARAS involves both increasing severity of hypertension and deteriorating renal function, often with a loss of renal size. Hence, the decision to consider renal revascularization commonly combines consideration of both the likelihood of salvage or the preservation of function, in addition to possible benefits regarding BP control.

Basal energy requirements for the kidney are met with less than 10% of blood flow, consistent with its filtration function. The term ischemic renal disease should be used with caution in this context.[61] Under normal conditions, cortical blood flow provides vastly more oxygenated blood than is needed for tissue metabolism. Postglomerular vasa recta deliver a portion of kidney blood flow to the medulla. The major metabolic workload of the kidney takes place in medullary segments as a function of solute transport, leaving the medulla normally with substantial oxygen desaturation.[62] The balance between regional oxygen saturation within the cortex and the medulla is carefully maintained over a variety of conditions.[63] Remarkably, blood flow to the kidney can be reduced gradually to levels sufficient to reduce kidney volume, activate pressor mechanisms including the RAAS, and lower GFR without measurably disturbing cortical or medullary oxygenation.[64] Lowered GFR is associated with a reduced filtered load of solutes and thereby reduced requirements for solute reabsorption.[63] Venous oxygen levels from poststenotic kidneys therefore can be higher than those from normally functioning kidneys due to reduced metabolic oxygen consumption.[64] Measurements of cortical and medullary deoxyhemoglobin by use of blood oxygen level–dependent magnetic resonance in human subjects confirm that both cortical and medullary oxygen saturation can be well preserved over a wide range of renovascular occlusion.[65] These observations may explain the relative stability of kidney function observed in recent prospective treatment trials for ARAS for many years, despite an obvious reduction in renal blood flow beyond the stenotic lesion.

There are limits to the renal capacity for adaptation, however. When vascular occlusion is sufficiently severe, cortical blood flow and oxygenation falls.[66] This in turn overwhelms the adaptive capacity of the medulla, with expanding hypoxia and activation of fibrogenic and inflammatory pathways.

Mechanisms underlying parenchymal renal damage differ from those responsible for generating hypertension. Remarkably, parenchymal fibrosis rarely develops in patients with fibromuscular disease with the exception of those experiencing renal infarction. This suggests that the activation of remodeling mechanisms in the poststenotic kidney partly is related to the atherosclerotic milieu itself, which produces microvascular proliferation and abnormal endothelial function within the kidneys.[67] Recent experimental studies underscore the development of magnified renal microvascular changes distal to a stenosis in the renal artery in the context of atherosclerosis.[68,69] An example of microvascular proliferation induced by cholesterol feeding (a surrogate for early atherosclerosis) and the subsequent rarefaction of renal small vessels beyond a main renal artery lesion is illustrated in Fig. 42.9. Numerous signaling pathways lead to the upregulation of cytokines and inflammatory mediators, including transforming growth factor (TGF)-β, within the poststenotic kidney.[70,71] Over time, rarefaction of the distal arterioles develops and is associated with fibrogenesis and a loss of viable function.[72,73] The sequence of events underlying the transition from a reversible loss of function beyond a vascular lesion to irreversible tissue fibrosis is not well understood. Atherosclerotic and inflammatory pathways can produce disturbances in endothelial function in small vessels that parallel tissue injury and accelerate cytokine signaling pathways.[74,75] At some phase, microvascular rarefaction occurs that accompanies a fall in tissue oxygenation and activation of fibrogenesis.[75] An irreversible loss of viable

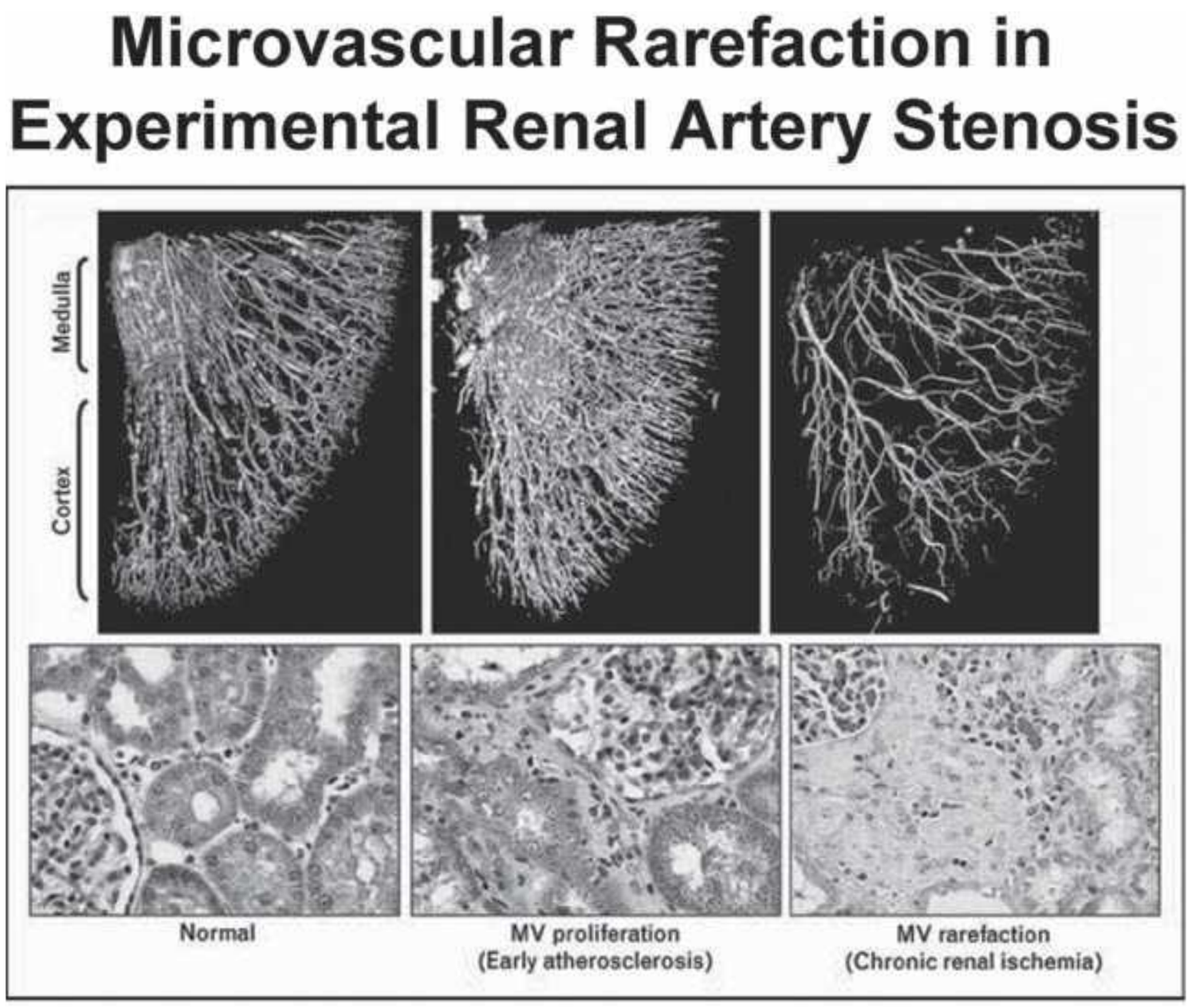


FIGURE 42.9 Reconstructions of the vascular structures using micro-computed tomography (CT) imaging in experimental renal artery stenosis. The atherosclerotic milieu produced by cholesterol feeding leads to microvascular (MV) proliferation and renders the animal especially susceptible to rarefication and obliteration of microvessels in the setting of high-grade renal artery stenosis. These changes eventually lead to interstitial fibrosis and a loss of kidney function. (From Lerman LO, Chade AR. Atherosclerotic process, renovascular disease and outcomes from bench to bedside. *Curr Opin Nephrol Hyper.* 2006;15:583–587, with permission.)

microcirculation may explain some of the limitations observed after the restoration of large-vessel patency (e.g., with renal revascularization). Occasionally, renovascular disease is associated with nephrotic range proteinuria that can regress after renal revascularization.[76] The mechanism of enhanced glomerular permeability in this context is unknown.

Circulatory Congestion and Flash Pulmonary Edema

Among other clinical manifestations, ARAS increasingly has been implicated in episodes of congestive heart failure and has been described as one of the cardiorenal syndromes.[47] Several mechanisms contribute to this disturbance, including (1) Impaired sodium excretion due to reduced renal perfusion pressure and activation of the RAAS that affects both stenotic and contralateral kidneys and (2) sustained, and sometimes rapid, increases in systolic arterial pressure can abruptly add to pressure overload of the left ventricle (Fig. 42.10).[77] Normally, the left ventricle compensates for changes in afterload with increased end-diastolic volume. This mechanism may be impaired, however, in patients with stiffened left ventricles due to left ventricular hypertrophy, precipitating in abrupt rises in end-diastolic pressure, left atrial, and pulmonary venous pressures. It is this abrupt rise in ventricular pressures that leads to sudden decompensation, often termed flash pulmonary edema, that was originally described by Pickering et al.[78] In addition, the activation of sympathetic nervous pathways magnifies these effects and alters the transcapillary capacities for gas exchange. Reports from several case series suggest that renal revascularization can interrupt this cycle and reduce reoccurrences.[79,80] In less dramatic cases, the presence of bilateral ARAS with reduced GFR may limit the effectiveness of diuretics and render congestive heart failure refractory to fluid removal short of external ultrafiltration.

Clinical Features of Renal Artery Stenosis

Renovascular Hypertension

In the 1970s, a cooperative study of RVH compared clinical characteristics of patients with surgically proven RVH with those of patients with primary hypertension. In this study, the average age of onset for fibrous renal artery disease as the cause of renovascular hypertension was 33 years, and 16% of these patients were younger than 20 years. For atherosclerotic renal artery disease as the cause of renovascular hypertension, the average age at onset was 46 years, and 39% of these patients were older than 50 years.[81] For these reasons, many argue that the clearly defined onset of hypertension below the age of 30 or above the age of 55 warrants the consideration of renovascular hypertension. Some features, such as the presence of an abdominal bruit, hypokalemia, and the absence of a family history of hypertension, were statistically more prevalent in RVH, but had little clinical predictive value. Recent studies suggest that for any level of office BP, patients with RVH may have higher nocturnal pressures and therefore higher overall pressure load as "non-dippers."[82] As a result, target organ manifestations are more severe, including left ventricular hypertrophy (Fig. 42.11). A series of patients with treatment-resistant hypertension indicate that elevated cholesterol, impaired renal function, lower body mass index (BMI), and smoking provide positive clues. In practical terms, none of these features is sufficiently sensitive or specific to offer diagnostic precision. As noted previously, RVH rarely may be associated with nephrotic-range

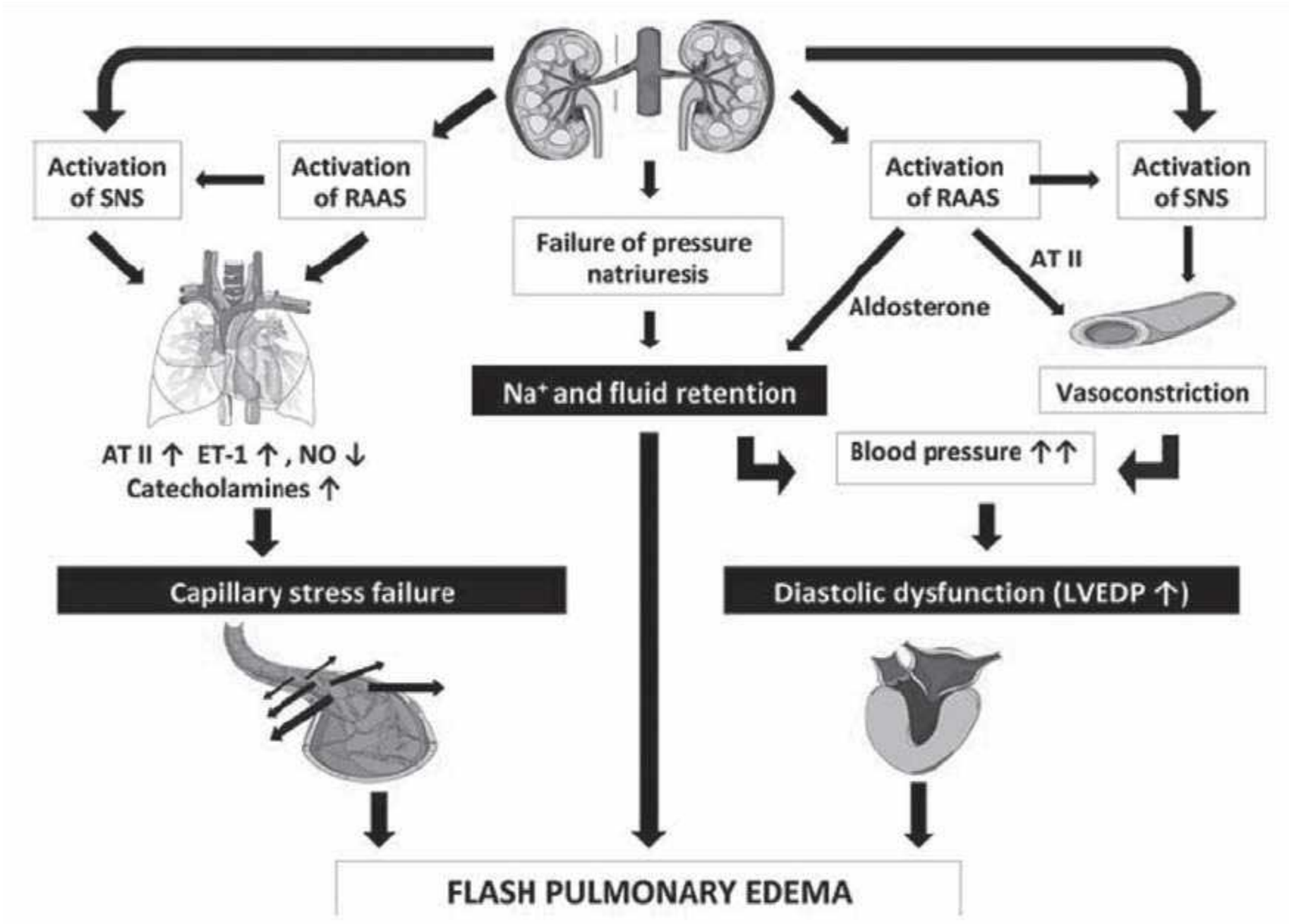


FIGURE 42.10 A schematic depicting the interplay of mechanisms leading to rapidly developing symptoms of left ventricular heart failure sometimes observed with renal artery stenosis. These include impaired volume excretion with bilateral atherosclerotic renal artery stenosis (ARAS), a rise in afterload and impaired left ventricular pump function, as well as disturbed capillary function within the pulmonary vasculature in part related to the activation of neurogenic and hormonal pathways. *SNS,* sympathetic nervous system; *RAAS*, renin-angiotensin-aldosterone system; *ATII*, angiotensin II; *ET-1*, endothelin 1; *NO*, nitric oxide; *LVEDP*, left ventricular end-diastolic pressure. (From Messerli FH, Bangalore S, Makani H, et al. Flash pulmonary oedema and bilateral renal artery stenosis: the Pickering syndrome. *Eur Heart J.* 2011;32(18):2231–2237, with permission.)

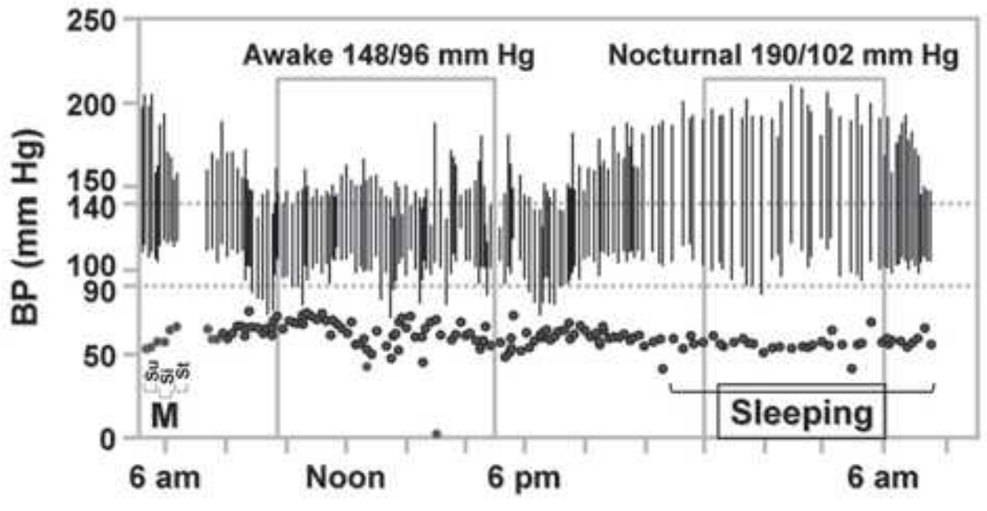


FIGURE 42.11 Ambulatory blood pressure (BP) monitoring in patients with renovascular hypertension commonly reveals a loss of nocturnal pressure fall, or in some cases, a complete reversal of the day–night pattern as shown here. This may be one of the features by which target-organ injury such as left ventricular hypertrophy is exaggerated as compared to patients with similar daytime blood pressure levels.[232]

proteinuria, which can regress with the correction of the vascular lesions.

BP elevations from RVH vary widely, often as a function of the rapidity of onset. Acute renal artery occlusion may only gradually produce an increase in pressure or it may produce a rapid increase in hypertension that may precipitate a hypertensive urgency or emergency. Before the current era of antihypertensive agents, 30% of Caucasian patients appearing in an emergency department with hypertensive urgency (defined as grade 3 or 4 hypertensive retinopathy) were ultimately found to have RVH. Hence, patients presenting with accelerated forms of hypertension should be considered candidates for renovascular hypertension (Table 42.4). Syndromes of polydipsia and accelerated hypertension with hyponatremia and hypokalemia, sometimes attributed to the dipsogenic actions of Ang II, also have been observed. Current antihypertensive medications have changed the clinical presentation of RVH, thus making them less severe.

Recent consensus documents regarding hypertension emphasize the need for effective populationwide BP control while limiting the number and expense of diagnostic studies.

42.4 Clinical Features Suggestive of Renovascular Disease

Clinical Clues

- Age at onset of hypertension (< 30 or > 55 years)
- Abrupt onset of hypertension
- Acceleration of previously well-controlled hypertension
- Hypertension refractory to an appropriate three-drug regimen
- Accelerated retinopathy
- Malignant hypertension/occasionally with hyponatremia
- Systolic–diastolic abdominal bruit
- Flash pulmonary edema
- Evidence of generalized atherosclerosis obliterans
- Acute renal failure with angiotensin-converting enzyme (ACE) inhibitor treatment

Laboratory Features of Renovascular Hypertension

- Early activation of renin-angiotensin-aldosterone system (RAAS)
- Paroxysmal symptoms: sympathetic nervous system activation
- Abnormal circadian rhythm: loss of nocturnal pressure fall
- Accelerated target organ damage
  - Left ventricular hypertrophy
  - Microvascular disease
  - Renal injury: fibrosis

Modified from Textor SC, Greco BA. Renovascular hypertension and ischemic renal disease. In: Floege J, Johnson RJ, eds. Comprehensive Clinical Nephrology. St. Louis, MO: Saunders/Elsevier; 2010: 451–468, with permission.

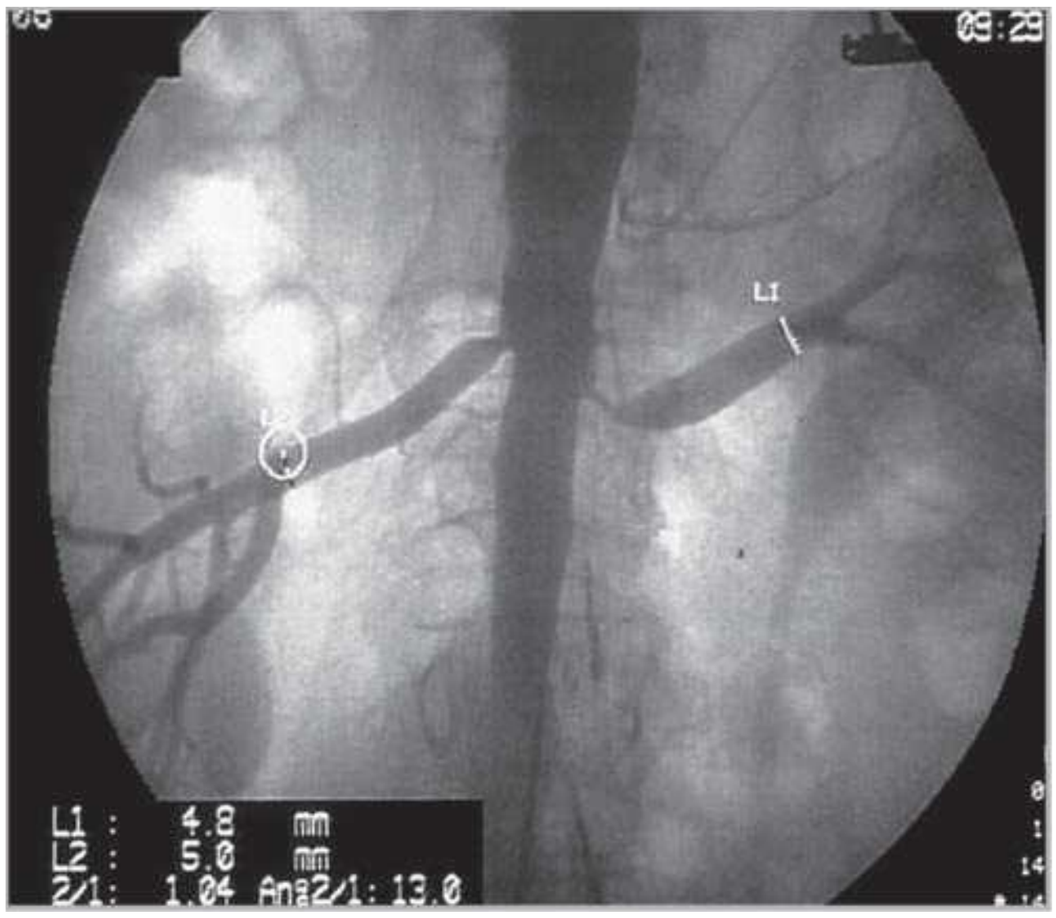


FIGURE 42.12 An aortogram revealing bilateral renal arterial disease performed during a coronary angiography. This individual was treated with antihypertensive drug therapy with excellent blood pressure (BP) control and normal kidney function and was identified only because imaging was included with coronary studies. Numerous series indicate that many such patients are otherwise never identified, but can be managed with medical therapy only. The number that progress to more advanced disease and/or loss of kidney function with current medical therapy may be less than 10% (see text).

When combined with the ambiguous results of prospective treatment trials in which medically treated individuals with ARAS fared as well as those treated with renal revascularization, some would argue that there is little evidence to support extensive diagnostic studies to identify all cases of renovascular hypertension. As a result, most patients with hypertension simply are treated and subjected to few laboratory investigations. For those who reach acceptable BP control without adverse effects, no further studies are performed. Hence, many if not most cases of true RVH are not detected (Fig. 42.12) unless hypertension becomes more difficult to treat or if renal dysfunction ensues. One important reason that RVH is less frequently detected is the availability of orally active antihypertensive agents that block the RAAS. Early studies beginning with captopril indicated that satisfactory BP control can be achieved in more than 86% of patients with RVH compared with less than 50% with previously available drugs. In recent years, the widespread application of ACE inhibitors and angiotensin receptor blockers (ARBs) for indications other than hypertension (e.g., congestive cardiac failure, diabetic nephropathy, and other proteinuric renal disease) increases the exposure of individuals with undetected renal artery stenosis to these drugs.

One result of these changes has been the emergence of distinctive clinical syndromes that merit a further evaluation in patients at risk for ARAS. These are summarized in Figure 42.1. As a result, patients who typically undergo a diagnostic evaluation and renal revascularization are a subset of the population of patients with RVH.

Deterioration of Renal Function During Antihypertensive Drug Therapy

The availability of effective and tolerable drugs for hypertension has meant that many patients are primarily treated with medical management (see the following). Because some patients with renovascular disease are functioning near the lower end of autoregulation, further pressure reduction may curtail blood flow further. Some authors argue that a rise in serum creatinine more than 30% above pretreatment levels should prompt the exclusion of large vessel ARAS.[83] The rapid deterioration of renal function following BP reduction with conventional antihypertensive agents or particularly following BP reduction with ACE inhibitors suggests the

presence of bilateral renal artery stenosis, or stenosis in a solitary functioning kidney.[84–88] In one series, more than half of patients demonstrating an acute elevation in the plasma creatinine concentration that was either unexplained or occurred shortly after the institution of therapy with an ACE inhibitor had main renal artery disease, whereas the remainder presumably had a disease of the intrarenal vessels due to nephrosclerosis.[88] Remarkably, most patients with renal artery stenosis tolerate ACE inhibition or ARB Rx with few adverse effects.[89]

Diagnostic Testing for Renovascular Hypertension and Ischemic Nephropathy

Goals of Evaluation

Given the array of diagnostic studies available and the need to focus the evaluation on patients most likely to benefit, it behooves clinicians to consider carefully the objectives of initiating expensive and sometimes ambiguous studies beforehand. As with all tests, the reliability and value of diagnostic studies depend heavily on the pretest probability of disease.[90] Furthermore, it is helpful to consider from the outset exactly what objective is to be achieved. Is the major goal to exclude high-grade renal artery disease? Is it to exclude bilateral (as opposed to unilateral) disease? Is it to identify stenosis and estimate the potential for clinical benefit from renal revascularization? Is it to evaluate the role of renovascular disease in explaining deteriorating renal function? The specific diagnostic studies may differ depending on which of these is the predominant clinical objective (Table 42.5).

Noninvasive diagnostic tests for renovascular hypertension and ischemic nephropathy remain imperfect. For the purposes of this discussion, diagnostic tests fall into the following general categories (Table 42.6): (1) physiologic and functional studies to evaluate the role of stenotic lesions particularly related to activation of the RAS, (2) perfusion and imaging studies to identify the presence and degree of vascular stenosis, and (3) studies to predict the likelihood of benefit from invasive maneuvers, including renal revascularization.

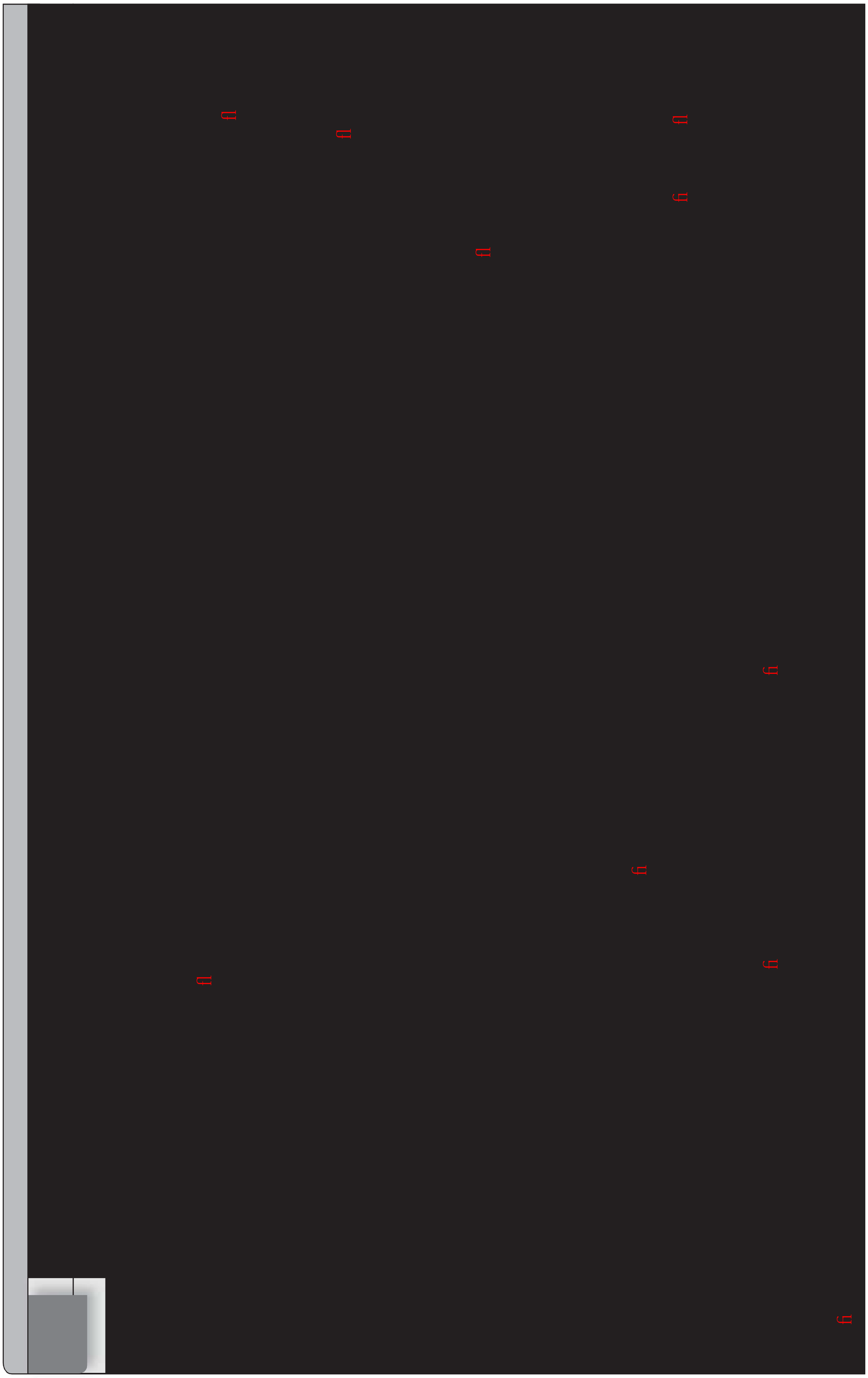
Physiologic and Functional Studies of the Renin-Angiotensin System

Efforts have been made for many years to link the measurement of activation of the RAS as a marker of underlying renovascular hypertension. Although these studies are promising when studied in patients with known renovascular hypertension, they have lower performance results as diagnostic tests when applied to wider populations, as we and others have reviewed.[91–93] PRA is modified by changes of sodium intake, volume status, renal function, and many medications. The sensitivity and specificity of measuring PRA are heavily dependent upon the a priori probability of renovascular hypertension. Although unilateral hypersecretion of renin is an expected finding in hemodynamically significant renal artery stenosis when renovascular hypertension is eventually proved, baseline PRA is elevated in only 50% of patients with renovascular hypertension.[92] Tabulation of the number of patients whose hypertension was cured or improved by surgical renal revascularization indicates that probably no more than 50% of patients with renovascular hypertension have elevated PRA.[94,95] In practice, the major utility of these studies often depends on their negative predictive value, specifically the certainty with which one can exclude significant renovascular disease if the test is negative. Because negative predictive value rarely exceeds 60% to 70%, these tests offer limited value in clinical decision making.

There are several reasons for the relatively low specificity and sensitivity of peripheral PRA in the diagnosis of renovascular hypertension. Most high-renin hypertension is not renovascular in origin; increased renin substrate can

42.5 Goals of Diagnostic and Therapeutic Intervention in Renovascular Hypertension and Ischemic Nephropathy

Goals of Diagnostic Evaluation
Establish presence of renal artery stenosis: location and type of lesion Establish whether unilateral or bilateral stenosis (or stenosis to a solitary kidney) is present Establish presence and function of stenotic and nonstenotic kidneys Establish hemodynamic severity of renal arterial disease Plan vascular intervention: degree and location of atherosclerotic disease
Goals of Therapy
I. Improved Blood Pressure Control Prevent morbidity and mortality of high blood pressure Improve blood pressure control and reduce medication requirement
II. Preservation of Renal Function Reduce risk of renal adverse perfusion from use of antihypertensive agents Reduce episodes of circulatory congestion ("flash" pulmonary edema) Reduce risk of progressive vascular occlusion causing loss of renal function: "preservation of renal function" Salvage renal function (i.e., recover glomerular filtration rate)



be due to estrogen intake, pregnancy, cortisol excess, intrarenal microvascular ischemia (parenchymal disease), or accelerated or malignant hypertension, and the 15% to 20% of patients with primary (essential) hypertension and high renin levels constitute the majority of high-renin hypertensives. Renin secretion fluctuates widely and is influenced by sodium intake, posture and sympathetic tone,[96] a variety of drugs, age, sex, and race.[97] The utility of peripheral PRA is reportedly enhanced when measured in the morning with the patient in the seated position and when indexed against urinary sodium excretion; when measured under these exacting circumstances, a high peripheral PRA is found in 75% to 80% of patients with proven renovascular hypertension.[98] Other investigators, measuring peripheral PRA under similar circumstances, failed to demonstrate significantly increased sensitivity of the peripheral PRA in predicting either the presence of ARAS and the response to therapy.[92,99,100]

The measurement of renal vein renin levels is a means to determine the role of a specific pressor kidney and has been widely applied in planning surgical revascularization for hypertension. Because surgical revascularization entails greater risks than those associated with current endovascular procedures, a substantial effort in the 1970s was directed to identify patients most likely to benefit.[101] These measurements are obtained by sampling renal vein blood and inferior vena cava blood individually. The level of the inferior vena cava is comparable to the arterial levels into each kidney and allows for the estimation of the contribution of each kidney to total circulating levels of plasma renin activity. Lateralization is defined usually as a ratio exceeding 1.5 to 2.0 between the renin activity of the stenotic kidney and the nonstenotic kidney. Some authors propose a detailed examination not only of the relative ratio between kidneys, but also the degree of suppression of renin release from the nonstenotic or contralateral kidney.[101] In general, the greater the degree of lateralization, the more probable that clinical BP reductions would follow after surgical revascularization. Results from many studies support the observation that large differences between kidneys identify high-grade renal artery stenosis. In 1976, Marks et al.[102] reviewed 21 published series encompassing 468 patients with unilateral renovascular disease who had been subjected to a broad spectrum of renin-stimulating maneuvers (e.g., sodium depletion, upright posture). They concluded that a lateralizing renal vein renin ratio predicted surgical cure or substantial improvement in the BP in 93% of patients. Remarkably, 57% of patients with a nonlateralizing renal vein renin ratio (less than 1.5 ipsilateral to contralateral) also benefited from surgery.[102] This later expanded to a review of 58 studies that suggested a ratio of 1.5 or more (stenotic kidney to contralateral kidney) as the diagnostic criterion predicting renovascular hypertension had a sensitivity of 80% and a specificity of 62%.[103] These observations have been extended recently through studies of renal vein measurements prior to considering a nephrectomy for refractory hypertension and advanced renovascular occlusive disease.[104] As with many tests of hormonal activation, study conditions are crucial and can change the interpretation of these tests. Strong and colleagues[105] demonstrated that nonlateralization can be changed to strongly lateralizing measurements by the administration of diuretics between sequential studies. A number of measures to enhance renin release and magnify differences between kidneys have been proposed, including sodium depletion with diuretic administration, hydralazine, tilt-table stimulation, or captopril.[106,107] Volume expansion with intravenous saline poses particular limitations due to the near universal application of this maneuver to limit nephrotoxicity associated with contrast imaging, which is usually performed with renal vein sampling.[108] Because of this variability with different testing conditions, recent series[109,110] concluded that the overall sensitivity of renal vein renin measurements was no better than 65% and that positive predictive value was 18.5%. For these and other reasons, renal vein assays are performed less commonly than before. A major additional factor is that the goals of renal revascularization have shifted substantially and are often directed toward the preservation of renal function, rather than BP control, per se. Nonetheless, the sum of cumulative data regarding renal vein renin determinations indicate that the renal vein renin ratio has a strong positive predictive value in forecasting favorable blood pressure responses to renal revascularization. On the other hand, many patients (at least 50%) demonstrating a nonlateralizing renal vein renin ratio have a marked improvement or cure of their hypertension following an intervention for the renal artery stenosis.

Many more patients are seen with bilateral renal arterial lesions in which both are suspected of participating in a loss of renal function. The most marked asymmetry is seen in patients who have complete occlusion of one renal artery, wherein renal vein renin levels from the side of the occluded artery may represent a combination of low flow through the kidney in addition to hypersecretion of renin.[111] In cases for which it is important to establish the degree of pressor effect of a specific kidney or site, such as before considering a nephrectomy of a pressor kidney, measurement of renal vein renin levels can provide strong supportive evidence. It may be argued that recent trials that suggest limited benefits from renal revascularization may prompt more extensive testing, including formal measurements of individual renin secretion before moving to interventional procedures.

Studies of Individual Renal Function

Serum creatinine, iothalamate clearance, and other estimates of total GFR are measures of overall renal excretory function and do not address differences between separate kidneys. A large body of literature addresses the potential for individual split renal function studies to establish the functional importance of each kidney in renovascular disease.

Split renal function studies classically utilized separate ureteral catheters to allow individual urine collection for the measurement of separate GFR, renal blood flow, sodium excretion, concentrating ability, and the response to blockade of

Ang II.[112] These studies demonstrate that the hemodynamic effects of renal artery lesions in fact do translate into functional changes, such as avid sodium retention, before major changes in blood flow occur. They emphasize that the autoregulation of blood flow and GFR can occur over a wide range of pressures in humans and may be affected in both stenotic and contralateral kidneys by the effects of Ang II. These studies require urinary tract instrumentation and provide only indirect information regarding the probability of benefit from revascularization. They are now rarely performed.

Separate renal functional measurements can be obtained less invasively with radionuclide techniques. These methods use a variety of radioisotopes (e.g., 99mTc-mercaptoacetyltriglycine [99mTc-MAG3] or 99Tc-diethlyene triamine penta-acetic acid [DTPA]) to estimate fractional blood flow and filtration to each kidney. The administration of captopril beforehand magnifies differences between kidneys, primarily by delaying excretion of the filtered isotope due to the removal of the efferent arteriolar effects of Ang II. Some authors advocate such measurements to follow progressive renal artery disease and its effect on unilateral kidney function as a guide to consider revascularization.[113] Serial measurements of individual renal function by radionuclide studies may allow a more precise identification of progressive ischemic injury to the affected kidney in unilateral renal artery disease than can be determined from the overall GFR. Recent studies indicate that single kidney GFR measurements by this method accurately reflect changes in three-dimensional volume parameters measured by magnetic resonance imaging (MRI).[114] These authors argue that demonstrating well-preserved parenchymal volume with a disproportionate reduction in single kidney GFR supports the concept of "hibernating" kidney parenchyma and might provide a predictive parameter for the recovery of kidney function after revascularization.[114]

Imaging of the Renal Vasculature

Advances in Doppler ultrasound, radionuclide imaging, magnetic resonance arteriography (MRA), and computed tomography (CT) angiography continue to improve the field of renovascular imaging. The details of these methods are beyond the scope of this discussion. They are addressed more fully elsewhere. What follows is a discussion of some of the specific merits and limitations of each modality as they apply to renovascular hypertension and ischemic nephropathy.[90]

Current practice favors limiting invasive arteriography to the occasion of endovascular intervention (e.g., stenting and/or angioplasty). Although for many, angiography remains the gold standard for evaluating the renal vasculature, its invasive nature, potential hazards, and cost make it most suitable for those in whom intervention is planned, often during the same procedure. As a result, most clinicians favor preliminary noninvasive studies. When noninvasive studies are equivocal, arterial angiography may be warranted to establish the presence of transstenotic pressure gradients, as recommended for treatment trials.[115,116]

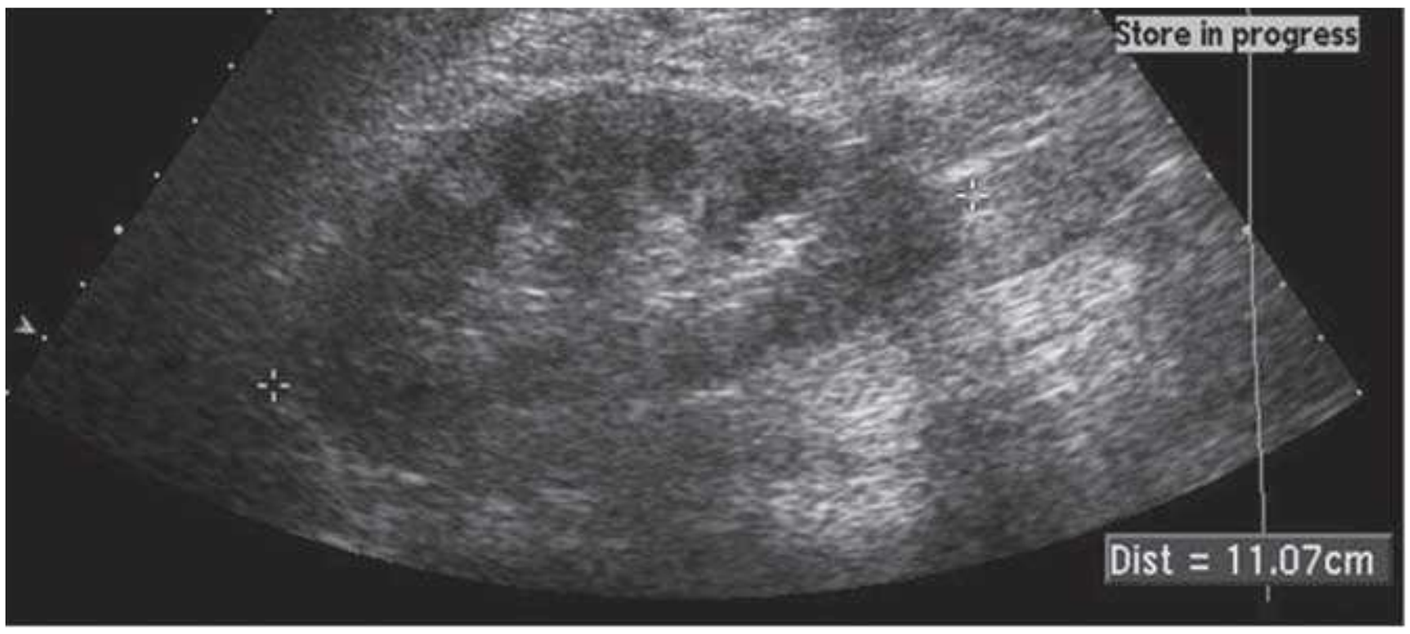
Noninvasive Imaging

Doppler Ultrasound of the Renal Arteries

Duplex interrogation of the renal arteries provides measurements of localized velocities of blood flow. This technique is available in most institutions and provides an inexpensive means for measuring vascular occlusive disease at sequential time points, both to establish the diagnosis of renal artery stenosis and to monitor its progression. After renal revascularization, Doppler studies are commonly used to detect restenosis and target vessel patency (Fig. 42.13A, B).[117,118] Its main drawbacks relate to the technical difficulties of obtaining adequate studies in obese patients and in those with complex vessels. The usefulness and reliability of Doppler ultrasound depend partly on the specific operator and the time allotted for optimal studies. These factors vary considerably between institutions. In our experience, results are most useful when positive (i.e., when a high grade renal artery velocity can be documented). Failure to identify a velocity increase (i.e., a negative duplex study) sometimes reflects an incomplete localization of the renal vessels.

Traditional primary thresholds for renal artery studies are a peak systolic velocity above 180 cm per second and/or a relative velocity above 3.5 as compared to the adjacent aortic flow.[119] Using these criteria, sensitivity and specificity with angiographic estimates of lesions exceeding 60% can surpass 90% and 96%, respectively, although not universally.[120] Increasing the threshold for peak systolic velocities reduces the rate of false positive estimates of stenosis. When main vessel velocities cannot be determined reliably, segmental waveforms within the arcuate vessels in the renal hilum can provide additional information. Damping of these waveforms, labeled as parvus and tardus, have been proposed as indirect signs of upstream vascular occlusive phenomena.[121] Recent studies challenge the use of angiographic estimates of stenosis as representing a gold standard altogether.[122] These authors argue that Doppler velocities correlate highly ($R = 0.97$) with a truer estimate of vascular occlusion, specifically stenosis, as determined by intravascular ultrasound.

Positive Doppler velocities in an artery clearly identified as the renal artery are rarely proven to be negative later. False negative studies are more common. In subjects with accessible vessels, Doppler ultrasound provides the most practical means of following vessel characteristics sequentially over time. A drawback of renal artery Doppler studies includes frequent failure to identify accessory vessels. Because the correlation between velocity and the degree of stenosis is only approximate, clinical trials such as Cardiovascular Outcomes for Renal Atherosclerotic Lesions (CORAL) have raised the diagnostic threshold for peak systolic velocity to 300 cm per second. This seems warranted, particularly when the risk of overdiagnosis of renal arterial lesions is high, as in the Stenting for Atherosclerotic Renal artery stenosis (STAR) trial, in which 18 out of 64 patients assigned to stenting were not treated because of insignificant renovascular disease at the time of angiography despite noninvasive estimates to



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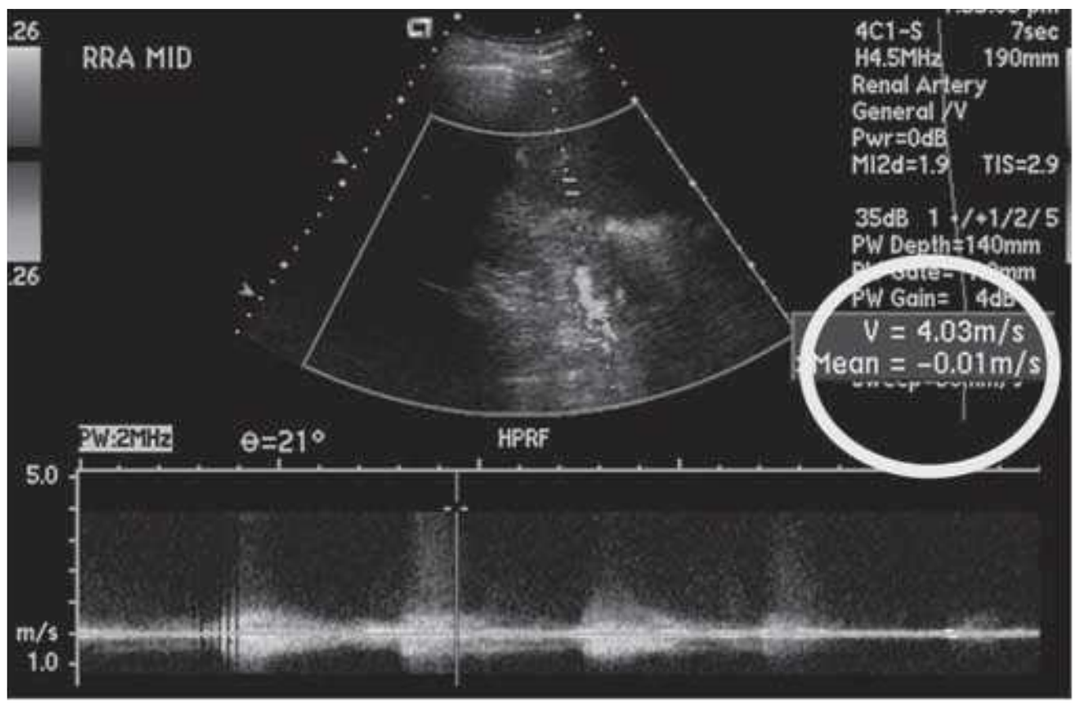

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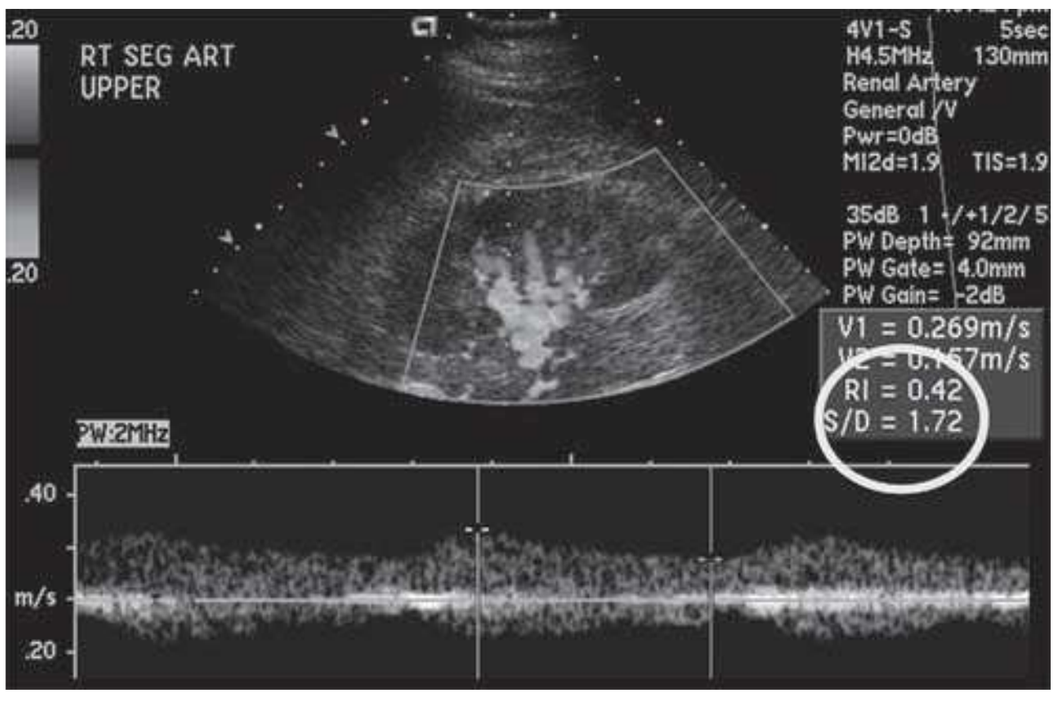


C

FIGURE 42.13 A and B: High-grade renal artery stenosis in a normal-sized kidney characterized by high peak systolic velocity (4.03 m per second) at the origin of the main vessel. **C:** Doppler velocity measured in the distal renal artery segments of the same kidney, illustrating delayed arrival with a blunted pulse wave (parvus and tardus) appearance characteristic of proximal stenosis. The sustained flow during diastole in this instance is expressed as low resistive index (RI) suggestive of favorable intrarenal hemodynamics (see text). (See Color Plate.)

the contrary.[123] A recent report suggests that a correlation with quantitative estimates of renal artery occlusion above 60% correlate best with velocities above 300 cm per second for native vessel disease and somewhat higher (395 cm per second) for in-stent restenosis.[124]

An additional feature of Doppler ultrasound allows for the characterization of small vessel flow characteristics within the kidney. The resistive index (RI) provides an estimate of the relative flow velocities in diastole and systole. In a study of 138 patients with renal artery stenosis, a RI above 80 provided a predictive tool for the identification of parenchymal renal disease that did not respond to renal revascularization (Fig. 42.14).[125] A sizable portion of the group with an elevated RI eventually progressed to renal failure. An RI less than 80 was associated with a more than 90% favorable BP response and stable or improved renal function. The authors emphasize that accurate predictive power depended on using the highest RI observed, even when present in the nonstenotic kidney. A subsequent study of 215 subjects with mean preintervention serum creatinine levels of 1.51 mg per deciliter failed to confirm the predictive value of RI measurements. Of 99 subjects with improved renal function after 1 year, 18% had an RI above 0.8 before intervention, whereas 15% of 92 subjects with no improvement had an index above 0.8 (not significant). In this series, the preintervention level of serum creatinine itself was the strongest predictor of improved renal function.[126] Additional studies indicate an overlap between levels of RI in those with and without clinical improvement after stenting. Nonetheless, low RI remained a predictor of clinical BP benefit after adjusting for age, sex, duration of hypertension, and other conditions.[127] In a series of 106 patients with ARAS undergoing revascularization, 70% of patients with an RI below 80 had a clinical benefit, as compared with only 20% of those with an RI above 80. Most clinicians agree that detecting a low RI indicates well-preserved vasculature within the kidney.[128]

Captopril Renography

Imaging the kidneys before and after the administration of an ACE inhibitor (e.g., captopril) provides a functional assessment of the change in blood flow and GFR to the kidney, related both to changes in arterial pressure and the removal of the efferent arteriolar effects of Ang II. The most commonly used radiopharmaceuticals are 99mTc, DTPA, and MAG3. The latter agent has clearance characteristics similar to Hippuran and is often taken as reflecting renal

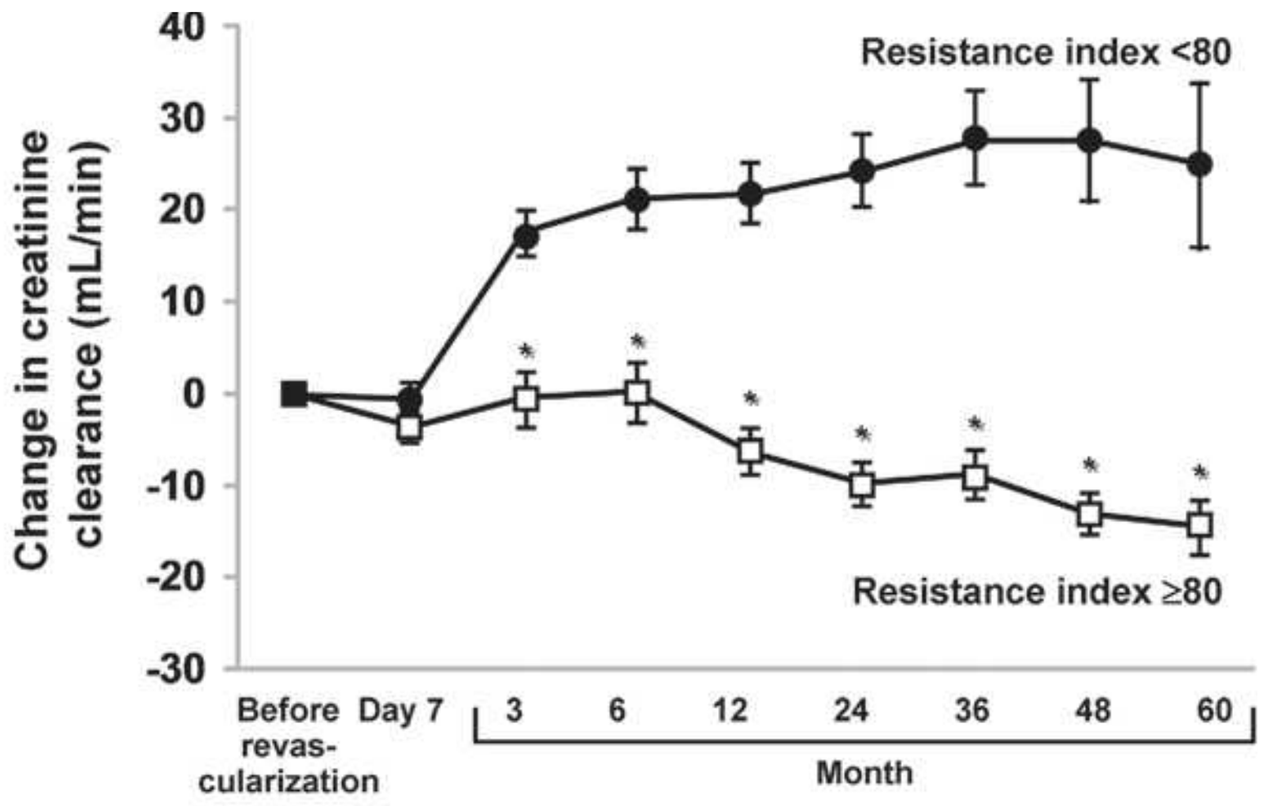


FIGURE 42.14 A resistive index (RI) obtained from 131 subjects undergoing renal revascularization. Those with relatively low resistive indexes (< 80) were more likely to have higher glomerular filtration rates (GFRs) during follow-up and were more likely to have a favorable blood pressure response than those with higher levels (> 80). These data suggest that preserved diastolic flow, which is the main determinant of RI, is a favorable sign for kidney recovery. (Adapted from Radermacher J, Chavan A, Bleck J, et al. Use of Doppler ultrasonography to predict the outcome of therapy for renal-artery stenosis. *N Engl J Med*. 2001;344:410–417, with permission.)

plasma flow. Both can be used, although specific interpretive criteria differ.[129] Both provide information regarding the size and filtration of both kidneys and the change in these characteristics after the inhibition of ACE allows inferences regarding the dependence of glomerular filtration on Ang II. Patient groups with the prevalence of renovascular disease rates between 35% and 64% of subjects suggest that sensitivity and specificity range between 65% and 96% and 62% and 100%, respectively.[129] Because of its high specificity, captopril renography can be applied to populations at low pretest probability with an expectation that a normal study will exclude significant renovascular hypertension in more than 96% of cases.[95] Some series report 100% accurate negative predictive values.[130]

These studies are less sensitive and specific for renovascular disease in the presence of renal insufficiency (usually defined as creatinine > 2.0 mg per deciliter). These performance characteristics deteriorate for patients who cannot be prepared carefully (i.e. withdrawal of diuretics and ACE inhibitors 4 to 14 days before the study).[129] It should be emphasized that renography provides functional information but no direct anatomic information (i.e., the location of renal arterial disease, the number of renal arteries, or associated aortic and/or ostial disease) (Fig. 42.15A,B). Some authors

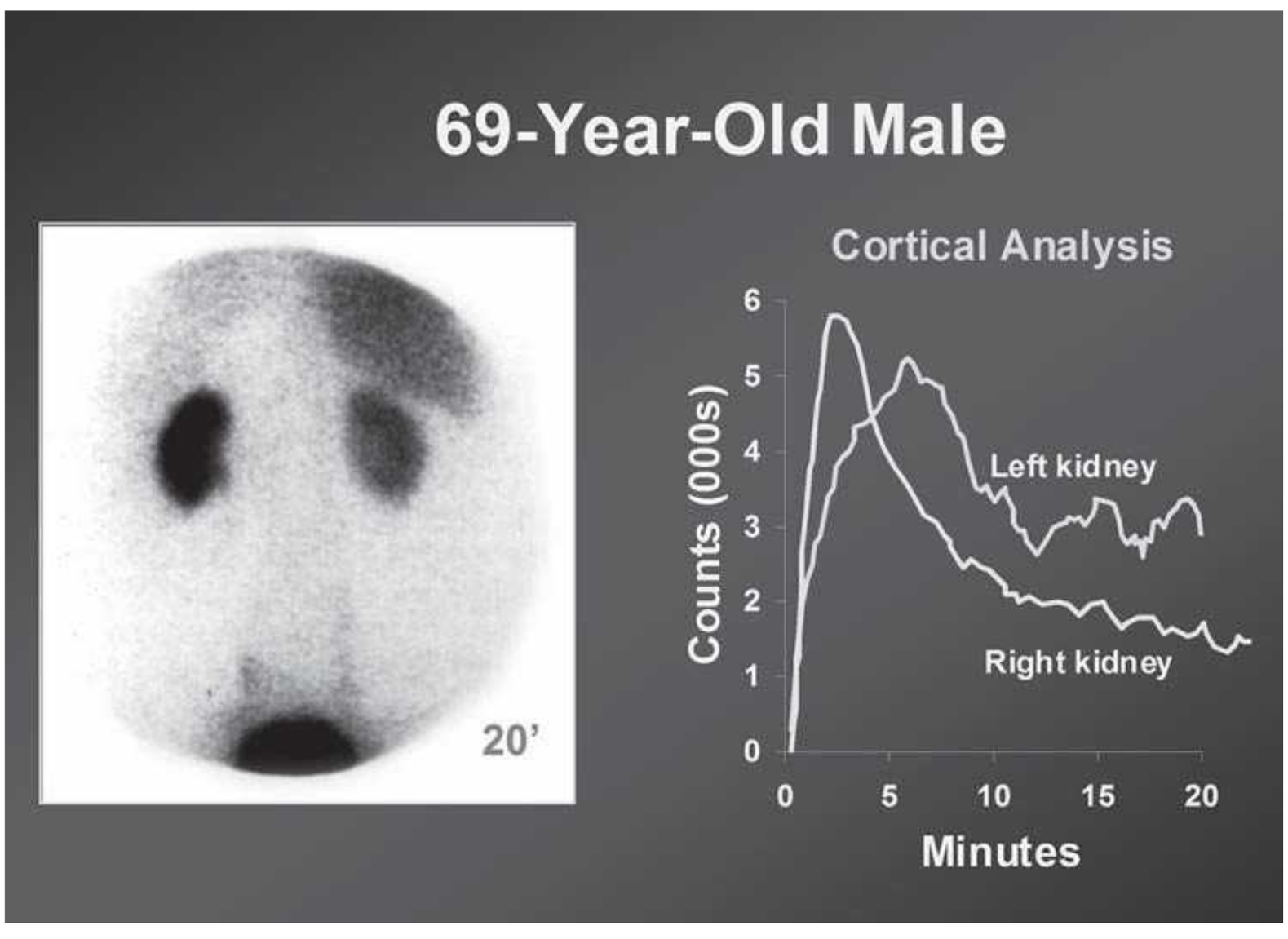


FIGURE 42.15 A radionuclide renogram illustrating asymmetry of flow and function between two kidneys in a patient with high-grade renovascular disease.

argue that renographic screening of patients using this technique is among the most cost-effective methods of identifying candidates for further diagnostic studies and superior to functional studies of the RAS. On the other hand, prospective studies of renovascular disease from the Netherlands did observe changes in the renogram during follow-up, but did not find captopril renography predictive of angiographic findings or outcomes.[39] A prospective study of 74 patients undergoing both renography and Doppler ultrasound evaluation before renal revascularization could identify only a limited predictive value of scintigraphy (sensitivity 58% and specificity of 57%) regarding BP outcomes.[131]

Under carefully controlled conditions, some authors argue that changes in renographic appearance correlate with changes in BP to be expected after revascularization. Changes in split renal function indicate that stenotic kidneys regain GFR after revascularization, sometimes with a decrement in contralateral GFR, thereby leaving overall kidney function unchanged.[132]

Computed Tomography Angiography

CT angiography using helical and/or multiple detector scanners and intravenous contrast can provide excellent images of both kidneys and the vascular tree. Resolution and reconstruction techniques render this modality capable of identifying smaller vessels, vascular lesions, and parenchymal characteristics, including malignancy and stones (Fig. 42.16A,B).[133] When used for the detection of renal artery stenosis, CT angiography agrees well with conventional arteriography (correlation 95%) and sensitivity may reach 98% and specificity may reach 94%.[134,135] Recent studies indicate that CT provides excellent accuracy regarding the evaluation of in-stent restenosis[136] and the evolving quantitative three-dimensional image analysis may improve on intra-arterial methods.[134] Although this technique offers a noninvasive examination of the vascular tree suitable for kidney donors with normal GFR, for example, it has the drawback of iodinated-contrast requirement. As a result, it is less than perfect for the evaluation of renovascular hypertension and/or ischemic nephropathy for patients with an impaired renal function. Concerns regarding toxicity associated with MRA contrast nonetheless encourage the wider use of multidetector CT as a diagnostic imaging test for patients suspected of renovascular disease. There are limitations, including the reduced visibility of vessel lumens in the presence of substantial calcium deposition. A single study comparing both CT angiography and MRA with intra-arterial studies in 402 subjects indicated substantially worse performance for the detection of lesions $>$ 50% stenosis.[137] In this particular study, CT angiography had a sensitivity of 64% and a specificity of 92%, whereas MRA had a sensitivity of 62% and a specificity of 84%. This was an unusual population with only 20% of the screened population having stenotic lesions, nearly half of which were FMD. The results of such studies reinforce the importance of careful patient selection for study and establishing in advance why imaging is being undertaken.[138]

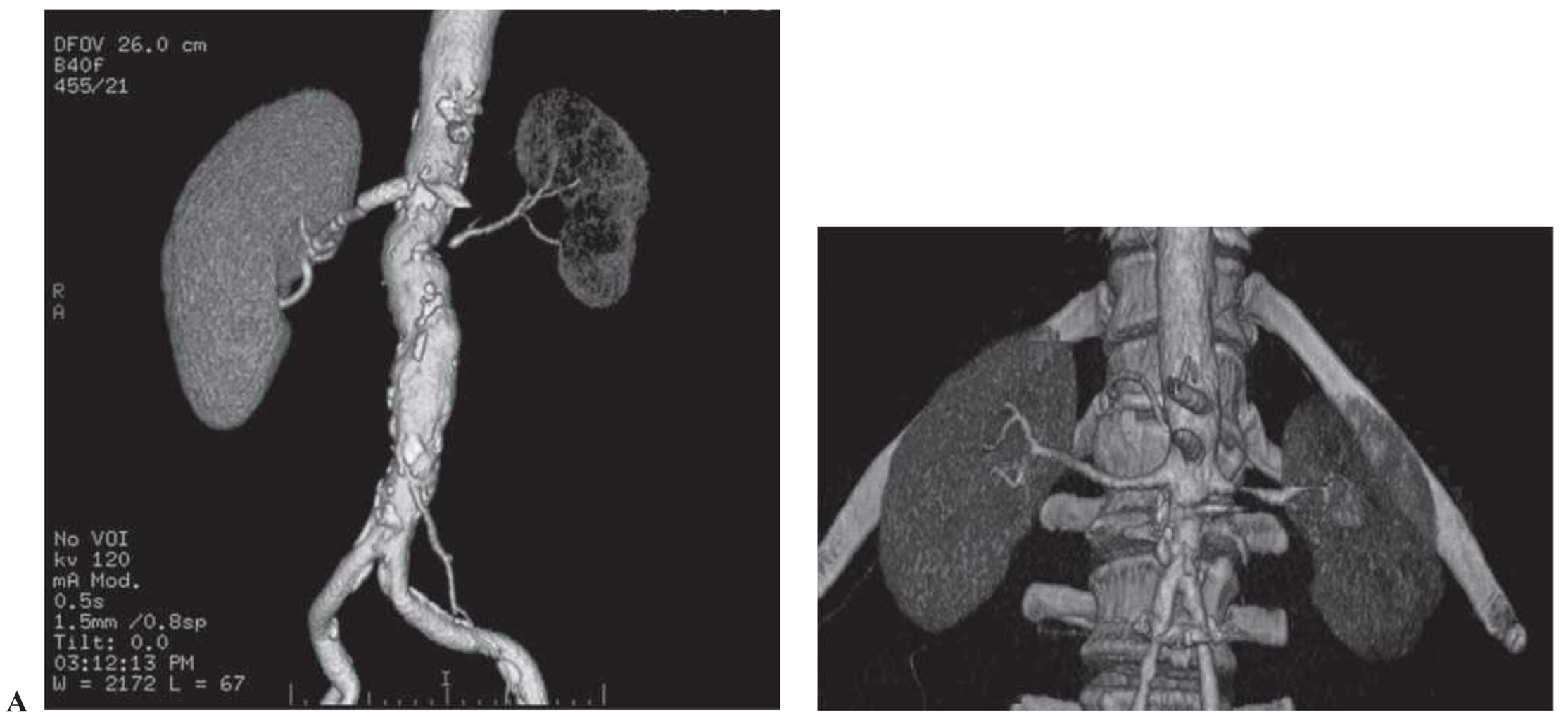


FIGURE 42.16 A and B: Computed tomography (CT) angiographic images with reconstruction can identify important elements of vascular anatomy and parenchymal function. **A:** A right renal artery stent with normal kidney parenchyma as compared with advanced renal artery stenosis affecting the left kidney, with evident loss of kidney size, perfusion, and function. **B:** A high-grade left renal artery stenotic lesion is evident with a moderate reduction in perfusion to the kidney as shown by its reduced size and the intensity of the nephrogram. (See Color Plate.)

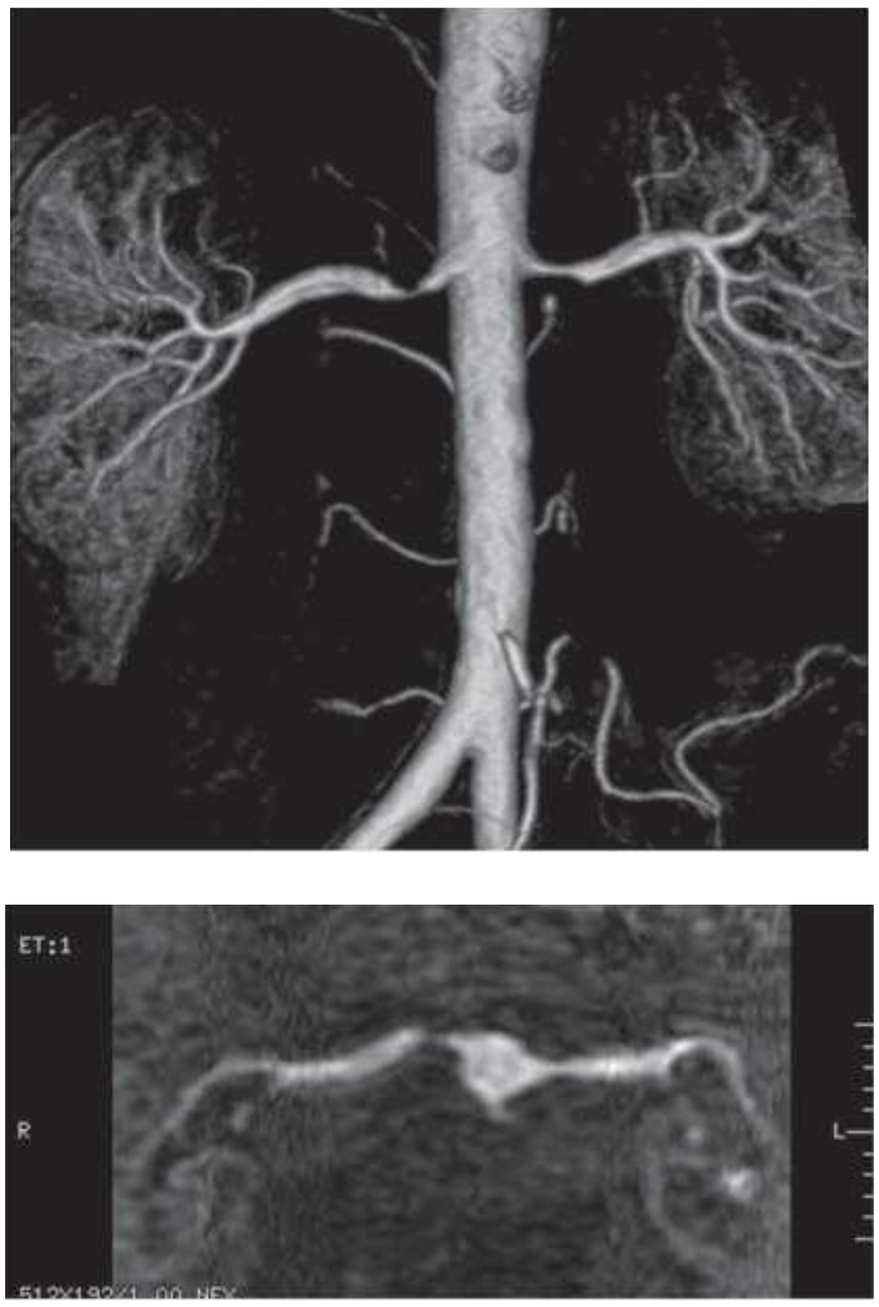
Magnetic Resonance Angiography

Gadolinium-enhanced images of the abdominal and renal vasculature have been a mainstay of evaluating renovascular disease in many institutions.[133,139] Comparative studies indicate that sensitivity ranges from 83% to 100% and specificity ranges from 92% to 97% in renal artery stenosis.[140,141] Meta-analyses of published literature[142] including 998 subjects support more than 97% sensitivity using gadolinium-enhanced imaging. The nephrogram obtained from gadolinium filtration provides an estimate of relative function and filtration, as well as parenchymal volume. The quantitative measurement of parenchymal volume determined by MRI appears to correlate closely with isotopically determined single kidney GFR in some institutions.[114] However, since 2006, concerns about the potential for gadolinium-based contrast to produce nephrogenic systemic fibrosis effectively have drastically reduced contrast-enhanced MR for patients with impaired kidney function in the United States.[143]

Technologic advances allow increasingly high-resolution vascular MRI without contrast in many patients. An example of MRA without contrast is shown in Fig. 42.17B. Drawbacks include the expense and the tendency to overestimate the severity of lesions, which in fact appear as a signal void.[144] The limits of resolution with current instrumentation make the detection of small accessory vessels limited, and quantitating fibromuscular lesions is difficult with current technology. Both of these are improving with newer generations of scanners. High spatial resolution three-dimensional contrast-enhanced MR scanners provide up to 97% sensitivity and 92% specificity for renal artery stenotic lesions.[145] Signal degradation in the presence of metallic stents renders MRA unsuitable for follow-up studies after endovascular procedures in which stents are used.

Functional Magnetic Resonance Imaging: Blood Oxygen Level–Dependent Magnetic Resonance

Blood oxygen level–dependent (BOLD) MR utilizes the properties of deoxygenated hemoglobin as a paramagnetic material that affects the local rates of magnetic polarization within tissues after a radiofrequency pulse (estimated as R2*).[146] These tools allow a real-time, noninvasive estimate of local oxygenation, which differs between the highly blood-perfused cortex and the much less perfused deeper medullary segments within the kidney.[147,148] Despite sufficient renal artery occlusion to reduce blood flow and GFR within the affected kidney by 30%, recent studies[61,64] indicate that overall cortical and medullary oxygenation in subjects treated with ACE inhibitors or ARBs maintain normal intrarenal patterns of oxygenation. In patients with more severe and/or long-standing reductions in blood flow, cortical oxygenation begins to deteriorate, reflecting overt hypoxia within the kidney associated with intrarenal fibrosis and inflammatory changes (Fig. 42.18).[66,149] Future studies hold the promise that such functional imaging may better identify those kidneys (1) at actual risk of ischemic injury and (2) that may benefit from restoring blood flow to recover function.[9]

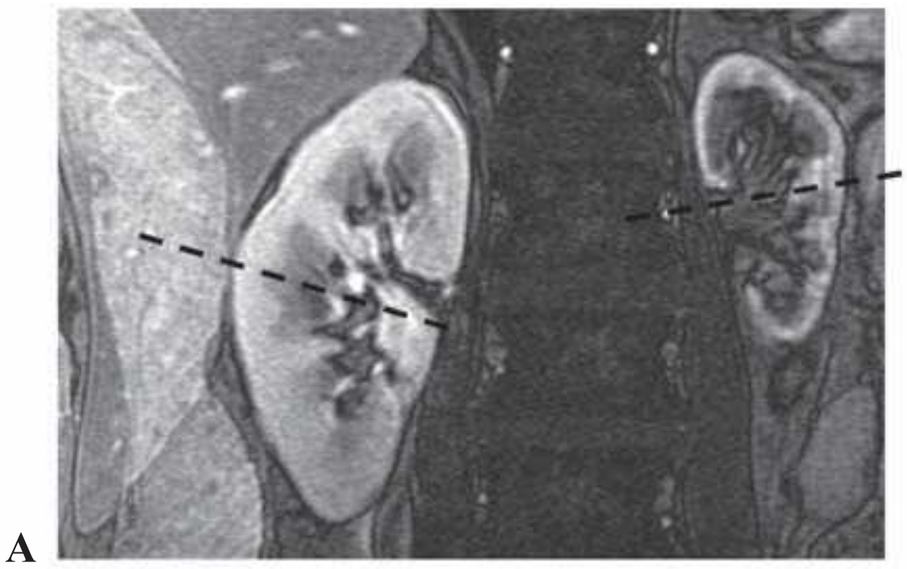


A **B**

FIGURE 42.17 Examples of magnetic resonance angiograms (MRAs) with **(A)** vascular reconstruction using gadolinium contrast. Continually improving MR imaging allows for the definition of smaller vessels with improved resolution and the definition of parenchyma without ionizing radiation. Use of gadolinium-based contrast agents has declined after concerns about nephrogenic systemic fibrosis (NSF) in patients with impaired glomerular filtration rates (GFRs). **B:** Time-of-flight imaging without contrast in the axial plane also can provide excellent definition of vascular occlusion, even at oblique angles from the aorta.

Invasive Imaging

Intra-arterial angiography currently remains the gold standard for the definition of vascular anatomy and stenotic lesions in the kidney. Often, it is completed at the time of a planned intervention, such as endovascular angioplasty and/or stenting. What is the current role of including angiography of the renal arteries during imaging of other vascular beds, such as drive-by angiography during coronary artery imaging? Several studies confirm that the prevalence of renal artery lesions exceeding a 50% lumen occlusion in patients with hypertension and coronary artery disease is high, usually between 18% and 24%.[150] Some individuals (7% to 10%) will have high-grade stenoses above a 70% occlusion



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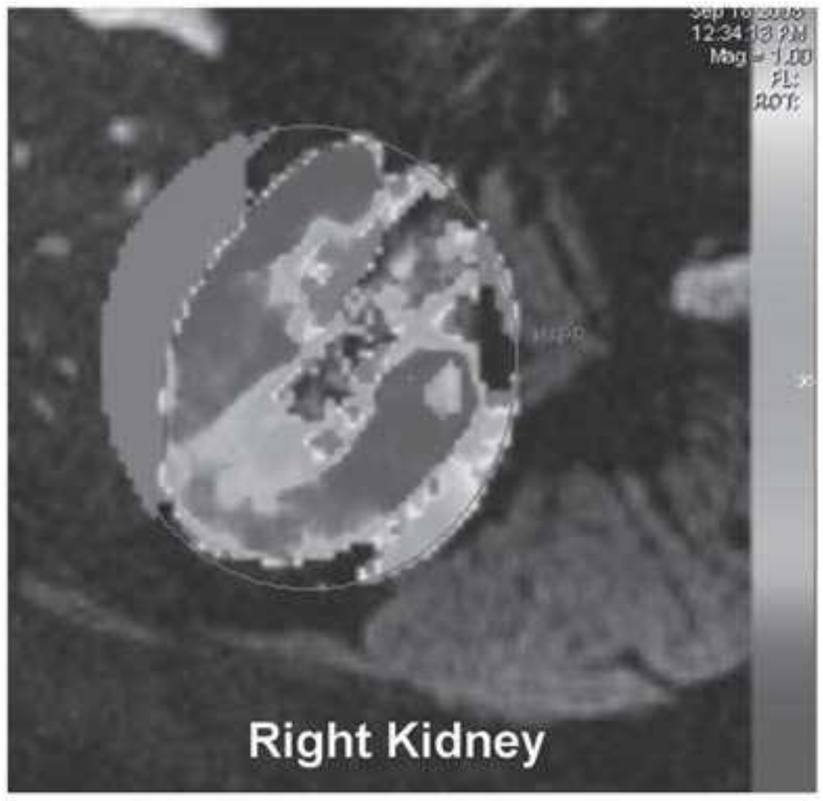

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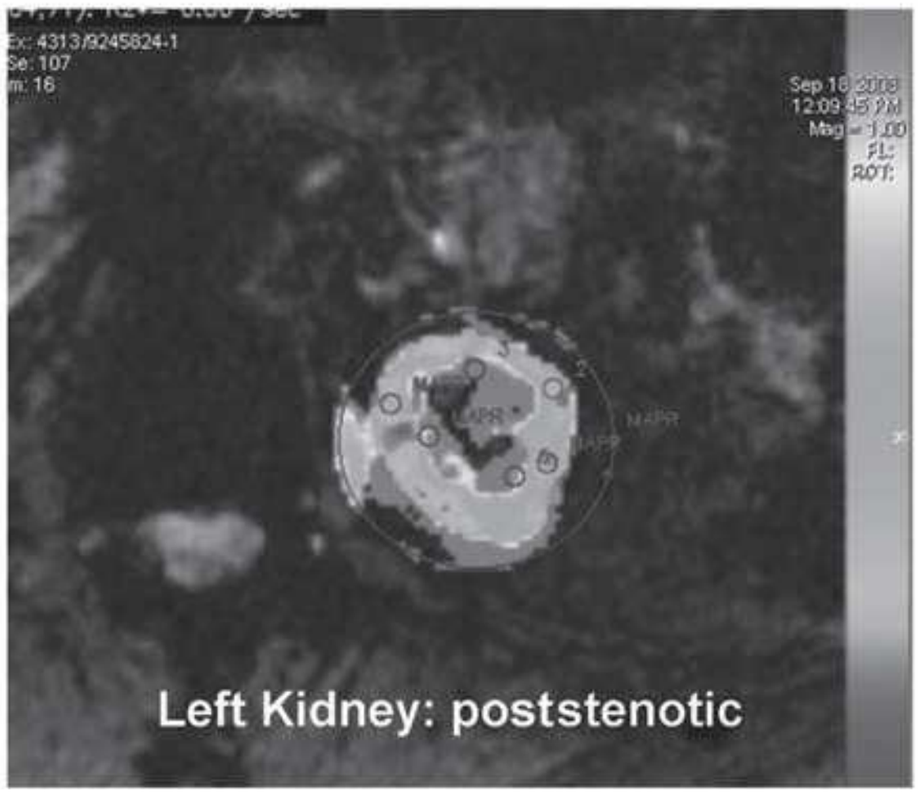

C

FIGURE 42.18 A, B, and C: Functional magnetic resonance (MR) using blood oxygen level–dependent (BOLD) imaging provides a measurement of tissue deoxyhemoglobin levels (expressed as R2* (sec^{-1})) illustrated in axial sections of a normal kidney **(B)** and the severely stenotic **(C)** kidney shown here. Maps of R2* in the normal kidney demonstrate abundant oxygenation in the kidney cortex (low R2* levels associated with blue-green regions) with a gradient to hypoxic regions in the medulla (higher R2* levels associated with orange-red). When blood flow is reduced sufficiently, cortical flow falls and the tissue becomes overtly hypoxic in the cortex and the zone of medullary hypoxia widens **(C)**. (Adapted from Reference (66) with permission.) BOLD MR represents one potential tool to identify vascular insufficiency leading to tissue hypoxia and may allow improved selection of patients for renal revascularization. (See Color Plate.)

and some will be bilateral. Accepting the fact that an arterial puncture and catheterization of the aorta and coronary vessels produces some risk, the added risk from including an aortography of the renal vessels appears to be small/almost negligible. Follow-up studies[12,151] of individuals with identified incidental renal artery lesions suggest that the presence of these lesions does provide an additive predictive risk for mortality. No data to this point suggest that combining screening angiography with renal revascularization changes that risk. Hence, endovascular procedures for such lesions should be confined to individuals with strong indications for renal revascularization, as even the most ardent advocates of catheter-based intervention have suggested.[150] Remarkably, studies of patients with identified ARAS during the preoperative screening indicate no increased risk for acute kidney injury (AKI) during or after cardiovascular surgery as compared to those without ARAS.[152]

Contrast toxicity remains an issue with conventional iodinated agents.[153] Intravascular ultrasound procedures have been undertaken using papaverine to evaluate flow reserve beyond stenotic lesions.[154] Various reports indicate that either a reduction or preserved flow reserve may identify a poststenotic kidney that may improve function after successful revascularization.[154,155] Previous studies of pressure gradients measured across stenotic lesions have failed to predict the clinical response to renal revascularization. Measurements using currently available low profile wire probes do, however, indicate a relationship between pressure gradients and the activation of the RAS.[43] Outcomes of patients with translesional pressure gradients measured after vasodilation suggest that a measurement of hyperemic systolic gradient above 21 mm Hg most accurately predicts high-grade stenosis (average 78% by intravascular ultrasound) and a beneficial response of BP after stenting.[156] The latter observation and the increasing reliability of technical measurements underscore the value of measuring gradients to establish a hemodynamic role for vascular lesions of marginal severity.

Management of Renal Artery Stenosis and Ischemic Nephropathy

Overview

Considering the variety of potential treatments and major differences in individual patient comorbid diseases and risks, clinicians need to formulate a clear set of therapeutic goals for each patient. Each treatment, ranging from medical therapy alone to surgical revascularization, carries both benefits and risks. The clinician's task is to weigh the role of each of these within the context of each patient's response and likely long-term outcome. Rarely is it obvious how best to proceed. In most cases, the long-term management of the patient with renovascular disease requires an integrated pharmacologic management of BP, treating cardiovascular risk factors, and the careful timing of renal revascularization. The objective of this section is to provide a framework by which to plan a balanced approach to the patient with unilateral or bilateral renal artery stenosis. It should be emphasized that the consideration of renal artery disease takes place in the broad context of managing other cardiovascular risk factors, including withdrawing tobacco use, reducing cholesterol levels, and treating diabetes and obesity.

Medical Therapy of Renovascular Disease

The overall goals of therapy are summarized in Table 42.5. Foremost among these is the statement by the Joint National Committee (JNC) of the National High Blood Pressure Education Program (NHBPEP): "The goal of treating patients with hypertension is to prevent morbidity and mortality associated with high blood pressure."[157] This task may include the effort to simplify or potentially eliminate long-term antihypertensive drug therapy. Several scenarios should be considered in the care of patients with renal artery stenosis and hypertension: (1) true renovascular hypertension, in which atherosclerotic or fibrous renal artery disease is the sole cause of the hypertension; (2) pure essential hypertension, in which atherosclerotic or fibrous renal artery disease is present but does not contribute to the hypertension at all; (3) essential hypertension with superimposed renal artery stenosis producing a renovascular contribution to the underlying essential hypertension; and (4) the hypertension of renal parenchymal disease, that is, chronic renal insufficiency with superimposed renal artery stenosis contributing to the hypertension (Fig. 42.19). Accordingly, the medical management of patients with presumed renovascular hypertension and renovascular disease is primarily an effort to control the BP. As will be summarized in the following, rarely does any specific revascularization procedure lead to the withdrawal of all antihypertensive therapy or to a true cure for hypertension. Hence, medical therapy at some level is a primary component of treatment for nearly all patients with renovascular disease. A second corollary is that a trial of medical therapy often becomes a practical test of the need for expanded diagnostic studies. Regarding this, some of the major criteria recommended by an American Heart Association writing group are summarized in Table 42.7.

A further goal is to prevent a loss of kidney function related to impaired renal blood flow. In some instances, renal revascularization is undertaken to allow for the improved management of salt and water balance in the process of managing patients with congestive cardiac failure. This may allow safer use of diuretic agents and ACE inhibitor/ARB classes of medication in patients with critical renal artery lesions to the entire renal mass. Because prospective, randomized trial information is limited and ambiguous for renovascular disease, each patient must be considered individually. What cannot be assumed is that renal revascularization prolongs

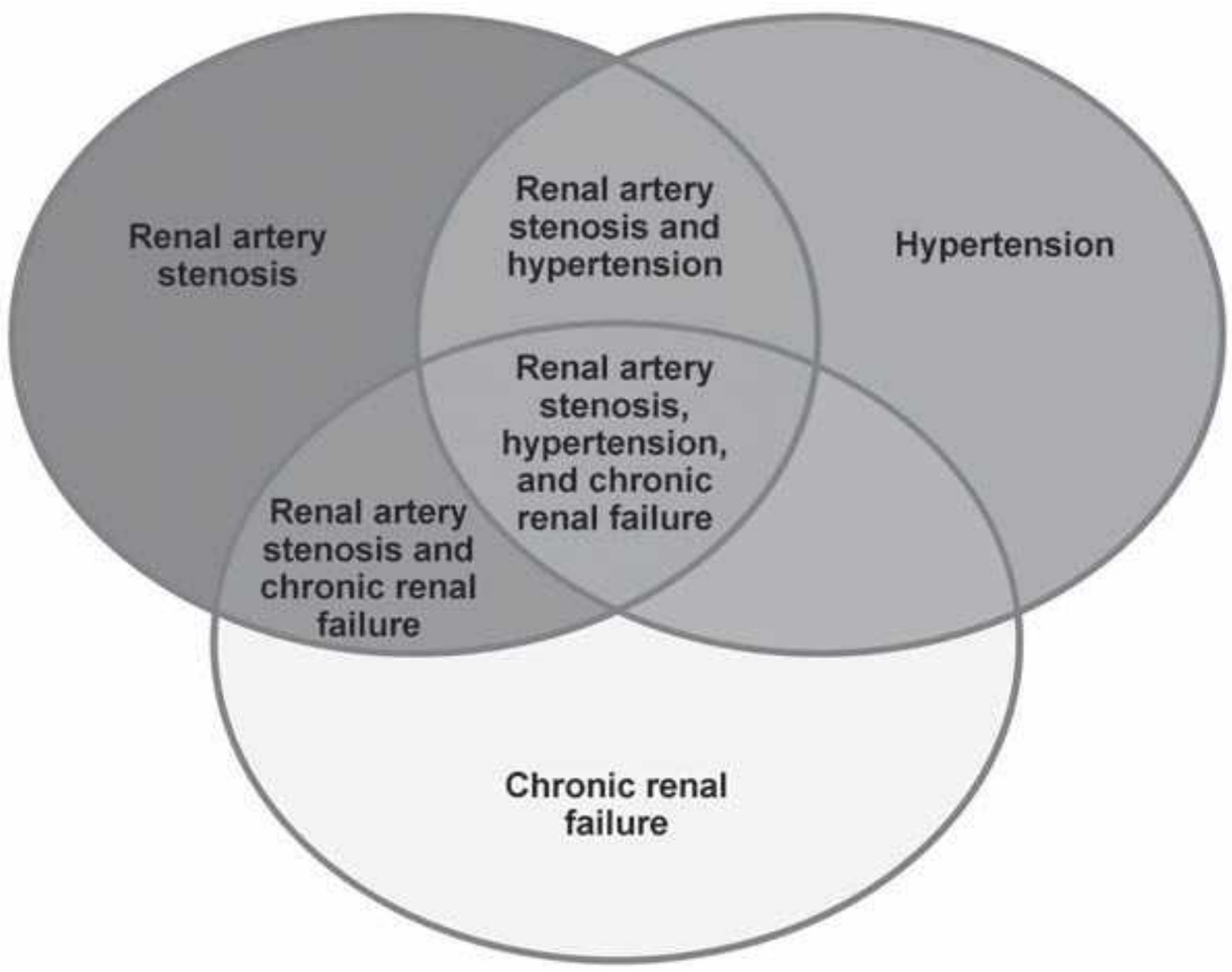

FIGURE 42.19 A Venn diagram illustrating the relationships between renal artery stenosis, hypertension, and chronic renal failure. The challenge to clinicians is to evaluate the relationship between these circles for an individual patient. Because all three are common and increase with age, it is no surprise that identifying patients with truly causal interconnections is difficult. In many respects, elucidating the role of renal artery stenosis remains a clinical judgment based on time, duration, and severity, in addition to the practical difficulties in managing both blood pressure and kidney function. (From Safian RD, Textor SC. Medical progress: renal artery stenosis. *N Engl J Med*. 2001;344:431–442, with permission.)

42.7 Classification and Therapies for Renal Artery Stenosis

A. Functional Classification for Atherosclerotic Renal Artery Stenosis

Grade I: Renal artery stenosis present, but no clinical manifestations (normal blood pressure and normal renal function)
Grade II: Renal artery stenosis present, but patients have medically controlled hypertension and normal renal function
Grade III: Renal artery stenosis present and patients have evidence of abnormal renal function, medically refractory hypertension, or evidence of volume overload

B. Factors Favoring Specific Therapies for Renal Artery Stenosis

Factors favoring **medical therapy and revascularization** for renal artery stenosis
- Progressive decline in GFR during treatment of hypertension
- Failure to achieve adequate blood pressure control with optimal medical therapy
- Rapid or recurrent decline in the GFR in association with a reduction in systemic pressure
- Decline in GFR during therapy with ACE inhibitors or ARBs
- Recurrent congestive heart failure in a patient in whom the adequacy of left ventricular function does not explain the cause

Factors favoring **medical therapy and surveillance** of renal artery disease
- Controlled blood pressure with stable renal function
- Stable renal artery stenosis without progression on surveillance studies (e.g., serial duplex ultrasound)
- Advanced age and/or limited life expectancy
- Extensive comorbidities that make revascularization too risky
- High risk or previous experience with atheroembolic disease
- Other concomitant renal parenchymal diseases that cause progressive renal dysfunction (e.g., diabetic nephropathy)

GFR, glomerular filtration rate; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker. Adapted from the writing group for Atherosclerotic Peripheral Vascular Disease Symposium.[5,234]

life or regularly prevents ESRD.[11,158] The Centers for Medicare and Medicaid Services (CMS) commissioned a formal review of strategies for managing atherosclerotic renal artery disease. The results were published in 2006 and concluded that "data were insufficient to conclude substantial benefit regarding blood pressure control, kidney function or mortality for atherosclerotic renal artery disease" in favor of either specific management strategy.[159] Since that review, several prospective randomized trials have been reported or remain in progress, albeit with limitations (see the following). None have provided compelling evidence supporting a major benefit for renal revascularization for all patients, although a subgroup of patients in each of these studies has benefited. As noted previously, the burden of atherosclerotic disease associated with renal artery stenosis is often widespread and the causes of death include a broad array of cardiovascular events. Both endovascular and surgical intervention in the aorta and renal vasculatures carry substantial risks that may accelerate morbidity and the loss of renal function. As a result, these measures must be considered within the entire context of patient management over time.

There is little question that the more severe the hypertension, the greater the likelihood that it has a renovascular component and the more one has to gain by successful revascularization to facilitate BP control. Furthermore, younger patients with fibromuscular renal artery diseases have less risk of procedural complications and may respond well to revascularization with either angioplasty or surgery. However, the results of renal revascularization in patients with atherosclerotic renal artery disease are less favorable, because many of these patients are older and almost certainly have coexisting primary or essential hypertension.[56]

Unilateral Versus Bilateral Renal Artery Stenosis

Consideration of these disorders differs in some respects. In this context, bilateral refers to the circumstances when the entire functional renal mass is affected by vascular occlusion. This may be associated either with bilateral stenoses or stenosis to a solitary functioning kidney. Not only are the putative mechanisms related to BP and volume control different in the presence of a nonstenosed, functioning contralateral kidney with unilateral disease (as outlined under pathophysiology, previously), but also the potential hazards of intervention and/or medical therapy differ. Patient survival is reduced in patients with bilateral disease or stenosis to a solitary functioning kidney. Progressive arterial disease in this group also poses the most immediate hazard of declining renal function. Patient survival depends on the extent of vascular involvement[160] regardless of whether renal revascularization is undertaken.

Management of Unilateral Renal Artery Stenosis. Most patients with atherosclerotic renal artery disease have preexisting hypertension. As a result, most are exposed to antihypertensive therapy before the lesion has been identified and may be treated successfully with only moderate medication use.[34,161] Such patients commonly come to clinical attention when recognizable clinical progression occurs or when imaging is undertaken for other reasons. Occasionally, clinical decision making is influenced strongly by concerns about the hazards of medical therapy and failing to achieve both BP control and failing to maintain adequate blood flow soon enough. Examination of the results of medical therapy alone is important before evaluating the role of vascular reconstruction or dilation.

Since the introduction of agents blocking the RAS, most patients (86% to 92%) with unilateral renal artery disease can achieve blood pressure levels < 140/90 mm Hg with medical regimens based on these agents. Recent treatment trials[162–165] for ARAS confirm that target BP levels often can be achieved with optimized medical therapy with or without renal revascularization. It must be understood that widespread application of these agents to patients with many forms of cardiovascular disease already ensures that many subcritical cases of renovascular disease are treated medically unbeknownst to the clinician. Several clinical reviews, including large administrative databases, suggest that treating renal artery stenosis with ACE inhibitors or ARBs is associated with a more favorable patient outcome than that observed with other antihypertensive regimens.[89,166,167] Comparing 1,857 (53%) patients given either ACE inhibitors or ARBs out of 3,570 patients treated for renovascular disease, Hackam and colleagues[166] reported lower rates of overall mortality, congestive heart failure, and chronic dialysis in the group treated with RAAS blockers. There were, however, slightly higher rates of hospitalization for acute renal failure (1.2 versus 0.6 events per 100 patient years).[166] An example of a patient with complex aortic disease and high-grade unilateral ARAS managed for many years with ACE inhibitor therapy and diuretics is illustrated in Fig. 42.20A.

Do the risks of treating unidentified renal artery stenoses with antihypertensive drug therapy pose a hazard to patients? This issue is at the crux of many clinical debates

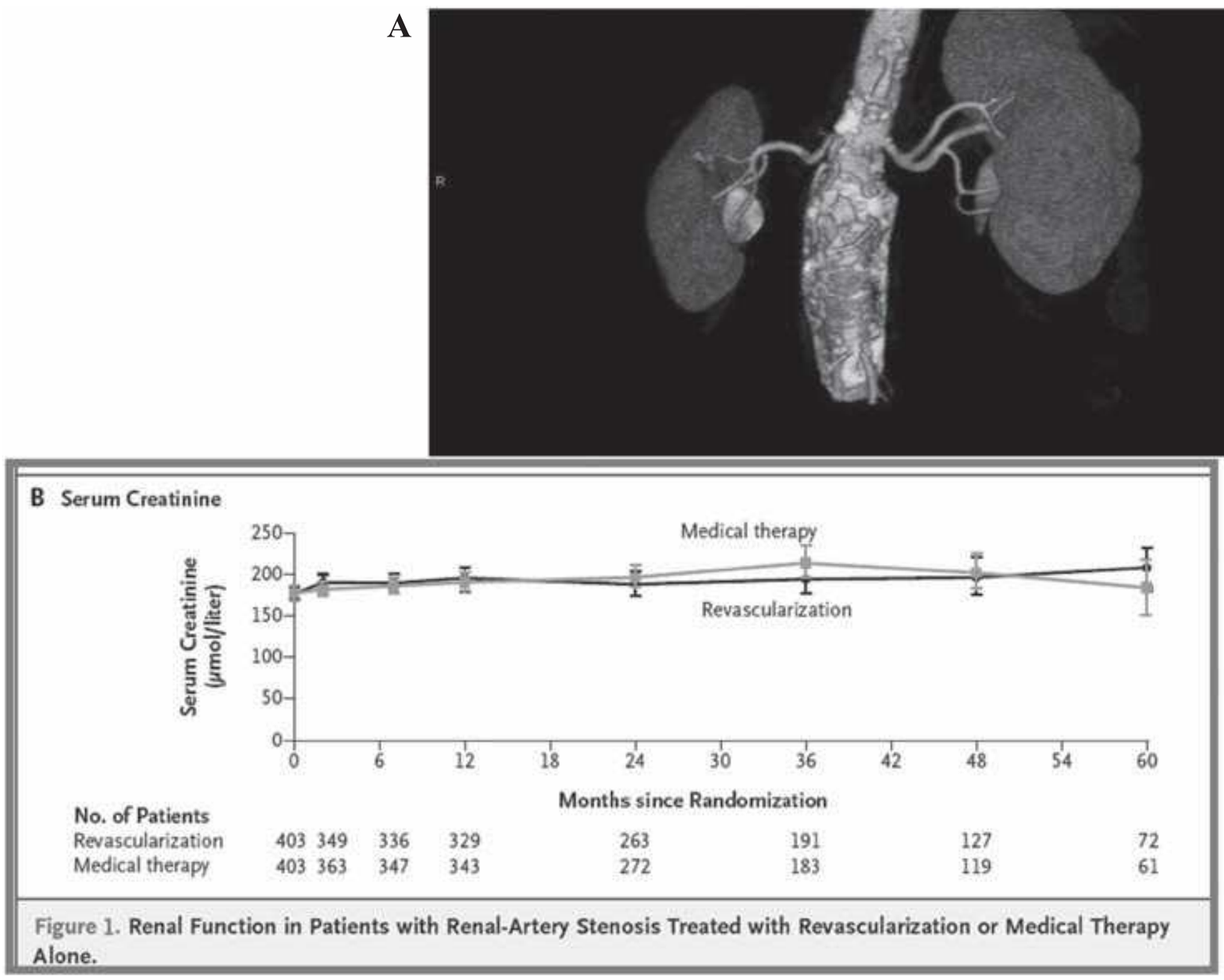

Figure 1. **Renal Function in Patients with Renal-Artery Stenosis Treated with Revascularization or Medical Therapy Alone.**

FIGURE 42.20 A and B: A computed tomography (CT) angiogram **(A)** obtained from a patient with high-grade renal artery stenosis leading to loss of parenchymal volume on the right kidney, but evidently normal blood flow and function on the left kidney. The aortic anatomy is complicated by an aneurysm formation and intramural thrombus that increases the hazards of endovascular stenting. This individual with unilateral renal arterial disease has been managed for years with stable renal function and blood pressure control with only modest antihypertensive drug requirements. Panel **B** reproduces the levels of serum creatinine for more than 400 patients in each group assigned to medical therapy with or without renal artery stenting in the ASTRAL trial. Patients assigned to this trial had moderate renal dysfunction that remained remarkably stable for the group as a whole during the follow-up approaching 5 years. Rates of progression to a renal endpoint were 16% to 20% in both groups. (From The ASTRAL Investigators. Revascularization versus medical therapy for renal-artery stenosis. *N Engl J Med.* 2009;361:1953–1962, with permission.)

(and prospective, randomized clinical trials) regarding the management of patients with renovascular hypertension. Early studies with experimental clip hypertension emphasized renal fibrosis and scarring, which occur in the stenotic kidney in animals treated with ACE inhibitors. It is well recognized that the removal of the efferent arteriolar effects of Ang II pose the possibility of a loss of glomerular filtration in a kidney with reduced renal perfusion. Experimental studies in 2K-1C rats indicate that the loss of kidney function is sometimes irreversible, although survival is improved in ACE inhibitor–treated animals as compared to minoxidil-treated ones.[168] The unique role of ACE inhibitors and ARBs must be understood in this regard. Any drug capable of reducing systemic arterial pressure has the potential to lower renal pressures beyond a critical stenosis. As a result, successful antihypertensive therapy in renovascular disease has the theoretical result of reducing blood flow to the poststenotic kidney sufficient to induce vascular thrombosis. The unique feature of agents that block the RAS is the reduction of efferent arteriolar resistance sufficient to lower transcapillary filtration pressures, despite preserved blood flow to the glomerulus. This property is central to the benefits of this class of agents in hyperfiltration states thought to accelerate renal damage in other settings. In the presence of renovascular disease, the fall in glomerular filtration beyond a stenotic lesion can be observed despite relatively preserved plasma flows. The fall in GFR heralds an approaching degree of critical vascular compromise before blood flow itself is reduced.[169] Studies[170] in renovascular hypertensive animals confirm that despite a reduction in filtration, renal structural integrity can be preserved and recovered after the removal of the clip and/or the removal of the ACE inhibitor. Hence, it is unlikely that ACE inhibitors or ARBs themselves pose a unique hazard beyond that attributable to a reduction in renal blood flow.

Recent studies of patients with ARAS treated with ACE or ARBs indicate that even with up to 30% reductions of renal blood flow beyond ARAS, actual tissue oxygenation can be remarkably well preserved. Gloviczki and colleagues[64] studied subjects under standardized conditions to measure regional blood flows within both stenotic and contralateral kidneys, as well as tissue oxygenation estimated by BOLD MR. Despite demonstrating high-grade stenosis with lateralizing renin activity and reduced blood flow in unilateral ARAS, both cortical and medullary levels of tissue oxygenation (as reflected by levels of deoxyhemoglobin) did not differ from either the contralateral kidney or from patients with essential hypertension under the same conditions. These authors argued that many such patients do not suffer true kidney hypoxia any more than essential hypertension or normal kidneys. Such observations support the relative safety of medical therapy and may partly explain the stability of kidney function during medical therapy over many years, as reported in recent trials such as Angioplasty and Stenting for Renal Atherosclerotic Lesions (ASTRAL) (Fig. 42.20B).[42] It should be emphasized that the same study format indicates that such an adaptation for oxygen delivery in a poststenotic kidney has limits. When the degree of vascular occlusion becomes sufficiently severe, cortical perfusion falls and overt hypoxia can appear in both the cortex and the medulla.[171]

It is important to recognize that the contralateral kidney supports total glomerular filtration despite reduced filtration in the stenotic kidney. Hence, changes in overall GFR may be small or undetectable. This may be interpreted in several ways. Some authors argue in favor of using split renal function measurements, such as radionuclide renal scans, to detect a loss of individual kidney function as a means of timing revascularization.[91] Depending on the circumstances, a loss of function in one kidney may be an acceptable price if one can assure the patient that the remaining kidney has adequate function and blood supply. The fall in GFR from a loss of one kidney represents a loss of GFR analogous to that of donating a kidney for renal transplantation or nephrectomy for malignancy. In such instances, the long-term hazard to the remaining kidney is small, although not negligible.[172,173] As the age and comorbid burden of the population at risk rises, the loss of one kidney may pose little additional hazard if overall glomerular filtration is adequate. The experience with ACE inhibition in trials of congestive cardiac failure is reassuring in this regard. Thousands of patients with marginal arterial pressures and clinical heart failure have been treated over many years with a variety of ACE inhibitors, and more recently, ARBs. These patients are at a high risk for undetected renal artery lesions as part of the atherosclerotic burden associated with coronary disease. Although a minor change in creatinine is observed in 8% to 10% of these individuals, a rise sufficient to lead to withdrawal of these agents under trial monitoring conditions occurs in only 1% to 2%.[169,174] Data from patients with a high risk of cardiovascular disease treated with ramipril included patients with creatinine levels up to 2.3 mg per deciliter. Those with creatinine levels between 1.4 and 2.3 mg per deciliter were at a higher risk for cardiovascular mortality and had a major survival benefit from ACE inhibition. A close follow-up of kidney function indicated that the withdrawal of ACE inhibition due to a deterioration of renal function was less than 5% and no greater than placebo.[175]

Should patients be evaluated for ARAS before major surgery, such as coronary artery bypass grafting (CABG)? Some raise concerns that these patients may be at a higher risk for AKI. A report[176] of 798 patients undergoing CABG surgery after having a diagnostic aortogram to detect renal artery stenosis indicated no increase in AKI related to the presence of ARAS.

Progressive Renal Artery Stenosis in Medically Treated Patients. The potential for vascular occlusive disease to worsen is central to the management of patients with renovascular disease. It may be argued that failure to revascularize the kidneys exposes the individual to the hazard of undetected, progressive occlusion, potentially leading to

total blood flow and/or an irreversible loss of renal function. A firm understanding of the data regarding progressive atherosclerotic disease of the kidney is important for planning both endovascular and surgical revascularization.

Atherosclerosis is a variably progressive disorder. Managing disorders of the carotid, coronary, aortic, and peripheral vasculatures all recognize the potential for progression, which occurs at widely different rates between individuals. Medical therapy of all of these disorders should incorporate measures aimed at an intensive reduction of risk factors, of which smoking cessation, BP control, and correction of dyslipidemias are paramount. Treatment of these risk factors reduces mortality rates related to cardiovascular disease.[177]

How does progressive renal artery occlusive disease affect the management of renovascular hypertension? Moderate anatomic progression alone does not reliably predict functional changes in terms of deteriorating BP control or renal function. In serial reports[41] of Duplex ultrasound studies from Seattle, a decrement in measured renal size by 1 cm (renal atrophy) developed in 5.5% of those with normal initial vessels and in 20.8% of those with baseline stenosis > 60% during a follow-up interval of 33 months. Changes in serum creatinine were infrequent but did occur in a subset of patients, particularly those with bilateral renal artery stenosis. These are in general agreement with early studies during medical therapy of renovascular hypertension in which 35% had a detectable fall in measured renal length, but only 8 out of 41 (19%) had a significant rise in creatinine levels during a follow-up of 33 months. The follow-up of the medical treatment arms during short-term studies fails to show major changes in kidney function, although the occasional loss of renal perfusion by radionuclide scan is observed.[39]

How often does the management of renal artery stenosis without revascularization lead to clinical progression, either in terms of refractory hypertension or advancing renal insufficiency? A follow-up of patients with incidentally identified renal artery stenosis is helpful in this regard. Review of peripheral aortograms identified 69 patients with high-grade renal arterial stenoses (> 70%) who were followed without revascularization for more than 6 months. Their long-term follow-up identified generally satisfactory BP control, although some required more intensive antihypertensive therapy during an average of 36 months follow-up.[178] Four patients eventually underwent renal revascularization for refractory hypertension and/or renal dysfunction. Five developed ESRD, of which only one was thought to be related to RAS directly. Overall, serum creatinine levels rose from 1.4 mg per deciliter to 2.0 mg per deciliter. These data indicate that many such patients can be managed without revascularization for years and that clinical progression leading to urgent revascularization develops in 10% to 14% of such individuals. Expansion of this data set to 160 individuals allowed for a comparison of different antihypertensive regimens. The rates of progression did not appear related to the introduction of ACE inhibitors, although the level of BP control improved in later years.[179] These observations are supported by a report of 126 patients with incidental renal artery stenosis compared to 397 patients matched for age. Measured serum creatinine levels were higher, and calculated (Cockcroft-Gault) GFR was lower in patients with RAS who were followed for 8 to 10 years. However, none of the patients progressed to ESRD.[180]

What Can We Learn From Prospective Randomized Trials of Medical Therapy in Renovascular Disease? As noted previously, advances in both medical therapy for hypertension and revascularization procedures have shifted the balance for therapy enormously. No fewer than six prospective, randomized trials conducted in several countries have attempted to address the relative advantages of medical therapy as compared to revascularization for ARAS, either with endovascular or surgical methods. Most of these trials have been small with short-duration follow-ups and inconclusive. Results of two recent trials have appeared in the general medical literature, including the largest single trial of 806 patients from the United Kingdom in ASTRAL.[42,123] The major results of these trials are summarized in Table 42.8. Although the number of antihypertensive drugs were slightly lower in revascularized groups, BP levels often did not differ. Kidney function was unchanged overall in these trials. Procedural complications, although not common, were occasionally severe. Overall, these data fail to demonstrate clinically important benefits to renal revascularization in the patients enrolled during follow-ups between 2 and 5 years. The U.S. trial addressing overall cardiovascular outcomes, CORAL, which completed enrollment at the beginning of 2010, is the largest and is the most stringent regarding entry criteria. Data from CORAL are not expected until late in 2012 at the earliest.

Negative conclusions from these trials have been unsatisfying and widely criticized.[1,2,181] Recruitment for randomized controlled trials (RCTs) has been difficult, in part due to intense preconceptions of practicing clinicians. Many of the patients enrolled in these trials were found to have mild—sometimes trivial—vascular disease, clearly reducing the power of the study. In the STAR trial, for example, nearly a quarter of patients assigned to renal revascularization did not have the procedure performed because of a lack of clinically evident vascular stenosis at the time of angiography.[123] It is clear that many patients with high-grade disease, sometimes to a solitary functioning kidney, were not included in these trials out of a conviction on the part of the clinician that revascularization should or should not be considered. The largest trial to date, ASTRAL, excluded those "that required surgery" or were "likely to need" revascularization within 6 months. No contemporary registry of exclusions is available, nor is there any defined criteria for requiring intervention. Some authors have argued that atherosclerotic renal artery stenosis, in particular, is confounded so heavily with other individualized patient factors and comorbid disease risks that it cannot be addressed responsibly in a prospective trial format.[182]

Some observations from these trials warrant comment, however. The size (Table 42.8) and power estimates on

42.8 Characteristics of 1,208 Patients Included in Prospective, Randomized Trials for Atherosclerotic Renal Artery Stenosis Subjected to Meta-Analysis[235]

	EMMA	SNRASCG	DRASTIC	ASTRAL	STAR	NITER
Total Number Pts.	49	55	106	806	138	43
Inclusion	HTN/ unilateral	Resistant HTN/CKD	Resistant HTN	HTN/CKD/ Uncertainty	CKD	Resistant HTN/CKD
Age (mean)	59.4	61.1	59.9	70.5	66.5	72
F/U mos (mean)	6	12	12	33.6	24	43
Initial creatinine	1.2	1.8	1.3	2.0	1.7	1.7
Bilateral (%)	0	51	23	53	48	52
BP change (SBP/DBP)						
Med + PTRA	−12/−10	−15/−10	−19/−11	−6/−3	−10/−7	−5/−3
Med only	−8/−10	−6/−1	−17/−1	−8/−4	−9/−4	−6/−7

EMMA, Essai Multicentrique Medicaments vs Angioplastie, France 1998; SNRASCG: Scottish and Newcastle Renal Artery Stenosis Collaborative Group, UK, 1998; DRASTIC: Dutch Renal Artery Stenosis Intervention Cooperative study group, Netherlands, 2000; ASTRAL: Angioplasty and Stenting for Renal Artery Lesions, UK, Australia, New Zealand, 2009; STAR: Stenting for Atherosclerotic Renal artery lesions, Netherlands, 2009; NITER: Italy, presented in abstract form; HTN, hypertension; CKD, chronic kidney disease; Pts, patients; F/U, follow-up; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PTRA, percutaneous transluminal renal angioplasty.
These authors concluded that, taken together, blood pressure reductions with modest reductions in medication requirements were evident in revascularized patients. They could not identify differences in kidney function, cardiovascular events, or mortality in these trials between groups treated medically with or without revascularization. It should be emphasized that these trials were short in duration, mostly quite small and heterogeneous in their characteristics, and many patients were not treated as assigned (see text).

the part of planners reflect vastly different expected rates of progression and clinical events. The STAR trial reported results from 140 subjects, whereas the goal for CORAL based on overall cardiovascular outcomes was more than 1,000 subjects.[183] As often occurs in randomized trials, the event rates were lower than expected. Although average estimates for the severity of renovascular disease in ASTRAL exceeded 70% occlusion, the trial cohort included 40% of subjects between 50% and 70% occlusion, many of whom had only a minor disease. This was confirmed at the time of angiography, at which point 68 of 403 (17%) assigned to stenting failed to be treated, because many of them had only trivial vascular disease. Importantly, many patients in these trials achieved acceptable BP levels during medical therapy. Renal function was remarkably stable in both arms in ASTRAL over years of follow-up, thus reaffirming the observation that many patients can be managed for years. Crossover rates from medical to revascularization arms were between 6% and 24%, primarily due to treatment failures.

These observations extend and confirm results of prospective trials of medical versus surgical intervention started in the 1980s and extended into the 1990s.[184] No differences in patient survival or renal function could be identified. Taken together, all of these studies indicate that rates of progression of renovascular disease are moderate and occur at widely varying rates. Often, such patients can be managed without revascularization for many years. The clinical issue in a specific patient frequently hinges on whether or not the risks of revascularization are truly less than the risks of progression.

Although these reports are informative, they leave many questions unanswered (Table 42.9). How often does suboptimal BP control in renovascular hypertension accelerate cardiovascular morbidity and mortality? Does one lose the opportunity to reverse hypertension successfully by delaying renal revascularization? How often will the expense and morbidity associated with complex drug regimens lead to inadequate treatment and/or adverse outcomes compared to restoring blood flow? These issues will depend on more selective, long-term prospective studies. It is equally clear that for many patients with progressive disease, optimal long-term stability of kidney function and BP control can be achieved by successful surgical or endovascular restoration of the renal blood supply.

42.9 Limitations of Clinical Trials in Atherosclerotic Renal Artery Stenosis

1. Patient selection
   a. Most severe cases not considered for randomization
   b. Most severe hypertension and progressive renal disease not included
   c. Limited selection of antihypertensive drug therapy
      i. Role of renin-angiotensin system blockade
      ii. Nonstandardized measurement of outcomes: BP levels
   d. Nonstandardized therapy for dyslipidemia/comorbid disease
2. Outcome measurement
   a. Widely variable definitions of BP goals, achieved levels
   b. Variable measurement of renal function
   c. Circulatory congestion/volume control/drug selection
   d. Crossovers from medical to interventional arms: 20%–44%
   e. Short duration of follow-up
3. Confounders limiting interpretation
   a. Roles and magnitude of comorbid disease: diabetes, preexisting cardiovascular disease
   b. Age/timing of intervention/timing of detection of disease
   c. Intermixing degree of renal involvement/tissue at risk: roles of unilateral and bilateral disease unevenly addressed
   d. Rates of development of disease, including renal dysfunction/BP changes
   e. Definition of treatment resistance

BP, blood pressure.
From Textor SC, McKusick MA, Misra S, Glockner J. Timing and selection for renal revascularization in an era of negative trials: what to do? Prog Cardiovasc Dis. 2009;52:220–228, with permission.

ENDOVASCULAR RENAL ANGIOPLASTY AND STENTING

The ability to restore vessel patency in high-risk patients with renovascular hypertension and ischemic nephropathy using endovascular methods undoubtedly represents a major advance in this disorder. Stenting allows the reversal of major degrees of vascular occlusion in more than 98% of cases, as illustrated in Fig. 42.21A,B. The restoration of blood flow to the kidney beyond a stenotic lesion seems to provide an obvious means to improve renovascular hypertension and to halt progressive vascular occlusive injury. For clinicians experienced in the field of renovascular hypertension, these developments represented a major breakthrough that hardly required testing in a controlled trial. As one author[185] argued in 2005, "A recent report on the use of parachutes to prevent death and major injury after jumping out of airplanes emphasized the fact that the parachute has never been subjected to a randomized controlled trial, even though numerous reports of survival after jumping without a parachute have been published."

The past 2 decades have been characterized by a major shift from surgical reconstruction toward preference for endovascular procedures. The total volume of renal revascularization procedures registered for the U.S. Medicare population above age 65 rose 62% from 13,380 to 21,600 between 1996 and 2000. This change reflects an increase in endovascular procedures by 2.4-fold, whereas surgical renovascular procedures fell by 45%. The trend continued with an estimate of 35,000 endovascular procedures in 2005 (Fig. 42.21C).[9,186] The transition to endovascular procedures also has enlarged and reconfigured the physician pool engaged in making decisions about renovascular hypertension. Interventional cardiologists increased their activity in this field nearly fourfold during this interval.

Revascularization of the kidney carries both benefits and risks, however. With older patients developing renal artery stenosis in the context of preexisting hypertension in the present era, the likelihood of a cure for hypertension is small, particularly in atherosclerotic disease. The true risks and benefits of these procedures are sometimes difficult to ascertain from published literature. They may vary between institutions depending on the technical expertise available. As noted in the following, methods of reporting results regarding clinical outcomes are inconsistent. Although complications are not common, they can be catastrophic, including atheroembolic disease and aortic dissection. Wide variability in the experience with peripheral endovascular procedures is reflected by the observation that their use, combining renal and lower extremity vascular stents, varies more than 14-fold between regions in the United States.[187] The probability of renal angioplasty within 30 days of cardiac catheterization has been fourfold higher when cardiologists perform the procedure than when interventional radiologists are responsible. Knowing when to pursue renal revascularization is central to the dilemma of managing renovascular disease.

Angioplasty for Fibromuscular Disease

Most lesions of medial fibroplasia are located at a distance away from the renal artery ostium. Many of these have multiple webs within the vessel, which can be successfully traversed and opened by balloon angioplasty. Experience in the 1980s indicated more than 94% technical success rates.[188] Some of these lesions (approximately 10%) develop restenosis for which repeat procedures have been used.[189] Clinical benefit regarding BP control has been reported in observational outcome studies in 65% to 75% of patients, although the rates of cure are less secure.[190] Cure of

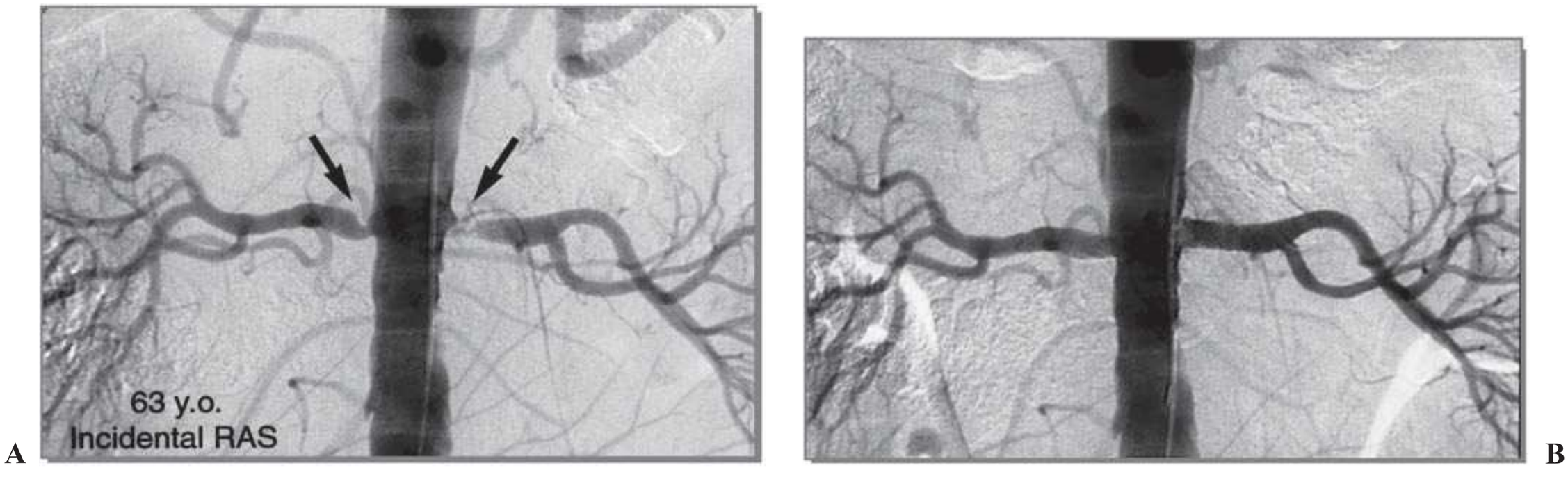

FIGURE 42.21 An angiogram obtained before **(A)** and after **(B)** renal artery stenting in an individual developing rapidly progressing renovascular hypertension. The ability to restore vascular patency using endovascular procedures has allowed many more patients to be treated than would have been considered for renovascular surgery. Development of these methods led to the striking expansion of renal artery stenting procedures, as reflected by the rise in Medicare claims submitted between 1996 and 2005 (see text) **(C).** *RAS*, renin-angiotensin system.

hypertension, defined as sustained BP levels less than 140/90 mm Hg with no antihypertensive medications, may be obtained in 35% to 50% of patients. Predictors of cure (normal arterial pressures without medication at 6 months and beyond after angioplasty) include lower systolic BP, younger age, and a shorter duration of hypertension.[191] A systematic analysis of treatment reports for fibromuscular dysplasia evaluated evidence from 47 series treated with angioplasty (1,616 patients) and 23 surgical series (1,014 patients).[192] Methods of evaluating BP outcomes and definitions of cure varied considerably in these reports. However, by selecting the criterion of BP $<$ 140/90 mm Hg after withdrawing antihypertensive drug therapy as a cure in contemporary terms, these authors found that only 36% of percutaneous transluminal renal angioplasty (PTRA)-treated patients and 54% of surgical patients achieved that response. Predictors of a favorable response included younger age and earlier date of publication. Major complications were lower with PTRA versus surgery (7% versus 15%). Hence, these authors concluded that renal revascularization even for FMD offers "moderate benefit."[192]

A large majority of patients with FMD are female. The age of detecting hypertension is usually younger than the series with atherosclerotic disease.[190] In general, such patients have relatively less aortic disease and are at less risk for major complications of angioplasty. Because the risk for major procedural complications is low and the potential antihypertensive drug treatment requirement is so long, most clinicians favor an early intervention for hypertensive patients with FMD with the hope of reduced antihypertensive medication requirements after successful angioplasty.

Angioplasty and Stenting for Atherosclerotic Renal Artery Stenosis

During the introduction of PTRA, it was soon evident that ostial lesions commonly failed to respond, in part because of extensive recoil of the plaque, which extended into the main portion of the aorta.[193] These lesions develop restenosis rapidly even after early success. Endovascular stents were introduced for ostial lesions in the late 1980s and early 1990s.[194]

The technical advantage of stents to achieve and maintain vessel patency is indisputable. An example of successful renal artery stenting is shown in Fig. 42.21. A prospective comparison between angioplasty alone versus angioplasty with stents indicates intermediate (6 to 12 months) vessel patency was 29% and 75%, respectively. Restenosis fell from 48% to 14% in stented patients.[193] As technical success

continues to improve, many reports suggest nearly 100% technical success in early vessel patency, although rates of restenosis continue to reach 14% to 25%.[150,195]

The introduction of endovascular stents has expanded the practice of renal revascularization, in part because of the improved technical patency that is possible with ostial atherosclerotic lesions as compared to angioplasty alone. It should be emphasized that much of the shift to endovascular procedures relates to their applicability in elderly patients and the widespread availability of interventional radiology.

What are the reported outcomes of patients undergoing renal artery stenting? These are commonly considered in terms of (1) BP control and (2) the preservation or salvage of renal function in ischemic nephropathy. Results from observational cohort BP studies after stenting face the same limitations as observed with angioplasty alone. Results during the follow-up from 1 to 4 years are summarized for representative series in Table 42.10. These have been reviewed elsewhere.[196] Typically, a fall in BP levels are in the range of 25 to 30 mm Hg systolic, the best predictor of which was the initial systolic BP.[197] Some authors report a 42% improvement in BP with fewer medications needed, although cures were rare and renal function was unchanged.[198] A careful attention to the degree of residual patency led to more than 91% patency at 1 year and 79% at 5 years in 210 patients with stents.[118] BPs were cured or improved in more than 80% of cases. In some cases, angina and recurrent congestive cardiac failure subsides.[199,200] As noted under the trials summarized previously, prospective randomized controlled trials have been less impressive regarding the benefits of angioplasty. The ambiguity of BP responses in these studies has produced widely different recommendations. These range from "we are left with whether renal angioplasty should be considered at all"[201] to a general conviction expressed within the interventional community that "open renal arteries are better than closed renal arteries" and that nearly all renal artery lesions should be opened (and probably stented).[150]

What results regarding the recovery of renal function can be expected after endovascular revascularization? Table 42.10 summarizes some of the recent series. In general, changes in renal function for atherosclerotic renal artery stenosis, as reflected by serum creatinine levels, have been small.[158] Remarkably, the changes in renal function in azotemic patients after surgical reconstruction are similar.[202,203] As we and others have observed, overall group changes in kidney function can be misleading. A careful evaluation of the literature indicates that three distinctly different clinical outcomes are routinely observed for patients with reduced GFR. In some instances (approximately 27%), revascularization produces meaningful improvements in kidney function. For this group, the mean serum creatinine level may fall from a mean value of 4.5 mg per deciliter to an average of 2.2 mg per deciliter. There can be no doubt that such patients benefit from the procedure and can avoid major morbidity (and probably mortality) associated with advanced renal failure. The bulk of patients (approximately 52%), however, have no measurable change in renal function. Whether such patients benefit much depends on the true clinical likelihood of progressive renal injury if the stenotic lesions were managed without revascularization, as discussed previously. Those without much risk of progression gain little. The most significant concern, however, is the group of patients whose renal function deteriorates further after a revascularization procedure. In most reports, this ranges from 19% to 25%.[158,204] In some instances,

42.10 Observational Outcomes of Renal Artery Stent Placement: Hypertension[118,193,194,198,204,206,236–240]

	Cured	Improved	No change
14 series n = 678 patients 98% technical success	Weighted mean: 17% Range: 3–68	Weighted mean: 47% Range: 5–61	Weighted mean: 36% Range: 0–61
Renovascular hypertension n = 472	12%	73%	15%
Renal Artery Stents: Effect on Renal Function in Azotemic Patients			
	Improved	**Stabilized**	**Worse**
14 series reporting "impaired renal function" n = 496 patients	Weighted mean: 30% Range: 10%–41%	Weighted mean: 42% Range: 32%–71%	Weighted mean: 29% Range: 19%–34%
Ischemic nephropathy n = 469	41%	37% no change	22%

this represents atheroembolic disease, or a variety of complications including vessel dissection with thrombosis.[205] Although less common with improved techniques, some of the complications associated with renal artery stenting can be clinically significant, as identified in Table 42.11. Hence, nearly 20% of azotemic patients face a relatively rapid progression of renal insufficiency and the potential for requiring renal replacement therapy, including dialysis and/or renal transplantation.[203,206,207] Possible mechanisms for deterioration include atheroembolic injury, which may be nearly universal after any vascular intervention[208] and acceleration of oxidative stress producing interstitial fibrosis.[209] Whether improving techniques, including the application of distal protection devices for endovascular catheters, will reduce these complications is not yet certain.

Several studies suggest that the progression of renal failure attributed to ischemic nephropathy may be reduced by endovascular procedures.[204,210] Harden et al.[204] presented reciprocal creatinine plots in 23 (of 32 patients), suggesting that the slope of loss of the GFR could be favorably changed after renal artery stenting. It should be emphasized that 69% "improved or stabilized," indicating that 31% worsened; these results were consistent with other series. These reports and a guideline document from the American Heart Association promote the use of breakpoint analysis to analyze and report the results of renovascular procedures. Caution must be applied regarding the use of breakpoints using reciprocal creatinine plots in this disorder, however. This concern was underscored by a report attempting to use estimated GFR (eGFR) changes compared to measured iothalamate GFR values to follow sequential changes in kidney function in atherosclerotic disease. Madder and colleagues[211] found that the staging of kidney disease—and even the direction of change, whether improving or deteriorating—in 254 patients was discordant between 28% and 40% of cases. Vascular disease does not affect both kidneys symmetrically, nor is it likely to follow a constant course of progression, in contrast to diabetic nephropathy, for example. As a result, a gradual loss of renal function with subsequent stabilization can be observed equally with unilateral disease, leading to total occlusion as well as successful revascularization. Perhaps the most convincing group data in this regard derive from serial renal functional measurements in 33 patients with high-grade (> 70%) stenosis to the entire affected renal mass (bilateral disease or stenosis to a solitary functioning kidney) with creatinine levels between 1.5 and 4.0 mg per deciliter. Follow-ups over a mean of 20 months indicate that the slope of GFR loss converted from negative (−0.0079 dL/mg/mo) to positive (0.0043 dL/mg/mo).[210] These studies agree with other observations that long-term survival is reduced in bilateral disease, and that the potential for renal dysfunction and accelerated cardiovascular disease risk is highest in such patients (see previous).

42.11 Complications After PTRA and Stenting of the Renal Arteries[205,206,227]

Minor: (most frequently reported)
- Groin hematoma
- Puncture site trauma

Major: (Reported in 71/799 treated arteries (9%)[227]
- Hemorrhage requiring transfusion
- Femoral artery pseudoaneurysm needing repair
- Brachial artery traumatic injury needing repair
- Renal artery perforation leading to surgical intervention
- Stent thrombosis: surgical or antithrombotic intervention
- Distal renal artery embolus
- Iliac artery dissection
- Segmental renal infarction
- Cholesterol embolism: renal
- Peripheral atheroemboli
- Aortic dissection[205]

Restenosis: 16% (Range: 0%–39%)

Deterioration of renal function: 26% (range: 0%–45%)

Mortality attributed to procedure: 0.5%

Procedure-related complications: 51/379 patients in 10 series: 13.5% (206)

Surgical Treatment of Renovascular Hypertension and Ischemic Nephropathy

Early experience with vascular disease of the kidney was based entirely on surgical intervention, either nephrectomy or vascular reconstruction, with the objective of surgical curability.[10] For that reason, much of the original data regarding split renal function measurements were geared toward identifying functionally significant lesions as a guide by which patients should be selected for a major surgical procedure. Surgical intervention is less commonly performed in the current era, in part because age and comorbid risks of patients with atherosclerotic disease commonly favor endovascular procedures when feasible.

Methods of surgical intervention have changed over the decades. A review in 1982 emphasized the role for ablative techniques, including the partial nephrectomy. Use of ablative operative means was guided by the difficulty of controlling BP during this period. They are less common since the expansion of tolerable medication regimens, as noted previously. The recent introduction of laparoscopic techniques, including the hand-assisted nephrectomy, may return attention to nephrectomies as a means to reduce medication requirements with low morbidity in high-risk patients.

Surgical series from the 1960s and early 1970s indicated that the cure of hypertension was present only in 30% to 40% of subjects, despite attempts at preselection. The survival of groups chosen for surgery appeared to be better than those chosen for medical management. This likely reflected the heavy disease burden and preoperative risks identified in those for whom surgery was excluded. The Cooperative Study of Renovascular Disease in the 1960s and 1970s examined many of the clinical characteristics of renovascular hypertension. These studies identified some of the limitations and hazards of surgical intervention and reported mortality rates of 6.8%, even in excellent institutions. The mean age in this series was 50.5 years. Definitions of operative mortality included events as late as 375 days after the procedure and may have overestimated the hazard. Had the authors considered only deaths within the first week, for example, the immediate perioperative mortality was 1.7%.[107]

The subsequent development of improved techniques for patient selection, including screening for coronary and carotid disease, for renal artery bypass, and endarterectomy, and for combined aortic and renal artery repair, represent major elements in the history of vascular surgery.[10] Several of the options developed for renal artery reconstruction are listed in Table 42.12. Most of these methods focus on reconstruction of the vascular supply for the preservation of nephron mass. A transaortic endarterectomy can effectively restore circulation to both kidneys. It requires aortic cross-clamping and may be undertaken as part of a combined procedure with aortic replacement. The identification and treatment of carotid and coronary disease led to reductions in surgical morbidity and mortality. By addressing associated cardiovascular risk before surgery, early surgical mortality falls below 2% for patients without other major diseases.

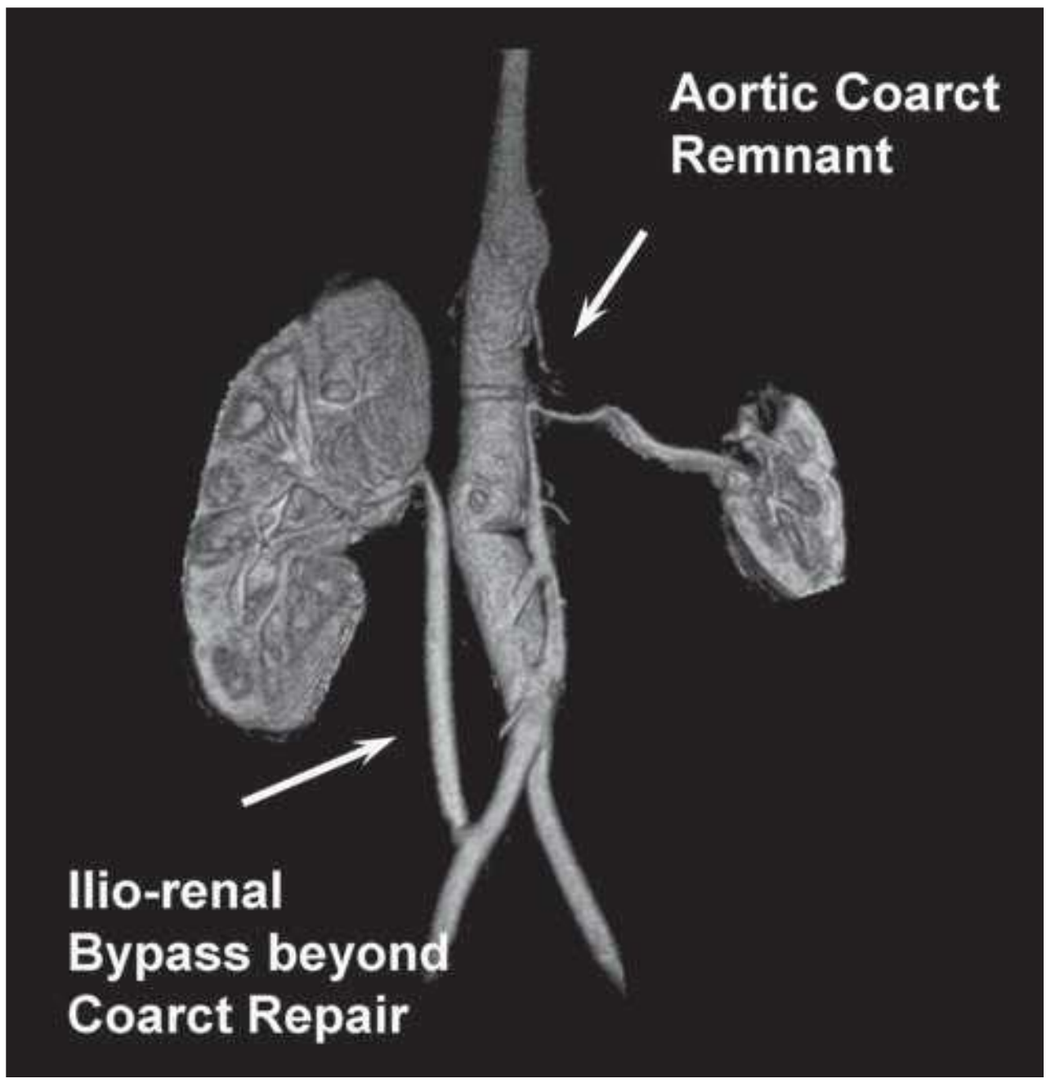

FIGURE 42.22 The surgical correction of renovascular disease. This is an example of late renal artery compromise after the correction of aortic coarctation in childhood. The tenuous status of renal circulation was corrected by construction of an ilio-renal bypass. These operative procedures now are reserved mainly for complex cases and repeated failures of endovascular stenting.

42.12 Surgical Procedures Applied to Reconstruction of the Renal Artery and/or Reversal of Renovascular Hypertension

Ablative Surgery: removal of a pressor kidney
- Nephrectomy: direct or laparoscopic
- Partial nephrectomy

Renal artery reconstruction (require aortic approach)
- Renal endarterectomy
- Transaortic endarterectomy
- Resection and reanastomosis: suitable for focal lesions
- Aortorenal bypass graft

Extra-anatomic procedures: (may avoid direct manipulation of the aorta)
- Require adequate alternate circulation without stenosis at celiac origin
- Splenorenal bypass graft
- Hepatorenal bypass graft
- Gastroduodenal, superior mesenteric, iliac-to-renal bypass grafts

Autotransplantation with ex vivo reconstruction

Modified from Libertino JA, Zinman L. Surgery for renovascular hypertension. In: Breslin DL, Swinton NW, Libertino JA, Zinman L, eds. Renovascular Hypertension. Baltimore, MD: Williams and Wilkins; 1982: 166–212.

Surgical reconstruction of the renal blood supply usually requires access to the aorta. A variety of alternative surgical procedures have been designed to avoid manipulation of the badly diseased aorta, including those for which previous surgical procedures make access difficult (Fig. 42.22). These include extra-anatomic repair of the renal artery using hepatorenal or splenorenal conduits, which avoid the requirement of manipulation of badly diseased aorta.[212] It should be emphasized that success with extrarenal conduits depends on the integrity of the alternative blood supply. Hence, a careful preoperative assessment of stenotic orifices of the celiac axis is undertaken before using either the hepatic or splenic arteries. The results of these procedures have been good, both in short-term and during long-term follow-up studies.[213] An analysis of 222 patients treated more than 10 years earlier indicated that these procedures were

performed with 2.2% mortality and low rates of restenosis (7.3%) and good long-term survival. The predictors of late mortality were age above 60 years, coronary disease, and previous vascular surgery.

The durability of surgical vascular reconstruction is well established. Follow-up studies after 5 and 10 years for all forms of renal artery bypass procedures indicate excellent long-term patency (above 90%) both for renal artery procedures alone and when combined with aortic reconstruction.[214] Results of surgery have been good despite increasing age in the reported series. Patient selection has been important in all of these reports. Although long-term outcome data are established for surgery, limited information is available for endovascular stent procedures, which are more prone to restenosis and technical failure. This proven record of surgical reconstruction leads some clinicians to favor this approach for younger individuals with longer life expectancies.

Some studies have compared endovascular intervention (PTRA without stents) and surgical repair. A single study of nonostial, unilateral atherosclerotic disease in which patients were randomly assigned to surgery or PTRA indicate that although surgical success rates were higher and PTRA was needed on a repeat basis in several cases, the 2-year patency rates were 90% for PTRA and 97% for surgery.[215] A prospective comparison of endovascular stents compared to open surgical renal revascularization argued that patency over 4 years was better with open surgical repair, but that otherwise, the outcome of the two procedures did not differ.[216]

In many institutions, surgical reconstruction of the renal arteries is most often undertaken as part of aortic surgery. Those with impaired renal function at the Mayo Clinic (creatinine ≥ 2.0 mg per deciliter) underwent simultaneous aortic and renal procedures in 75% of cases.[202] Recent experience indicates that combining renal revascularization with aortic repair does not increase the risk of the aortic operation. As with endovascular techniques, the results regarding changes in renal function include improvement in 22% to 26%, no change (some consider this stabilization) in 46% to 52%, and progressive deterioration in 18% to 22% (Fig. 42.23). Using intraoperative color flow Doppler ultrasound allows for the immediate correction of suboptimal results and improved long-term patency.[217] Despite good results, open operations for renal artery revascularization continue to decline. A review of the National Inpatient Sample indicates relatively high mortality rates (approximately 10%) overall, leading the authors to support lower risk endovascular methods where possible or a referral to high volume surgical centers.[218] For experienced centers using current techniques, operative risk is below 4% in good risk candidates.[219,220] Factors for higher risk include advanced age,

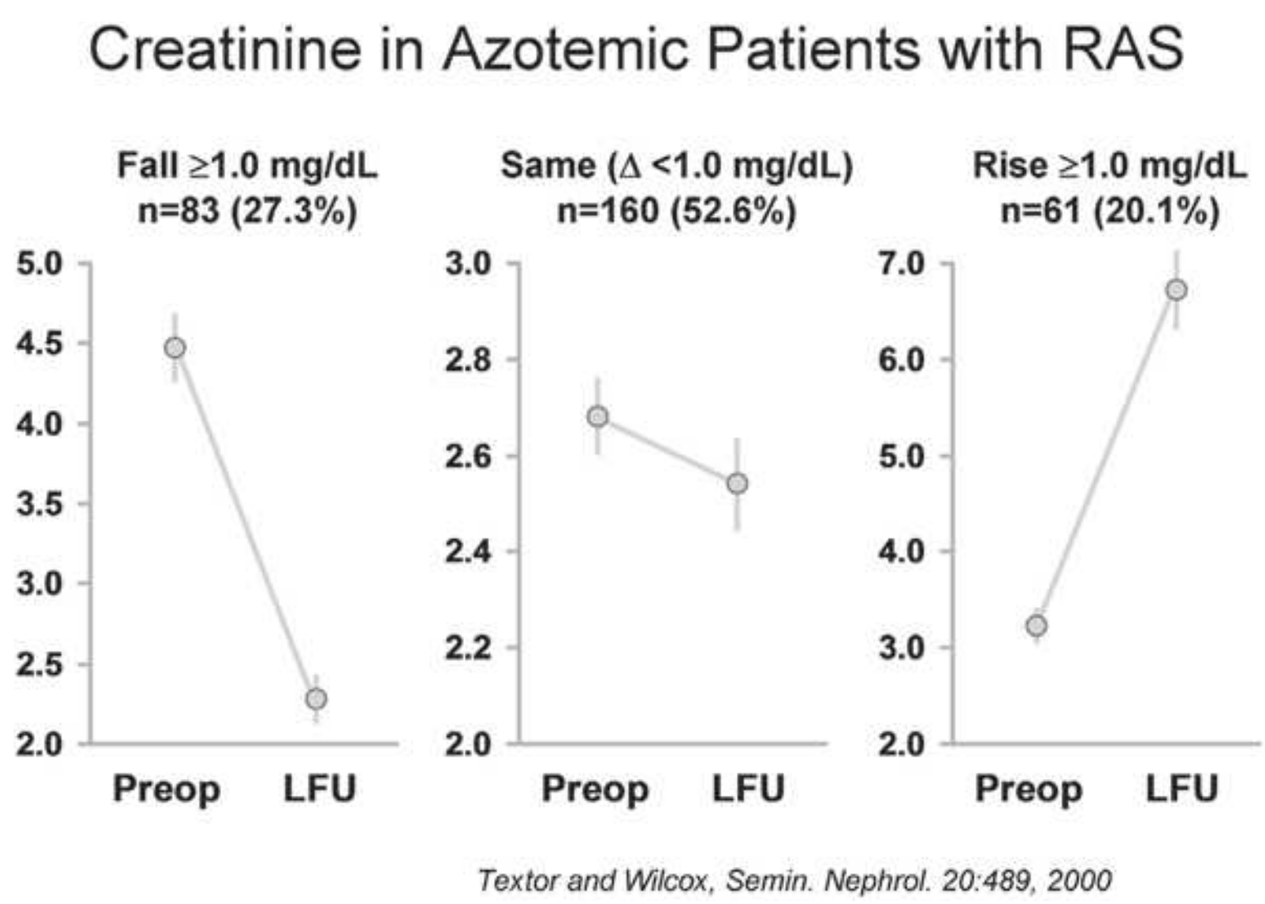

FIGURE 42.23 Renal functional outcomes following surgical revascularization of more than 300 patients with serum creatinine above 2.0 mg per deciliter. Although mean values for the entire group were unchanged, these figures obscured the fact that meaningful clinical improvement (defined as a fall in creatinine ≥ 1.0 mg per deciliter) was evident in 27.3%, and creatinine remained stable in 52.6% (thereby avoiding progression). These outcomes were counterbalanced in this cohort by 20.1% experiencing a distinct worsening of kidney function (defined as a rise in creatinine of more than 1 mg per deciliter). These outcomes have been remarkably reproducible in azotemic patients regardless of the method of revascularization and have been attributed to a variety of effects, including atheroembolic complications.[158] The identification of subjects likely to benefit from revascularization remains a primary challenge in this disorder. RAS, renal artery sclerosis; LFU, last follow-up. (From Textor SC, Wilcox CS. Renal artery stenosis: a common, treatable cause of renal failure? *Annu Rev Med.* 2001;52:421–442, with permission.)

elevated creatinine (above 2.7 to 3.0 mg per deciliter) and associated aortic or other vascular diseases. In some cases, nephrectomy of a totally infarcted kidney provides major improvement in BP control at low operative risk. The introduction of laparoscopic surgical techniques makes nephrectomy technically easier in some patients for whom vascular reconstruction is not an option. These series reflect widely variable methods of determining BP benefits, as discussed in the following.

Studies in patients with bilateral renal artery lesions or vascular occlusions to the entire renal mass indicate that the restoration of blood flow can lead to preservation of renal function in some cases.[221] Most often, this has been undertaken when a clue of preserved blood supply, sometimes from capsular vessels, is evident by renography. Occasionally, revascularization can lead to functional recovery that is sufficient to eliminate the need for dialysis.

Predictors of Likely Benefit Regarding Renal Revascularization

Identifying patients who are most likely to improve their BP and/or renal function after renal revascularization remains an elusive task. As noted previously, functional tests of renin release, such as the measurement of renal vein renin levels, have not performed universally well. Many of these studies are most useful when positive (e.g., the likelihood of benefit improves with more evident lateralization), but have relatively poor negative predictive value; that is, when such studies are negative, outcomes of vessel repair may still be beneficial. As a clinical matter, the recent progression of hypertension remains among the most consistent predictors of improved BP after intervention.

Predicting favorable renal functional outcomes is also difficult. Either surgical or endovascular procedures are least likely to benefit those with advanced renal insufficiency, which is usually characterized by serum creatinine levels above 3.0 mg per deciliter. Nonetheless, occasionally, patients with recent progression to far advanced renal dysfunction can recover GFR with durable improvement over many years. Small kidneys, as identified by a length less than 8 cm, are less likely to recover function, particularly when little function can be identified on radionuclide renography.[222] Reports of the renal resistance index as measured by Doppler ultrasound in 5,950 patients indicated that the identification of lower resistance was a favorable marker for both an improvement in GFR and BP, whereas an elevated resistance index was an independent marker of poor outcomes (Fig. 42.14).[125] None of these is absolute, and recent studies identify favorable outcomes in some patients with adverse predictors.[223] Some authors suggest that detecting abnormalities in fractional flow reserve, as measured by translesional flows and gradients after dilation with papaverine, may predict benefits of revascularization.[156,224] Recent deterioration of kidney function or hypertension portends a more likely improvement with revascularization.

Complications of Renal Artery Angioplasty and Stenting

Atherosclerotic plaque is commonly composed of multiple layers with calcified, fibrotic, and inflammatory components. The physical expansion of such a lesion applies considerable force to the wall of the artery and may lead to cracking and the release of small particulate debris into the bloodstream. Effective balloon angioplasty and stenting requires applying optimal techniques for limiting the damage to blood vessels during the procedure. A review[206] of 10 published series with 416 stented vessels indicated that significant complications arise in 13% of cases, not counting those that led to the need for dialysis. These include several of the events listed in Table 42.11, including hematomas and retroperitoneal bleeding requiring transfusion. Renal function deteriorated in these series on average 26% of the time and 50% of subjects (7 out of 14) with preprocedure creatinine levels above 400 mc/mol progressed to advanced renal failure requiring dialysis.[206] Most complications are minor, including local hematomas and false aneurysms at the insertion site. Occasional severe complications develop, including aortic dissection,[205] stent migration, and vessel occlusion with thrombosis.[225] Local renal dissections can be managed by the judicious application of additional stents. Mortality related directly to this procedure is small, but has been reported in 0.5% to 1.5% of patients.[206,223]

Restenosis remains a significant clinical limitation. Rates vary widely between 13% and 30%, most often developing within the first 6 to 12 months.[206,226–228] The most recent series reported 13% to 16% restenosis, sometimes leading to repeat procedures.

SUMMARY

Renovascular disease is common, particularly in older subjects with other atherosclerotic disease. It can produce a wide array of clinical effects, ranging from asymptomatic incidentally discovered disease to accelerated hypertension and progressive renal failure. With improved imaging and older patients, significant renal artery disease is detected more often than ever before. It is incumbent upon the clinician to evaluate both the role of renal artery disease in the individual patient and the potential risk/benefit ratio for renal revascularization. An algorithm to guide treatment and reevaluation of patients with atherosclerotic renal artery stenosis is presented in Fig. 42.24. The application of this strategy relies heavily on considering comorbid risks and the evolution of both BP control and kidney function over a period of time. Managing cardiovascular risks and hypertension are the primary objectives of medical therapy. For most patients, the realistic goals of renal revascularization are to reduce medication requirements and to stabilize renal function over time. Patients with bilateral disease or stenosis to a solitary functioning kidney may have a lower risk of circulatory congestion (flash pulmonary edema or its equivalent) and

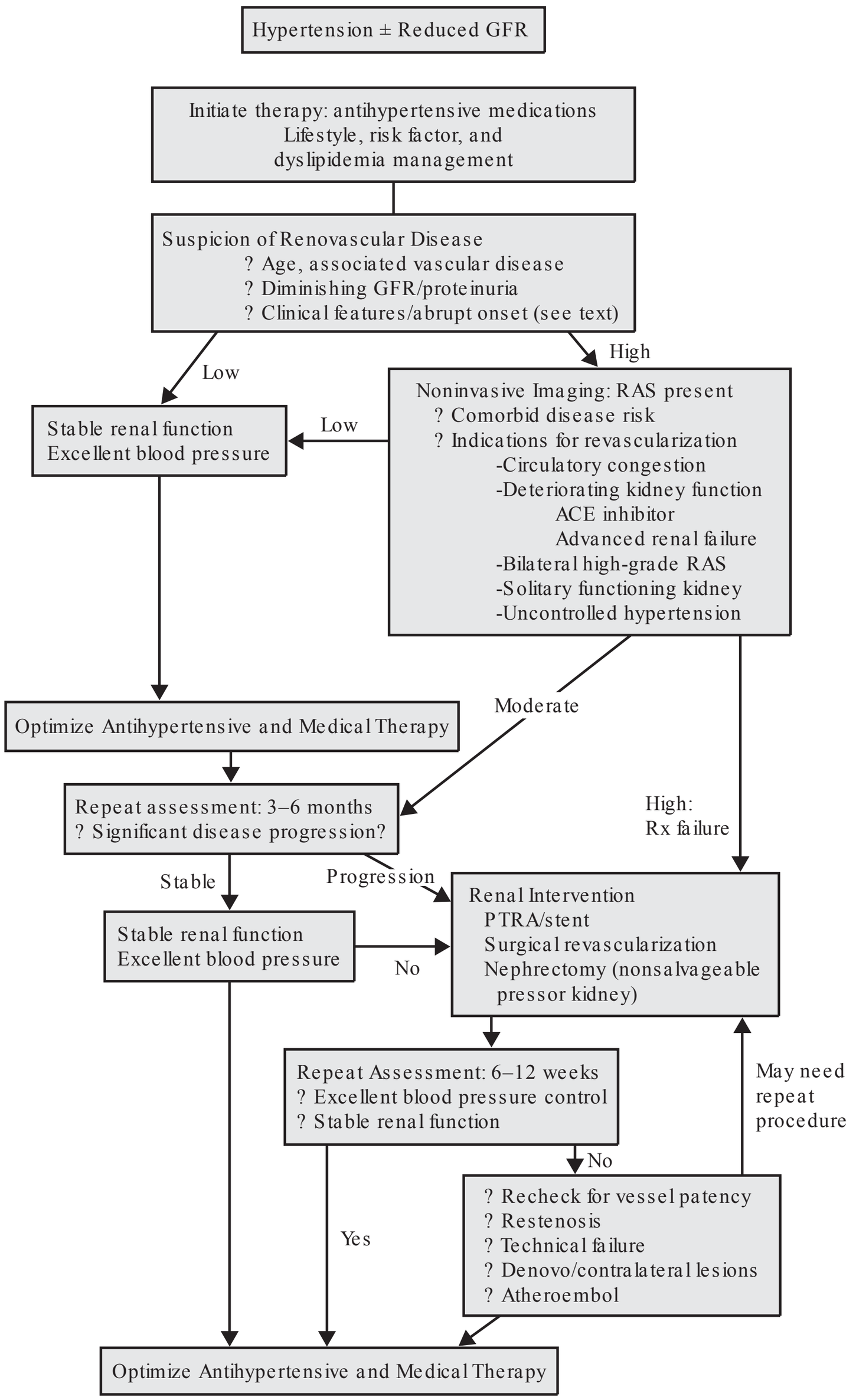

FIGURE 42.24 An algorithm proposed for the identification and management of renovascular disease and ischemic nephropathy. In general, antihypertensive drug therapy is a primary mode of therapy. If kidney function and blood pressure are stable and controlled with a tolerable regimen, there may be little to gain from an extensive evaluation for renovascular disease. If there is high suspicion for developing renovascular disease and/or a commitment to intervene, then more extensive imaging is appropriate. Indications for renal revascularization should be clearly defined before this process proceeds. If kidney function, blood pressure control, or circulatory congestion is inadequate and the individual is prepared to accept the hazards of revascularization, this may be appropriate. As with any vascular disease, periodic follow-up for disease progression and/or recurrence is warranted (see text). GFR, glomerular filtration rate; RAS, renal artery sclerosis; ACE, angiotensin-converting enzyme; PTRA, percutaneous transluminal renal angioplasty. (From Textor SC. Renovascular hypertension and ischemic nephropathy. In: Brenner BM, ed. *Brenner and Rector's: The Kidney*. Philadelphia, PA: Saunders; 2008: 1528–1566, with permission.)

a lower risk for advancing renal failure after revascularizing the kidney. It is essential to appreciate the risks inherent in either surgical or endovascular manipulation of the diseased aorta. These include a hazard of atheroembolic complications and the potential deterioration of renal function related to the procedure itself (estimated at 20% for patients with preexisting kidney dysfunction). Hence, the decision to undertake these procedures should include consideration of whether the potential gain warrants such risks. In many cases, improved BP and the recovery of renal function justify the costs and hazards completely. A follow-up of both BP and renal function is important, particularly because of the

potential for restenosis and/or recurrent disease. Optimal selection and timing for medical management and revascularization depend largely on the comorbid conditions for each patient.

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CHAPTER

43 Hypertension due to Primary Aldosteronism and Related Disorders

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INTRODUCTION

Distinct hypertensive syndromes related clearly to mineralocorticoid overproduction are increasingly being recognized. Primary aldosteronism (PA), first described by Conn in 1955,[1] is characterized by hypertension, hypokalemia, suppressed plasma renin activity (PRA), and increased aldosterone production. Reversal of the clinical manifestations by the surgical removal of a right adrenal adenoma established the relationship among aldosterone overproduction, hypokalemia, and hypertension for the first time.

Three heritable forms of aldosteronism are known today. FH-1 is a glucocorticoid-remediable form of aldosteronism (GRA). Two glucocorticoid-resistant forms have been described: FH-II, the familial occurrence of aldosterone-producing adenoma (APA), or bilateral adrenal hyperplasia and FH-III, the familial occurrence of massive bilateral adrenal hyperplasia.

Excessive production of deoxycorticosterone (DOC) results from (1) disorders of steroid production (congenital adrenal hyperplasia), (2) generalized glucocorticoid resistance, and (3) DOC-producing adrenocortical tumors.

A group of disorders simulating PA but with a low production of aldosterone or other mineralocorticoids has also been described. These disorders occur as a result of increased activation of mineralocorticoid receptors (MR). The list includes (1) apparent mineralocorticoid excess (AME), (2) cortisol excess, (3) gain-of-function mutation of ENaC of distal renal tubules (Liddle syndrome), and (4) hypertension exacerbated by pregnancy due to a mutation of mineralocorticoid receptors.

PRIMARY ALDOSTERONISM

PA is the most common and important cause of secondary hypertension. The urgency in identifying PA early and treating it is heightened by data from studies demonstrating that persistent elevation of aldosterone levels can result in end organ damage. In addition, findings from animal studies indicate that cardiovascular and renal injury occur independently of blood pressure levels.[2] Cross-sectional studies in humans demonstrate early subclinical end organ damage that includes an increase in carotid intima media thickness and worsening in pulse wave velocity, both gold standard measures of arterial wall stiffness. Further, these studies have shown reduced endothelial function when compared to matched controls with essential hypertension. Several studies have also demonstrated clear evidence of increased left ventricular hypertrophy, diastolic dysfunction, myocardial fibrosis, and albumin excretion rate when compared to those with primary hypertension.[3–10] It appears that these deleterious end organ effects could potentially be ameliorated with early and appropriate medical and surgical intervention.[11]

Pathophysiology of Mineralocorticoid-Induced Hypertension

Aldosterone, a major mineralocorticoid hormone, has potent effects on unidirectional transepithelial sodium transport. Inappropriately elevated aldosterone levels drive sodium and water retention, which increases circulatory volume and cardiac output; the latter, in turn, is reflexively normalized by vasoconstriction, resulting in hypertension. It is reasoned that the increase in total peripheral resistance to maintain the elevated arterial blood pressure occurs later, following vascular autoregulation.[12] However, hypervolemia is not a universal finding in patients with PA.[13] Many patients have either low or normal intravascular volume and there is no correlation between arterial blood pressure and plasma or total blood volume in either men or women with untreated PA. The potent effects of aldosterone on salt and water retention reasonably suggest that hypervolemia might have had a role in initiating the pressure rise and then may have been superseded or even partially reversed by other mechanisms. One could speculate that a secondary rise of resistance after an initial increase in cardiac output would establish new levels of equilibrium. The accumulated evidence favors the conclusion that aldosterone, by producing functional changes in the arterial wall, is responsible for the

initial vasoconstrictive response and the sustained and progressive hypertensive state that follows. Most experimental evidence from intact animal studies suggests that mineralocorticoids both increase membrane permeability to sodium and elevate intracellular sodium concentration, which in turn decreases calcium efflux.[14] By partially depolarizing the muscle cell membrane, the abnormalities of cation turnover lead to vasoconstriction and elevated vascular resistance. Such changes also increase metabolic activity and provide an early signal for vascular smooth hypertrophy, which when combined with rising blood pressure, could lead to thickening of the media and so raise the wall-to-lumen ratio. This structural adaptation implying enhanced reactivity could be crucial for both potentiating and maintaining the hypertensive process.[15] The study suggests that an increase in systemic vascular resistance leading to hypertension could occur independent of changes in intravascular volume.

Prevalence

The prevalence of PA has remained debatable as studies have been fraught with several limitations, which include bias in patient selection and reliance on tests that are not regarded as confirmatory for the diagnosis of PA.[16] A large prospective clinical trial, the PAPY (PA Prevalence in Hypertensives) Study, demonstrated that PA involves at least 11.2% of consecutive patients with newly diagnosed hypertension. Although the patients underwent a thorough workup that allowed the investigators to definitively establish the presence or absence of PA, this may still be an overestimation of PA prevalence in patients with hypertension as study participants were enrolled from specialized hypertension clinics.[17] Gordon and coworkers[18] reported the incidence of 12% of PA in 199 patients referred to their clinic for hypertension. The clue that a much lower prevalence of PA exists in patients with hypertension is provided by another study by Douma et al.[19] that evaluated patients with resistant hypertension, which showed that 11.3% of patients in this group had PA based on the confirmatory salt suppression test. Based on the prevalence of PA in patients with resistant hypertension in the general hypertensive population and the lower prevalence of PA in milder forms of hypertension, we could assume that the prevalence of PA in the general unselected hypertensive population is much lower than currently thought. Therefore, the actual prevalence of PA among unselected hypertensives is still unknown, but can be estimated from the data of Mosso et al.,[20] considering the relative proportion of the different grades of hypertension in the general population is around 4%.

Case Detection of Primary Aldosteronism

A high index of suspicion for PA must be entertained in patients who develop (1) spontaneous or unprovoked hypokalemia; (2) severe hypokalemia (<3 mEq per liter), which does not normalize even with potassium replacement or addition of potassium-sparing diuretics; (3) potassium levels that do not normalize even after discontinuation of diuretics for 4 weeks; (4) resistant hypertension with no other evidence of secondary cause; (5) hypertension with adrenal adenoma; (6) and those who have a family history of PA, early onset hypertension, or cerebrovascular accident at a young age (<40 years).

Serum Potassium

Traditionally, spontaneous hypokalemia (serum K <3.5 mEq per liter) has been regarded as the most effective screening test to diagnosis PA. But data from our studies and others have shown that a substantial number of patients with PA do not present with hypokalemia.[13] The reported prevalence of normokalemia in PA is variable. Conn reported a 7.6% prevalence of normokalemia in his series of 145 patients.[21] In the PAPY study, only 9.6% of patients with PA were found to have spontaneous hypokalemia.[17] In a study by Douma et al.,[19] 45.6% of patients with PA demonstrated hypokalemia. Others have shown a normal serum potassium concentration in 7% to 38% of reported cases.[21,22] In addition, 10% to 12% of patients with proven adrenocortical tumors may not develop hypokalemia during short-term salt loading. A normal serum potassium does not rule out PA; however, spontaneous hypokalemia associated with renal potassium wasting (UkV≥30 mEq per liter) has a high sensitivity and specificity in the diagnosis of PA.

Plasma Aldosterone Concentration:Plasma Renin Activity Ratio (ARR)

The plasma aldosterone concentration (PAC):plasma renin activity (PRA) ratio is considered the best screening test for PA. This technique is highly sensitive but has a high false-positive rate (about 30% to 50%) because PRA, the denominator, can be very low (as low as 0.1 ng per milliliter per hour) in some laboratories. Accordingly, a minimum PRA of 0.65 ng per milliliter per hour is recommended in calculating the ratio.[23] In published studies, the screening cutoff values vary from 7.2 to 100 ng per deciliter per nanogram per milliliter per hour; consequently, there is wide variation in the sensitivity (64% to 100%) and specificity (87% to 100%) of the test.[24] Reported ratios are all laboratory dependent. In a large, multicenter, prospective trial the accuracy of ARR for pinpointing patients with aldosterone-producing adenomas (APA) was close to 80%.[25] In addition, study results demonstrated a highly significant within-patient correlation ($r=0.69$; $P< .0001$) and reproducibility (coefficient of determination: 0.47). Better diagnostic accuracy is obtained if the absolute PAC is included as a second criterion in combination with the ARR. In a retrospective study,[26] the combination of a PAC:PRA ratio >30 and a PAC value >20 ng per deciliter had a sensitivity of 90% and a specificity of 91% for APA.[26] At the Mayo Clinic, a PAC:PRA ratio of ≥20 and a PAC >15 ng per deciliter were found in more than 90% of patients with surgically confirmed APA.[27]

Several factors such as time of day, diet, posture, method of blood collection, and plasma potassium level may affect

ARR sensitivity and specificity. Other factors that may affect ARR sensitivity and specificity include age, gender, race, diabetes mellitus, and use of oral contraceptive agents. Most elderly and diabetic patients have low levels of PRA. About 40% of essential hypertensive patients have low PRA and about 27% of untreated patients with PA may have nonsuppressed PRA.[13] Pizzolo and coworkers[28] reported that oral contraceptive administration may increase ARR, contributing to the diagnostic inaccuracy in women. Only 36.9% of women with positive ARR had confirmed PA by intravenously administered salt.

Blood samples are best obtained in the morning in an ambulatory seated patient. Ideally, all antihypertensive medications should be discontinued 2 to 3 weeks before ARR testing, but in many patients, this is not feasible. Although some drugs may alter the accuracy of the PAC:PRA ratio, they are not usually an issue in patients with PA. Thiazide diuretics, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers can actually improve the diagnostic discriminatory power of the PAC:PRA ratio, whereas beta-adrenergic blockers and central alpha-2 agonists suppress PRA and may give false-positive results,[29] especially if the absolute PAC cutoff is not used. If blood pressure control is an issue, alpha-1-adrenoreceptor blockers and/or a nondihydropyridine calcium channel blocker (such as verapamil) may be used. Furthermore, specific aldosterone antagonists may stimulate plasma renin activity, giving rise to false-negative results.

Confirmatory Test(s)

The diagnosis of PA can often be established with relative ease. In the hypertensive patient receiving no treatment who demonstrates significant hypokalemia ($<$3 mEq per liter) with renal potassium wasting (24-hour urinary potassium $>$30 mEq), PRA below 1 ng per milliliter per hour, and elevated plasma or urinary aldosterone values, the diagnosis of PA is unequivocal and may not undergo salt suppression testing. Often, however, the diagnosis is not obvious because of equivocal values. In such cases, multiple measurements are needed during salt loading. Cleveland Clinic patients ingest a normal diet with additional salt added (1 tsp table salt) to food each day for 5 consecutive days. On the 5th day of increased dietary salt, 24-hour urine is collected for sodium, potassium, aldosterone, and creatinine. On the morning of the 6th day, blood is drawn for basic metabolic panel, aldosterone, and renin activity. A 24-hour urinary aldosterone $\geq$14 μg per day (mean +2 standard deviations above values obtained in essential hypertensives) is definitive evidence of a nonsuppressible aldosterone production as long as the 24-hour urinary sodium is $\geq$200 mEq. The development of hypokalemia (serum K $<$3.5 mEq per liter) with renal potassium wasting (UkV $\geq$30 mEq per liter) provides additional evidence of inappropriate aldosterone production. An aldosterone excretion greater than 14 μg per 24 hours following salt loading distinguishes most patients with PA from those with primary hypertension; only 7% of patients with PA have aldosterone excretion values that fall within the range obtained in primary hypertension.[13]

Because most classes of antihypertensive medications affect plasma aldosterone levels and PRA values, antihypertensive treatment should be modified 4 to 6 weeks before the salt loading test. Long-acting calcium channel blockers and the alpha-adrenoreceptor antagonist doxazosin may be used during this period to control blood pressure when required.

Other aldosterone suppression testing has been described.[30] These include intravenous sodium chloride loading, captopril stimulation, or fludrocortisone suppression with the measurement of PAC. The captopril stimulation test operates on the principle that ACE inhibition is without effect when PRA is suppressed and plasma aldosterone is elevated while PRA increases and plasma aldosterone decreases in patients with secondary aldosteronism. However, the specificity of the test is markedly compromised by the large number of hypertensive patients with suppressed PRA (i.e., in low renin essential hypertension, the elderly, diabetics, and African Americans).

Differentiation of Subtypes

The two major causes of PA are bilateral adrenal hyperplasia (BAH), accounting for 65% to 70% of PA patients and aldosterone-producing adenoma (APA) accounting for 30% to 35% of PA. Unilateral hyperplasia and familial hyperaldosteronism accounts for 3% to 4% of patients with PA.[31] It is important to differentiate those with APA because this is a potentially curable condition with surgical intervention.

Biochemical. Adenomas are likely to be present in a patient with spontaneous hypokalemia ($<$3 mEq per liter) and plasma 18-hydroxycorticosterone levels greater than 100 ng per deciliter. In addition, hyperaldosteronism resulting from a unilateral adrenal abnormality is exquisitely responsive to adrenocorticotropic hormone (ACTH) and not to angiotensin II infusions.[32] A plasma 18-hydroxycorticosterone level $<$100 ng per deciliter or a postural increase in plasma aldosterone, or both, are usually associated with adrenal hyperplasia but do not completely rule out the presence of an adenoma.[13] However, a postural decrease in PAC has a high positive predictive value for the diagnosis of APA because a postural decrease in PAC does not occur in hyperplasia.

Localization of Aldosterone Hypersecreting Adrenal Gland.

Adrenal Computed Tomography Scan. All patients diagnosed with PA should undergo an adrenal computed tomography (CT) scan as the initial study in subtype differentiation.[33] A high resolution CT scan with contrast with fine cuts (2.5 to 3 mm) is the imaging technique that displays the best sensitivity and specificity; it is generally more available and less costly than magnetic resonance imaging (MRI). Adrenal

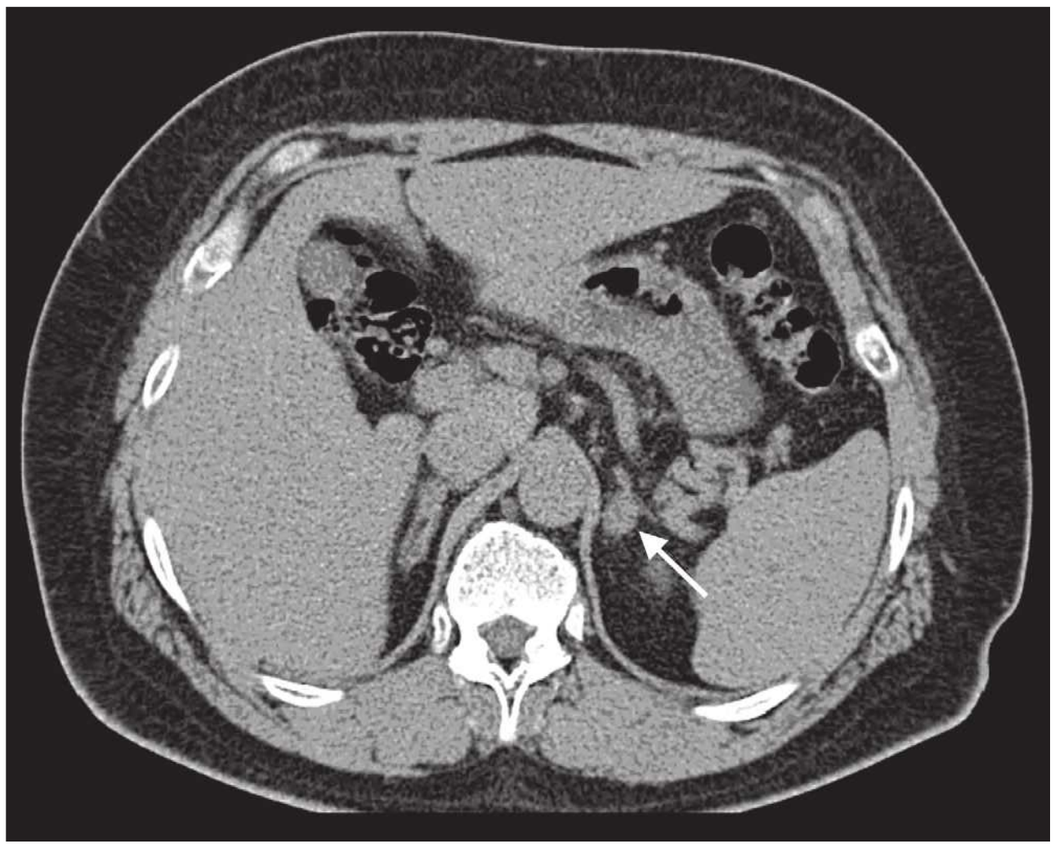

FIGURE 43.1 A 1.5 cm left adrenal adenoma (*arrow*) in the noncontrast enhanced computed tomography examination. The mass has an attenuation value <10 H and a calculated washout rate >50%. These are radiologic features consistent with an adenoma. Adrenal venous sampling (results shown in Table 43.1) provided evidence of a unilateral, left aldosterone-producing tumor.

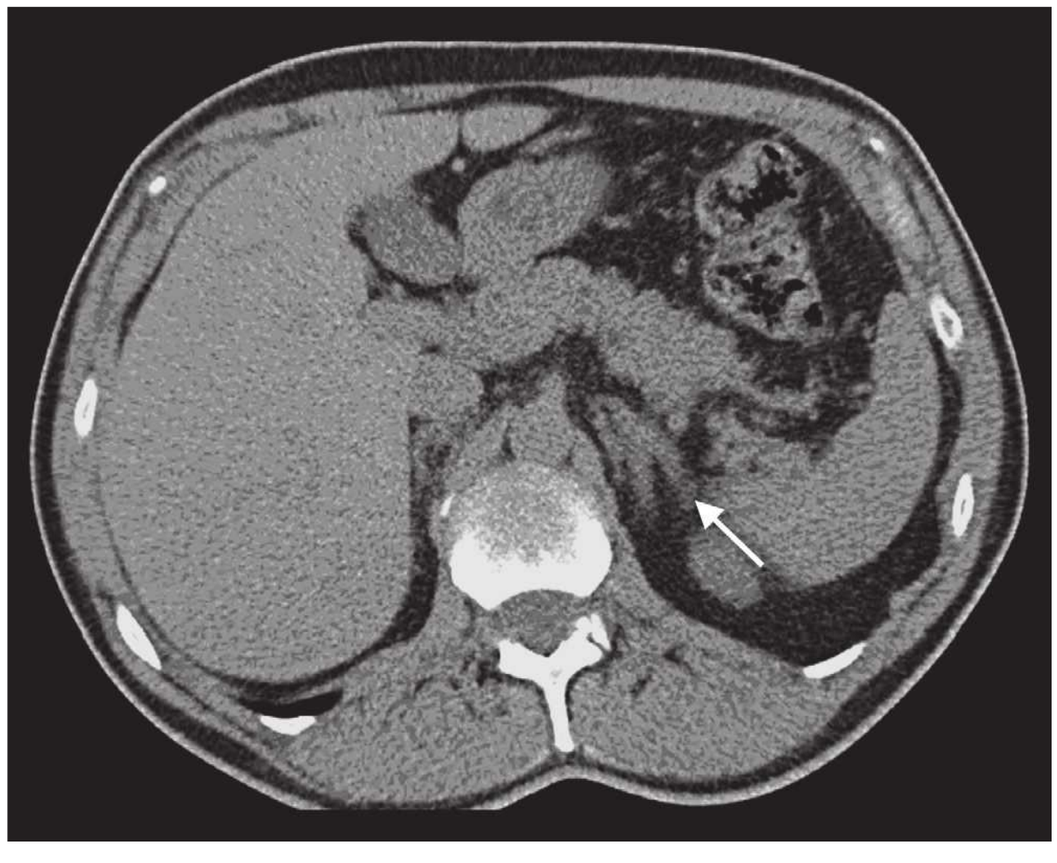

FIGURE 43.2 A noncontrast enhanced adrenal computed tomography scan of a patient with clinical characteristics suggestive of primary aldosteronism. There is marked thickening of both limbs of the left adrenal gland without discrete nodules. The right adrenal gland was reported as normal. Adrenal venous sampling (results shown in Table 43.2) showed bilateral secretion of aldosterone, indicating bilateral adrenal hyperplasia.

cortical adenomas typically have low X-ray attenuation (≤10 HU) in the noncontrast enhanced CT examination (Fig. 43.1). By adding a CT examination approximately 15 minutes after the start of the intravenous contrast enhancement, the washout rate of the iodine contrast medium from the tumor is typically faster (40%) in benign cortical adenomas compared to nonadenomas. Adenomas that are 1.5 cm or larger in diameter can accurately be detected with this procedure. Only 60% of adenomas that measure 1.0 to 1.4 cm are detected, whereas nodules that are less than 1 cm in size are more likely to be missed by a CT scan. Reported sensitivity rates of localizing adenomas by CT scans are between 75% and 80%.[34]

Adrenal Vein Sampling for Aldosterone. The Endocrine Society Guidelines[33] recommend that all patients for whom treatment is practicable and desired should undergo adrenal vein sampling (AVS). There are exceptions to this recommendation. A patient <40 years of age with spontaneous hypokalemia, PAC 15 to 20 ng per deciliter, PRA <1 ng per milliliter, with a solitary adrenocortical macroadenoma (>1 cm) that is discrete, uniform, of low attenuation (HU ≤10), has a contrast washout >40%, and a morphologically normal contralateral adrenal gland needs no further evaluation and should be referred for surgery. Older patients with PA with CT findings demonstrating bilateral morphologically normal or abnormal glands or unilateral microadenoma should be sent for AVS (Fig. 43.2). There is debate as to whether older patients with PA with adrenal CT characteristics identical to the 40-year-old patient described previously should undergo AVS for subtype characterization.

AVS is technically difficult and requires skill and expertise. Even allowing for publication bias from large centers of excellence, success rates are variable ranging from 42%[35] to 75%[36] to 98%[37] for successful bilateral cannulation. During AVS, continuous Cosyntropin is employed to increase adrenal blood flow and to augment aldosterone secretion from an APA. Blood samples are collected from the adrenal veins and the inferior vena cava (IVC) (peripheral sample) for the determination of cortisol and aldosterone concentrations. An adrenal vein:peripheral vein cortisol ratio of at least 3:1 indicates successful adrenal vein catheterization with 100% reproducibility. An aldosterone/cortisol (A/C) adrenal vein over an A/C contralateral adrenal vein of at least 4, plus an A/C contralateral adrenal vein/A/C IVC less than 1, indicates lateralization.[38] Recent data from an Italian center of excellence on a cohort of 44 cases where the diagnosis of APA was not in doubt and using an optimal lateralization ratio (as previously) demonstrated sensitivity and specificity rates of 80% and 75%, respectively (Tables 43.1 and 43.2).[38] A diagnostic approach to patients at risk for PA is shown in Figure 43.3.

11C-Metomidate Positron Emission Tomography/Computed Tomography Scan. Another imaging modality that targets the adrenal cortex is positron emission tomography (PET) using the tracer 11C-metomidate (MTO).[39] MTO binds to the 11β-hydroxylase enzyme in the adrenal cortex and is not taken up in noncortical tumors. The use of MTO-PET for imaging and the characterization of adrenocortical tumors has demonstrated high sensitivity and

43.1 Results of Adrenal Vein Sampling for Plasma Aldosterone and Cortisol Concentrations in the Patient with a Left Adrenal Nodule (Shown in Figure 43.1)

Site	Aldosterone (ng/dL)	Cortisol (μg/dL)	A/C ratio
Right	426	615	0.69
Left	15,230	545	27.94
IVC (peripheral)	65	17	3.82

Adrenal vein sampling was performed with constant infusion of Cosyntropin (50 μg/hr). The adrenal vein:IVC cortisol ratios exceed 3:1 indicating successful cannulation of both adrenal veins. Lateralization to the left is evidenced by an A/C ratio from the left that is ≥4 times greater than that from the right. Suppression of aldosterone secretion from the right adrenal gland is shown by the A/C ratio from the right/A/C ratio in IVC of <1.0 (0.18). A/C, aldosterone/cortisol; IVC, inferior vena cava.

specificity in differentiating adrenocortical from nonadrenocortical tumors.[40,41] Hennings and coworkers[41] evaluated 212 MTO-PET examinations in 173 patients in correlation with 75 histopathologic examinations in 73 patients. Sensitivity was 89% and specificity was 96% for MTO-PET in proving adrenocortical origin of the lesions. Pheochromocytomas, metastases to the adrenal gland, and nonadrenal masses were all MTO negative. A high 11C-MTO tumor uptake, quantified as the standard uptake values (SUV) 15 to 45 minutes after tracer administration, indicated an adrenocortical adenoma with hormonal overproduction. The SUV ratio between the tumor and the contralateral gland was significantly higher in all hormonally secreting adenomas. Burton and coworkers[42] tested the accuracy of 11C-MTO PET-CT scans in 15 patients with an adenoma and successful AVS study. They found that MTO qualitatively distinguished small tumors (>5 mm) from normal adrenal in all patients. They concluded that MTO offers a noninvasive technique to visualize subcentimeter adrenal adenomas and differentiate functional from nonfunctional adrenal tumors.

However, the technique is complicated and the tracer is very expensive to synthesize. The 20-minute half-life of 11C-MTO makes transportation of the tracer to other centers impossible. However, given an easier, cheaper, and more stable labeling procedure, MTO-PET could be an alternative to AVS for subtype differentiation in PA.

43.2 Results of Adrenal Vein Sampling for Plasma Aldosterone and Cortisol Concentration in the Patient with Left Adrenal Gland Hyperplasia (Shown in Figure 43.2)

Site	Aldosterone (ng/dL)	Cortisol (μg/dL)	A/C ratio
Right	3,007	1,257	2.39
Left	6,814	1,253	5.44
IVC (peripheral)	55	31	1.79

Adrenal vein sampling was performed with constant infusion of Cosyntropin (50 μg/hr). The adrenal vein:IVC cortisol ratios exceed 3:1 indicating successful cannulation of both adrenal veins. The A/C ratio from the left/A/C ratio from the right is <4 (2.28) indicating no lateralization. In addition, the A/C ratio from the right/A/C ratio in peripheral vein is >1.0 (1.34), indicating nonsuppression of aldosterone secretion from the right adrenal gland. A/C, aldosterone/cortisol; IVC, inferior vena cava.

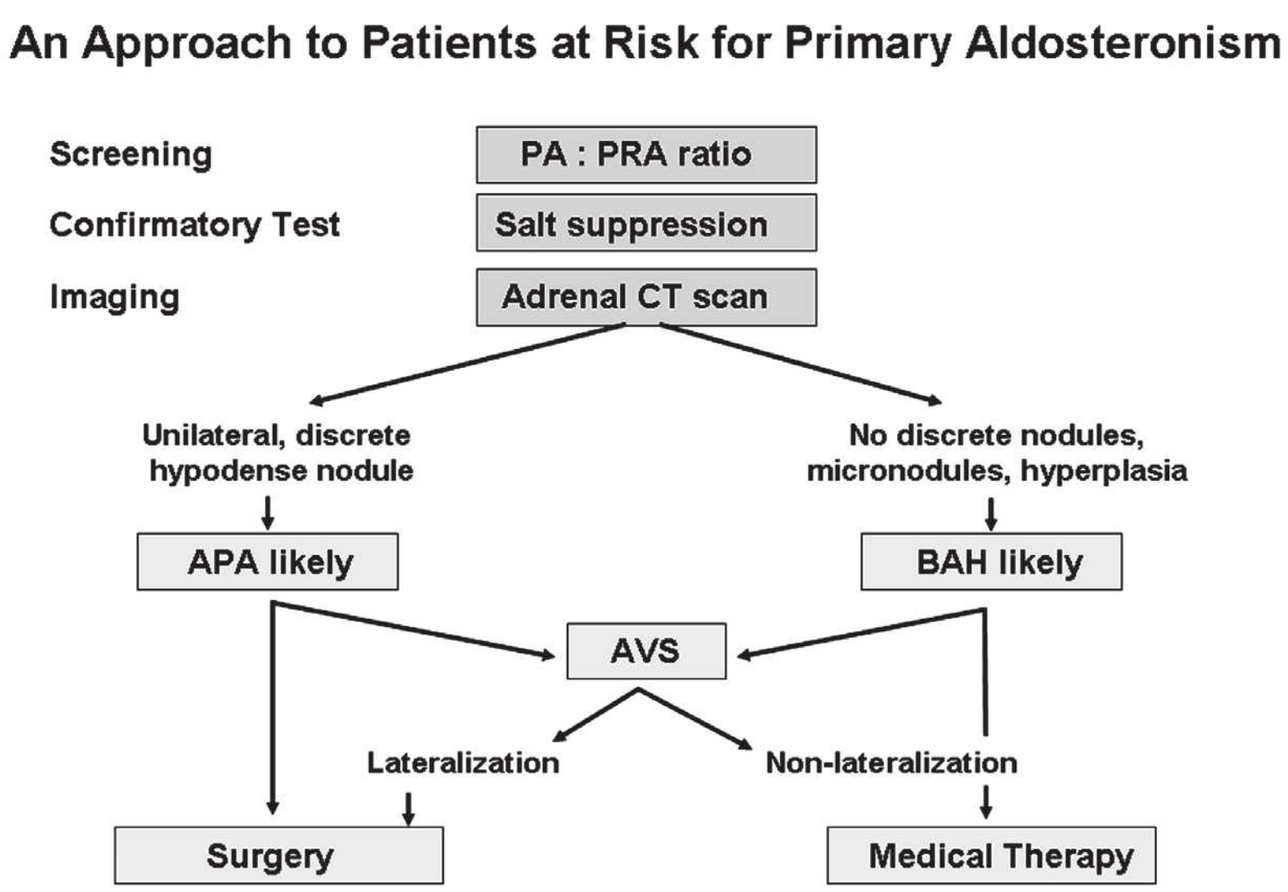

FIGURE 43.3 An approach to patients at risk for primary aldosteronism. *PA*, plasma aldosterone; *PRA*, plasma renin activity; *APA*, aldosterone-producing adenoma; *BAH*, bilateral adrenal hyperplasia; *AVS*, adrenal vein sampling.

Treatment

Medical

Medical therapy for the management of PA is directed not only at controlling blood pressure, but also at protecting end organs from the deleterious effects of excess aldosterone levels.[43] Medical therapy is indicated for those with bilateral adrenal hyperplasia or with an adenoma who are poor candidates for surgical intervention. Surgery is the treatment of choice for those with APA, although medical therapy remains an option because these tumors rarely undergo malignant transformation.[43] The hypertension associated with primary aldosteronism is dependent on excess salt and water retention and is best treated by sustained salt and water depletion (Fig. 43.4).[44] The usual doses of hydrochlorothiazide (12.5 to 50 mg per day) or furosemide (80 to 160 mg per day) in combination with an aldosterone antagonist spironolactone (100 to 200 mg per day) provides for an adequate control of blood pressure and a correction of hypokalemia within 2 to 4 weeks. Additional antihypertensive medications may be required for improved blood pressure control.[45] Among potassium sparing diuretics, spironolactone, an aldosterone antagonist, is the preferred agent because of the adverse cardiovascular effects of prolonged exposure to aldosterone. Several studies have documented a mean reduction of systolic blood pressure by 25% and diastolic blood pressure by 22% with spironolactone therapy in patients with primary hyperaldosteronism.[46,47] The most common side effects of spironolactone include dose-dependent breast and nipple tenderness and gynecomastia. Decreased libido and erectile dysfunction in men and menstrual abnormalities and breast tenderness in women have also been reported.

Eplerenone is a highly selective mineralocorticoid antagonist with efficacy similar to spironolactone.[46] It does not have androgen and progestin receptor activity and is better tolerated and a reasonable alternative in those patients who experience adverse effects with spironolactone. Parthasarathy and coworkers[47] conducted a study comparing the antihypertensive effect of eplerenone and spironolactone in patients with PA. They found a significantly greater antihypertensive effect from spironolactone than that from eplerenone. Alternatively, potassium-sparing diuretics such as triamterene or amiloride combined with hydrochlorothiazide may be used when aldosterone antagonist use is limited by its adverse effects (spironolactone) or prohibitive cost (eplerenone).

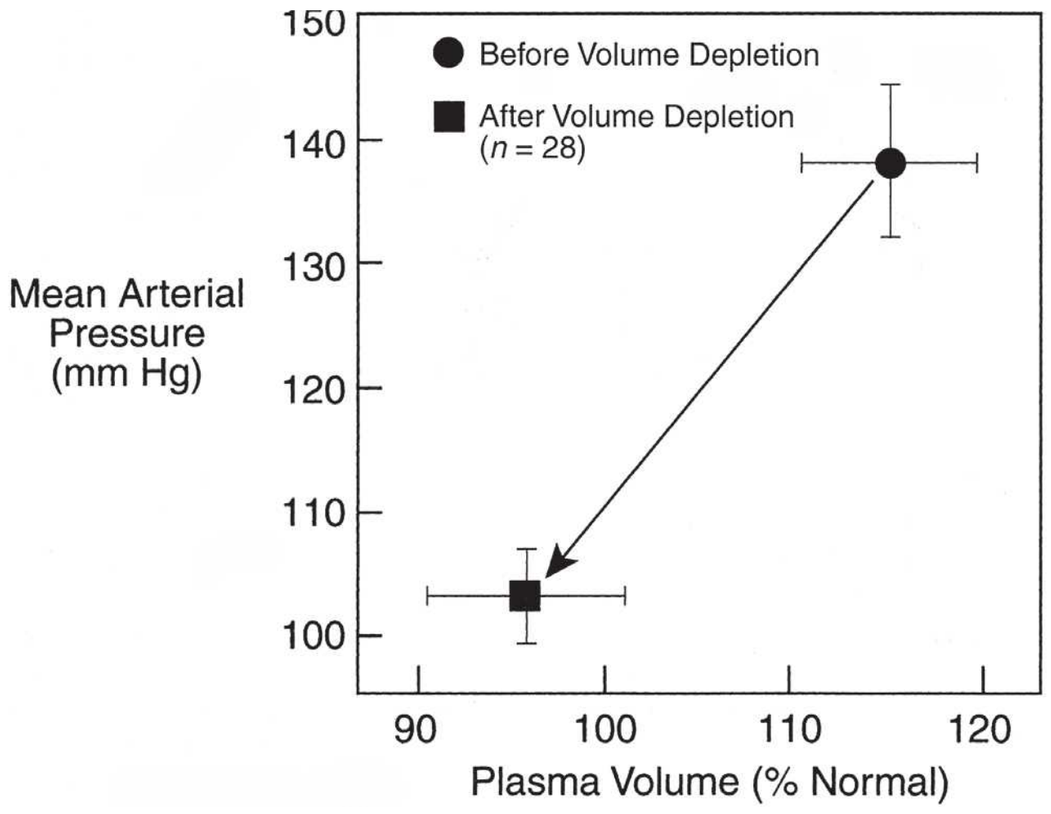

FIGURE 43.4 The effect of diuretic therapy on the blood pressure of patients with primary aldosteronism. Spironolactone (100 mg twice daily) and hydrochlorothiazide (50 to 100 mg per day) were added to current therapy. Blood pressure and plasma volume values were obtained after 8 to 12 weeks of continued therapy. Mean arterial pressure was significantly reduced in all. For the group as a whole, it fell from 138 +/− 2 to 103 +/− 9 (SEM) mm Hg ($P < .01$). Associated with reductions in mean arterial pressure were decreases in plasma volume (from 114% +/− 3% to 97% +/− 2% (SEM), $p < 0.01$. (From Bravo EL. Primary aldosteronism. Issues in diagnosis and management. *Endocrinol Metab Clin North Am*. 1994;23(2):217–282.)

Surgical

In unilateral APA, a laparoscopic adrenalectomy should be considered to minimize risk and postoperative recovery time. In those with bilateral adrenal adenomas, the surgical removal of both adrenal glands is not an option because the adverse metabolic and cardiovascular consequences of adrenal insufficiency are more difficult to treat than the hypertension caused by hyperaldosteronism. Postadrenalectomy, serum potassium levels normalize in all patients. Elevated blood pressure improves to normal levels in 35% to 50% of patients following adrenalectomy and, in those patients who remain hypertensive following surgery, there is a reduction in the number of antihypertensive medications required.[47] In a recent study that followed patients with unilateral APA, there was significant improvement in quality of life after undergoing adrenalectomy.[48] Patients undergoing surgery should receive medical therapy with an aldosterone antagonist for 8 to 10 weeks preoperatively to normalize blood pressure and serum potassium concentrations.

Clinical Outcomes. Significant lowering of blood pressure levels and the normalization of serum potassium occur with both medical[49] (spironolactone) and surgical intervention. After removal of the solitary adenoma, one-third of all cases are cured and free of all therapy, 75% of cases improved with the reduction of antihypertensive therapy, and 100% will have a reversal of hypokalemia. Factors reported to predict cure after an adrenalectomy are response to spironolactone therapy, younger age, shorter duration of hypertension, family history of hypertension in at least one first-degree relative, preoperative use of at least two antihypertensive agents, higher ARR, and 24-hour urinary aldosterone levels.[50,51] A recent study suggests that a unilateral adrenalectomy may be beneficial in carefully selected patients with BAH.[49]

OTHER HYPERTENSIVE DISORDERS ASSOCIATED WITH THE OVERPRODUCTION OF MINERALOCORTICOIDS

Glucocorticoid-Remediable Aldosteronism

Glucocorticoid-remediable aldosteronism (GRA), also known as familial hyperaldosteronism type 1 (FH-1), is an inherited autosomal disorder that mimics an APA. It is characterized by a strong family history of hypertension and moderate-to-severe hypertension often associated with early death from hemorrhagic stroke as the result of ruptured intracranial aneurysms. In a retrospective review of the International Registry of GRA, 48% of all GRA pedigrees and 18% of all GRA patients had cerebrovascular complications.[52] The frequency of intracranial aneurysms is similar to that of adult polycystic kidney disease, and it has been recommended that screening with MRI angiography should be performed in affected patients beginning at puberty, and every 5 years thereafter.

This disorder is caused by a genetic mutation that results in a chimeric gene product that fuses nucleotide sequences of the 11β-hydroxylase[53] and is regulated by ACTH (Fig. 43.5). In addition, the chimeric gene allows for the

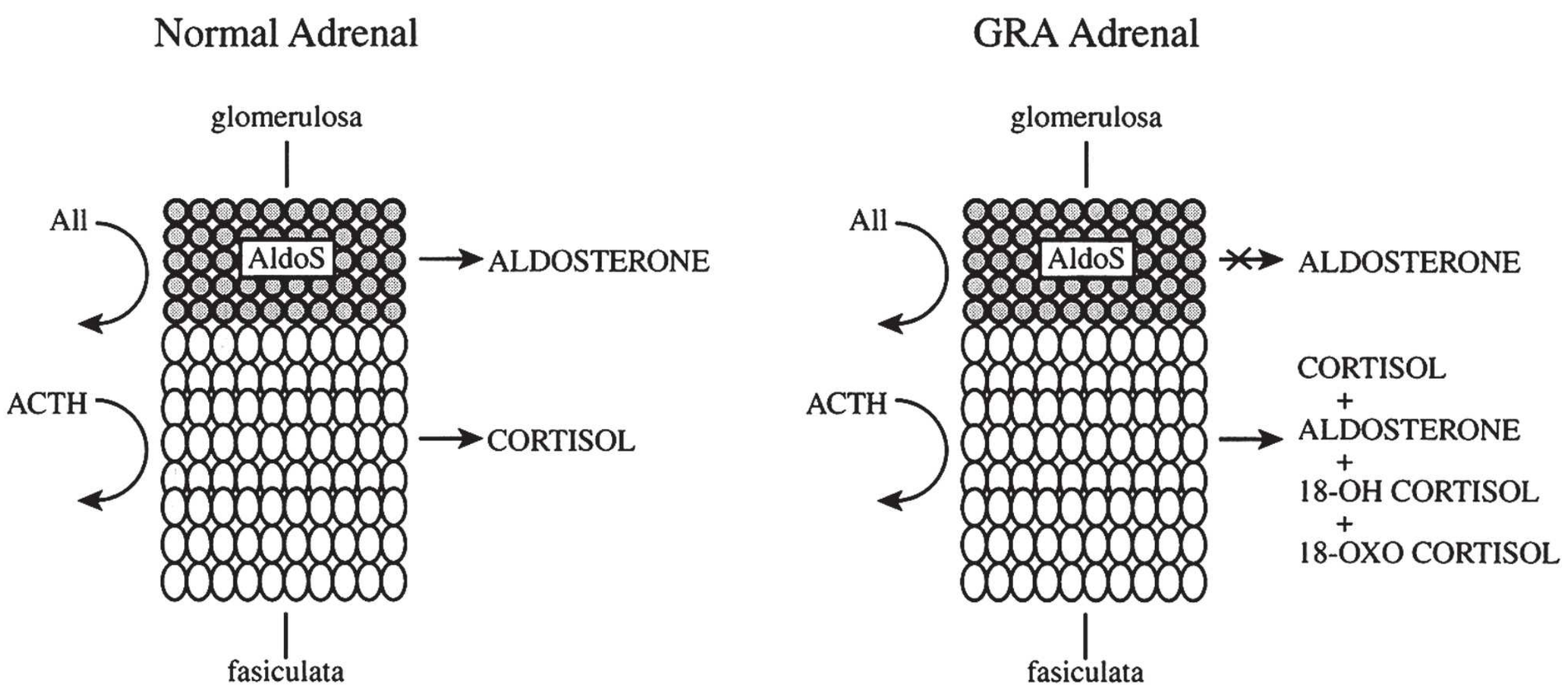

FIGURE 43.5 A model of the physiologic abnormalities in the adrenal cortex in glucocorticoid-remediable aldosteronism (GRA). In the normal adrenal gland, AldoS activity is present only in the adrenal zona glomerulosa. Aldosterone is produced in the glomerulosa layer under the regulation of angiotensin II, and cortisol is secreted from the adrenal fasciculata under the regulation of adrenocorticotropic hormone (ACTH). Steroid 11β-hydroxylase is involved in the biosynthesis of both of these hormones and is expressed in both tissues. The gene is under positive control of ACTH in fasciculata. Ectopic activity of the enzyme in fasciculata results in the metabolism of cortisol to 18-hydroxycortisol and 18-oxocortisol, as well as production of aldosterone from high levels of corticosterone present in fasciculata. These mineralocorticoids are under the control of ACTH, and can consequently be suppressed by exogenous glucocorticoids. (From Lifton RP et al., *Nature Genetics*. 1992;2:66–74.)

ectopic expression of aldosterone synthase enzyme activity in the ACTH-regulated zona fasciculata, which normally secretes only cortisol, but now also secretes aldosterone. Aldosterone secretion is positively and solely regulated by ACTH, not by potassium or angiotensin II. As a consequence, exogenous administration of low-dose glucocorticoid (which suppresses aldosterone secretion in affected individuals) reverses the clinical manifestations of the syndrome.

As in PA, PRA is suppressed, and increased aldosterone production is nonsuppressible by salt loading. Most patients with GRA are not hypokalemic; therefore, serum potassium lacks sensitivity as a screening test for this disorder. Because aldosterone secretion is solely regulated by ACTH, the dexamethasone suppression test has been employed as a diagnostic test. However, the test has a high false-positive rate. For example, Fogari and coworkers[54] reported that only one of eight of their patients with PA that tested positive with the dexamethasone suppression test had the chimeric gene for GRA. Direct genetic screening for the presence of the gene duplication in GRA is 100% sensitive and specific. It is recommended for patients with PA without a radiographic evidence of tumor, for young hypertensive individuals with suppressed PRA (especially children), and for at-risk individuals in affected families. Treatment with low-dose glucocorticoids, amiloride, and aldosterone receptor antagonists effectively controls elevated blood pressure in GRA.

Congenital Disorders of Steroid Hormone Production

P450C11β and 17α deficiencies cause hypertensive variants of congenital adrenal hyperplasia (CAH). Both enzyme deficiencies result in reduced cortisol production with subsequent overproduction of ACTH. In turn, ACTH drives the zona fasciculata to increase the production of precursor steroids with an accumulation of 11–deoxycorticosterone (DOC), a potent mineralocorticoid, leading to hypertension and hypokalemia with suppressed PRA and, unlike PA, virtual absence of aldosterone production. In both enzyme deficiency disorders, inhibiting ACTH release with glucocorticoids decreases DOC production, which results in the normalization of blood pressure and the serum potassium concentration.

In 11β-hydroxylase deficiency, there is a shunting of precursor steroids into the androgen pathway, resulting in the increased formation of androgens, which produce virilization in females or precocious puberty with advanced masculinization in males.[55] This enzyme deficiency clusters in exon 6 to 8 of the CYP11β1 gene and accounts for 15% of cases of CAH in Muslim and Jewish Middle Eastern populations.

By contrast, patients with 17α-hydroxylase deficiency present with hypogonadism in addition to hypertension, hypokalemia, suppressed PRA, and absent aldosterone production.[56] This enzyme deficiency reduces the production of all adrenal and gonadal androgens, resulting in a form of hypergonadotropic hypogonadism and abnormalities of sexual development. The hypogonadal consequence accounts for most of the clinical features of the disorder. Females with this disorder have primary amenorrhea, disproportionately long limbs relative to the trunk, and absent secondary sexual characteristics. Male patients have either ambiguous external genitalia or a female phenotype (male pseudohermaphroditism). A large number of random mutations can cause 17α-hydroxylase deficiency, making genetic diagnosis difficult.

Glucocorticoid Resistance

Cortisol synthesis is regulated through a negative feedback loop in which cortisol feeds back on the pituitary to inhibit ACTH secretion. In generalized inherited glucocorticoid resistance, cortisol remains ACTH dependent but is reset to a higher level than normal.[57] Because the peripheral tissues and pituitary are equally resistant to cortisol, affected individuals do not develop features of Cushing syndrome despite marked elevations in circulating cortisol levels. An ACTH-dependent increase in DOC and in adrenal androgens occurs. The clinical presentation is characterized by virilization and precocious puberty (due to excess adrenal androgens) and by hypertension and hypokalemia (due to excess DOC).

Two strategies are used to treat generalized glucocorticoid resistance. The first employs high amounts of glucocorticoids, such as dexamethasone, to suppress adrenal stimulation by ACTH. Alternatively, mineralocorticoid or androgen antagonists can be used.

HYPERTENSION DUE TO ACTIVATION OF MINERALOCORTICOID RECEPTORS

Syndrome of Apparent Mineralocorticoid Excess

Mineralocorticoid receptors (MR) in the distal nephron of the kidney have equal affinity for their two ligands—aldosterone and cortisol—but are protected from cortisol by the presence of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which inactivates cortisol by converting it to cortisone, preventing full MR occupancy by cortisol despite 100- to 1,000-fold plasma concentrations greater than those of aldosterone (Fig. 43.6).[58] The 11, 18 hemiacetal structure of aldosterone protects it from the action of 11β-HSD2 so that aldosterone has unimpeded access to the receptors. When this mechanism is defective, intrarenal levels of cortisol increase, causing its inappropriate access to MR.[59] The resulting antinatriuresis and kaliuresis leads to hypertension and hypokalemia. Biochemically, PRA is suppressed and aldosterone production is markedly decreased. Elevations in urinary free cortisol excretion and in the ratio of the urinary metabolites of cortisol to those of cortisone, as well as prolongation of the half-life of cortisol, are noted. Plasma cortisol concentrations usually are not elevated.

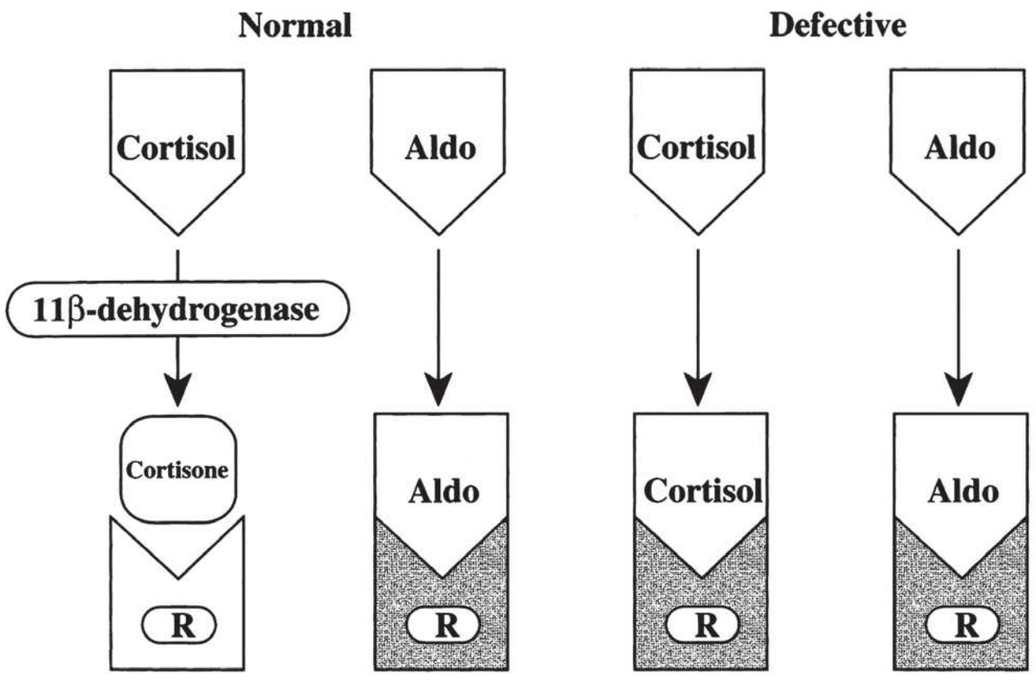

FIGURE 43.6 Enzyme-mediated receptor protection. Normal 11β-hydroxysteroid dehydrogenase converts cortisol to inactive cortisone, protecting mineralocorticoid receptors (R) from cortisol and allowing selective access for aldosterone (Aldo). When 11β-dehydrogenase is defective, cortisol gains inappropriate access to mineralocorticoid receptors with resulting antinatriuresis and kaliuresis. (From Walker BR, Edwards CR. *Endocrinol Metab North Am*. 1994;23:359–377.)

The hypertensive syndrome is reversed by spironolactone or dexamethasone and is exacerbated by the administration of physiologic doses of cortisol.

The recessively inherited apparent mineralocorticoid excess (AME) is caused by a genetic deficiency of 11β-HSD2.[60] Cortisol-mediated excessive mineralocorticoid action results in early-onset severe hypertension, failure to thrive, hypokalemia, suppressed PRA, and low aldosterone levels. The phenotype–genotype of AME may vary widely from mild to severe hypertension depending on the effect of mutation on 11β-HSD2.

Excessive licorice ingestion is known to cause an acquired form of AME. Licorice contains glycyrrhizinic acid, which is hydrolyzed to glycyrrhetinic acid, an inhibitor of 11β-HSD2. This results in an AME-phenotype, including hypertension, hypokalemia, suppressed PRA, and low plasma aldosterone levels. However, because licorice consumption does not always lead to the hypertensive syndrome, Miettinen and coworkers[61] reasoned that genes influencing licorice action may partly determine susceptibility to its side effects. In preliminary studies in human volunteers, those investigators found that a mutation of the 11β-HSD2 gene does not appear to constitute a common cause for licorice-induced hypertensive syndrome. Their studies suggest that subtle variants of the α, β, and λ subunits of the ENaC may render some individuals sensitive to licorice-induced metabolic cardiovascular alterations.

Cushing Syndrome (Resulting from Ectopic Adrenocorticotropic Hormone Excess)

The recognizable causes of Cushing syndrome include Cushing disease (72%), ectopic ACTH excess (12%), adrenal adenoma (8%), carcinoma (6%), and hyperplasia (4%). The typical clinical presentation of Cushing syndrome includes truncal obesity, moon facies, hypertension, plethora, muscle weakness and fatigue, hirsutism, emotional disturbances, and typical purple skin striae. Carbohydrate intolerance or diabetes, amenorrhea, loss of libido, easy bruising, and spontaneous fracture of ribs and vertebrae may also be encountered. Patients with ectopic ACTH excess may not have the typical manifestations of cortisol excess, but they may present with hyperpigmentation of the skin, severe hypertension, and marked hypokalemic alkalosis.

The incidence of hypokalemic alkalosis in the ectopic ACTH syndrome is greater than 90%, compared with only 10% in Cushing syndrome of other causes.[62] It is widely supposed that corticosterone or 11-DOC is responsible for mineralocorticoid excess, but poor correlation exists between the levels of these steroids and the degree of hypokalemia. A better predictor of hypokalemia is the level of cortisol.[63,64] Several studies suggest that the ratio of cortisol to cortisone metabolites is increased in all forms of Cushing syndrome. Ulick and associates[65] advanced the hypothesis that excessive circulating cortisol overwhelms the enzyme, thus escaping conversion of cortisol to cortisone and gaining inappropriate access to Mrs. Walker and coworkers[66] demonstrated a negative correlation between the extent of impairment of 11β-hydroxysteroid dehydrogenase and plasma potassium concentration in 26 patients with Cushing syndrome, 9 of whom had higher cortisol-to-cortisone ratios than the 15 patients with pituitary Cushing and the 2 patients with adrenal adenomas.

The determination of a 24-hour urinary free cortisol concentration is the best available test for documenting endogenous hypercortisolism.[67] A level of higher than 100 μg per 24 hours suggests excessive cortisol production. There are virtually no false-negative results. False-positive results may be obtained in non-Cushing hypercortisolemic states (e.g., stress, chronic strenuous exercise, psychiatric states, glucocorticoid resistance, malnutrition). If a differentiation between pituitary and ectopic sources of ACTH cannot be made based on plasma levels alone, pharmacologic manipulation of ACTH secretion should be performed. The overnight dexamethasone suppression test requires only a blood collection for serum cortisol the morning after the patient has taken a 1.0-mg dose of dexamethasone at 11 p.m. the previous evening. In physiologically normal subjects, cortisol levels at 8 a.m. will be suppressed to 5.0 μg per deciliter or less.

When the syndrome has been diagnosed by appropriate biochemical testing, the cause must be identified. A radioimmunoassay of plasma ACTH is the procedure of choice for pinpointing the basis of hypercortisolism, but this test is not available in many hospitals. In patients with ACTH-independent Cushing syndrome, ACTH levels have usually been suppressed to less than 5 pg per milliliter. In contrast, patients with the ACTH-dependent form tend to have either normal or elevated levels of ACTH, usually higher than 10 pg per milliliter. In patients with Cushing disease

43.3 Humoral Characteristics of Mineralocorticoid-Dependent Hypertension

Disorder	Aldosterone	Cortisol	Androgen
Primary aldosteronism (PA)	High	Normal	Normal
Glucocorticoid-remediable aldosteronism (GRA)	High	Normal	Normal
Excess deoxycorticosterone production			
– 11β-hydroxylase deficiency	**Low**	**Low**	**High**
– 17α-hydroxylase deficiency	**Low**	**Low**	**Low**
– Glucocorticoid resistance	**Low**	**Very high**	**High**
Apparent mineralocorticoid excess	Low	Normal	Normal
Ectopic ACTH excess	Low	Very high	Normal

ACTH, adrenocorticotropic hormone.

(i.e., basophilic pituitary microadenomas), ACTH release can be inhibited only at much higher doses of dexamethasone (2 mg every 6 hours for 2 days). The established criterion for the test is that suppression of the 24-hour urine and plasma steroids to less than 50% of baseline indicates pituitary Cushing syndrome (i.e., Cushing disease). Failure to suppress these concentrations to less than 50% of baseline is considered consistent with an ectopic source of ACTH or ACTH-independent Cushing syndrome. The best way to differentiate pituitary ACTH excess from the ectopic production of ACTH is with the inferior petrosal sinus procedure for ACTH concentration, which is invasive and carries its own risks.[68] The test has been characterized in the literature as having 100% sensitivity and 100% specificity. The criterion currently used after corticotropin-releasing hormone administration is that the ACTH gradient between the inferior petrosal sinus and the peripheral site will be greater than 2 if the patient has Cushing disease.

Table 43.3 shows the humoral characteristics of mineralocorticoid-dependent hypertension.

Liddle Syndrome

Liddle syndrome is a rare autosomal dominant disorder with variable penetrance. Patients with Liddle syndrome clinically present with hypertension, hypokalemia, and metabolic alkalosis at a relatively young age. However, some patients with Liddle syndrome are not hypokalemic[69] at presentation. Sporadic cases of Liddle syndrome have also been described.[70] Thus, the absence of hypokalemia at presentation and/or the absence of a family history do not preclude the diagnosis. In the absence of hypokalemia, a positive family history of hypertension at a young age, with some members being hypokalemic, should lead to the suspicion of the genetic disorder.

The defect in Liddle syndrome results from the constitutive activation of amiloride-sensitive epithelial sodium channels (ENaC) on distal renal tubules, which causes excessive sodium reabsorption. This channel is composed of at least three subunits and is normally regulated by aldosterone. The mutations causing Liddle syndrome have been localized to genes on chromosome 16p12 that encodes the β and λ subunits of ENaC.[71] Deletions or substitutions in a short proline-rich segment of the intracytoplasmic C-terminus result in the inability of these subunits to bind with an intracellular protein ligase (Nedd4) that normally removes the luminal sodium channel from the cell surface in response to decreased circulating aldosterone.[72] Failure to remove sodium channels results in an increased number of ENaC at the renal distal apical cell surface. These mutations in "gain-of-function" result in excessive sodium reabsorption (leading to hypertension and suppressed PRA) and increased potassium secretion (producing hypokalemia and metabolic alkalosis). Aldosterone levels are undetectable, and antagonism of MR with specific aldosterone receptor antagonists have no effect on either blood pressure or serum potassium. Hypertension and hypokalemia are effectively treated with sodium deprivation or potassium-sparing agents that block the collecting tubule sodium channels (amiloride or triamterene).[73] Genetic testing is the most reliable method of establishing the diagnosis of Liddle syndrome.[74]

Activating Mutations of Mineralocorticoid receptors

In 2000, Geller and coworkers[75] described a group of women with early onset hypertension (<20 years of age), which was markedly exacerbated by pregnancy. Hypertension in these patients could be very severe and unresponsive to antihypertensive therapy. There were no proteinuria, edema, or neurologic changes, excluding preeclampsia. Hypertension was often accompanied by hypokalemia (with renal potassium-wasting), and suppressed PRA and aldosterone levels, which normally increase 10-fold in pregnancy, were undetectable.

These investigators found a mutation in the MR, S810L, which results in constitutive MR activity and alters specificity, with progesterone and other steroids, lacking 21-hydroxyl groups that are normally MR antagonists, becoming potent agonists of MR L80. Similarly, spironolactone, another MR antagonist commonly used in the treatment of patients with hypertension and hypokalemia, is also a potent agonist of MR L810 and is contraindicated in MR L810 carriers. The hypertension is promptly reversed by delivery.

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CHAPTER

44 Malignant Hypertension and Other Hypertensive Crises

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THE CLINICAL SPECTRUM OF SEVERE HYPERTENSION

The vast majority of hypertensive patients are asymptomatic for many years until complications due to atherosclerosis, cerebrovascular disease, or congestive heart failure develop. In a minority of patients this "benign" course is punctuated by a hypertensive crisis.

A hypertensive crisis is defined as the turning point in the course of an illness at which acute management of the elevated blood pressure plays a decisive role in the eventual outcome. The haste with which the elevated blood pressure must be controlled varies with each crisis. However, the crucial role of hypertension in the disease process must be identified and a plan for management of the blood pressure successfully implemented if the outcome is to be optimal. The absolute level of blood pressure is not the most important factor in determining the existence of a hypertensive crisis. In children, pregnant women, and other previously normotensive individuals in whom moderate hypertension develops suddenly, a hypertensive crisis can occur at a diastolic blood pressure normally well tolerated by adults with chronic hypertension. Furthermore, in adults with only mild to moderate hypertension, a crisis can occur when there is concomitant acute end-organ dysfunction involving the heart or brain.

Approximately 1% to 2% of patients with hypertension will have a hypertensive emergency at some time in their life. A recent study explored changes in the frequency of hospitalizations and in-hospital mortality for hypertensive emergencies before and after the publication of the Seventh Joint National Committee (JNC7) on the prevention, detection, evaluation, and treatment of high blood pressure.[1] Using the Nationwide Inpatient Sample from 2000 to 2007, adult patients hospitalized with a diagnosis of hypertensive emergency were identified based on International Classification of Diseases, 9th revision, clinical modification codes. A total of 456,259 hospitalizations with the diagnosis of hypertensive emergency occurred from the start of calendar year 2000 to the end of calendar year 2007. Analysis revealed that the frequency of hospitalizations in the United States with a hypertensive emergency increased about 1.11% over this time period from 101/100,000 population in 2000 to 111/100,000 population in 2007. Despite this increase in hospitalizations, the all-cause in-hospital mortality rate for hypertensive emergencies decreased from 2.8% in the pre-JNC7 era to 2.6% in the post-JNC7 era (odds ratio [OR] 0.91, 95% confidence interval [CI] 0.86–0.96). The authors conclude that although the number of patients with hypertensive emergency increased from 2000 to 2007, the mortality rates decreased significantly after publication of the JNC7 guidelines. The spectrum of hypertensive crises and other categories of severe hypertension are outlined in Table 44.1.

Malignant hypertension is a clinical syndrome characterized by marked elevation of blood pressure with widespread acute arteriolar injury. The clinical sine qua non of malignant hypertension is the finding of hypertensive neuroretinopathy. Hypertensive encephalopathy is a medical emergency in which cerebral malfunction is attributed to the severe elevation of blood pressure. It is one of the most serious complications of malignant hypertension. However, hypertensive encephalopathy can also occur in the absence of malignant hypertension (neuroretinopathy). Hypertensive encephalopathy can develop in the setting of severe hypertension of any cause, especially when acute blood pressure elevation occurs in previously normotensive individuals with eclampsia, acute glomerulonephritis, pheochromocytoma, or drug withdrawal hypertension. Clinical features include severe headache, blurred vision or blindness, nausea, vomiting, and mental confusion. If aggressive treatment is not initiated, stupor, convulsions, and death can ensue within hours. There is a prompt and dramatic clinical response to antihypertensive therapy.

On occasion, hypertension that is not in the malignant phase (hypertensive neuroretinopathy is absent) may still qualify as a hypertensive crisis when acute end-organ dysfunction occurs in the presence of even moderate hypertension. The term benign hypertension with acute complications includes hypertension complicating acute

44.1 The Clinical Syndromes of Severe Hypertension

Hypertensive crises

- Malignant hypertension (hypertensive neuroretinopathy present)
- Hypertensive encephalopathy
- Benign hypertension with acute complications (acute organ system dysfunction but no hypertensive neuroretinopathy)
  - Acute hypertensive heart failure (acute diastolic dysfunction with pulmonary edema)
  - Atherosclerotic coronary vascular disease
    - Acute myocardial infarction
    - Unstable angina
  - Acute aortic dissection
  - Active bleeding including postoperative bleeding
  - Central nervous system catastrophe
    - Hypertensive encephalopathy
    - Intracerebral hemorrhage
    - Subarachnoid hemorrhage
    - Severe head trauma
  - Catecholamine excess states
    - Pheochromocytoma crisis
    - Monoamine oxidase inhibitor-tyramine interactions
    - Antihypertensive drug withdrawal syndromes
    - Phenylpropanolamine overdose
  - Preeclampsia and eclampsia
  - Poorly controlled hypertension in a patient requiring emergency surgery
  - Severe postoperative hypertension
  - Scleroderma renal crisis
  - Miscellaneous hypertensive crises
    - Severe hypertension complicating extensive burn injury
    - High-dose cyclosporine in children after bone marrow transplantation
    - Autonomic hyperreflexia in quadriplegic patients
    - Severe hypertension with acute rejection or transplant renal artery stenosis in renal allograft recipients
    - Hypoglycemia in patients receiving β-adrenergic receptor blockers

(continued)

44.1 The Clinical Syndromes of Severe Hypertension (continued)

Benign hypertension with chronic stable complications (chronic end-organ dysfunction but no hypertensive neuroretinopathy)
Chronic renal insufficiency due to primary renal parenchymal disease
Chronic congestive heart failure with diastolic dysfunction
Atherosclerotic coronary vascular disease
Stable angina
Previous myocardial infarction
Chronic cerebrovascular disease
Transient ischemic attacks
Prior cerebrovascular accident
Severe uncomplicated hypertension (severe hypertension without hypertensive neuroretinopathy or end-organ dysfunction)

pulmonary edema (acute diastolic dysfunction), acute myocardial infarction or unstable angina, acute aortic dissection, active bleeding, or central nervous system (CNS) catastrophe (hypertensive encephalopathy, intracerebral or subarachnoid hemorrhage, or severe head trauma). In each case, adequate control of the blood pressure is the cornerstone of successful therapy.

Catecholamine excess states—such as pheochromocytoma crisis, monoamine oxidase inhibitor–tyramine interactions, use of sympathomimetic drugs (cocaine, amphetamines, phencyclidine, or high-dose phenylpropanolamine), and abrupt withdrawal of antihypertensive medications (clonidine, methyldopa, or guanabenz)—can produce life-threatening hypertensive crises. The clinical presentation usually includes marked elevation of blood pressure with headache, diaphoresis, and tachycardia. With the severe acute elevation of blood pressure a number of complications can occur, including hypertensive encephalopathy, intracerebral hemorrhage, and pulmonary edema due to acute left ventricular diastolic dysfunction. Thus, catecholamine-related hypertensive crises require prompt recognition and control of blood pressure to avert disaster.

Preeclampsia is a hypertensive disorder unique to pregnancy that usually presents after the 20th week of gestation with proteinuria, edema, and hypertension. Eclamptic seizures may ensue and without treatment may result in death. Eclampsia is considered to be a subtype of hypertensive encephalopathy.[2]

Poorly controlled hypertension in a patient requiring emergency surgery is a hypertensive crisis because of the increased cardiovascular risk that accompanies inadequate preoperative blood pressure control. Surgical manipulation of the carotid arteries or open heart surgery (especially coronary artery bypass) is occasionally followed by severe hypertension in the immediate postoperative period. Severe postoperative hypertension represents a crisis requiring immediate blood pressure control because it can cause hypertensive encephalopathy or intracerebral hemorrhage, or jeopardize the integrity of vascular suture lines and thereby lead to postoperative hemorrhage.

In patients with progressive systemic sclerosis, scleroderma renal crisis can occur with sudden onset of hypertension that may enter the malignant phase. There is a rapid progression to end-stage renal disease (ESRD) within days to weeks unless the vicious cycle of hypertension, renal ischemia, and activation of the renin–angiotensin–aldosterone axis is interrupted.

Severe acute hypertension can also occur in patients with extensive burns or children receiving high-dose cyclosporine for allogeneic bone marrow transplantation. In quadriplegic patients, hypertensive crises may develop due to autonomic hyperreflexia resulting from stimulation of nerves below the level of the spinal cord injury. Hypertensive crises due to autonomic hyperreflexia can also develop in Guillain-Barré syndrome. Hypertensive crises may also complicate acute rejection or transplant renal artery stenosis

in patients with renal allografts. In each of these conditions, a sudden increase in blood pressure may cause acute pulmonary edema, hypertensive encephalopathy, cerebrovascular accident, and death.

On the other hand, severe hypertension or the presence of hypertensive complications does not always imply the existence of a hypertensive crisis requiring immediate control of the blood pressure. Patients with benign hypertension (no hypertensive neuroretinopathy) and chronic stable end-organ dysfunction do not require emergent reduction of blood pressure, although a long-term lack of adequate blood pressure control often results in further deterioration of end-organ function. The term benign hypertension with chronic stable complications includes hypertension occurring in the setting of primary renal parenchymal disease with chronic kidney disease, chronic congestive heart failure, atherosclerotic coronary vascular disease (stable angina pectoris or prior myocardial infarction), or chronic cerebral vascular disease (prior transient ischemic attacks or cerebrovascular accident).

It is important to emphasize that the finding of severe hypertension does not always imply that a hypertensive crisis is present. In patients with severe hypertension that is not accompanied by acute end-organ dysfunction or evidence of malignant hypertension (hypertensive neuroretinopathy) eventual complications due to stroke, myocardial infarction, or congestive heart failure occur over a time frame of months to years rather than hours to days. Although long-term control of blood pressure can prevent these complications, a hypertensive crisis cannot be diagnosed, as there is no evidence that acute reduction of blood pressure results in any improvement in short-term or long-term prognosis. Severe uncomplicated hypertension is defined by a diastolic blood pressure higher than 115 mm Hg without evidence of malignant hypertension (no hypertensive neuroretinopathy) or signs of acute end-organ dysfunction. Although this is not a true hypertensive crisis as defined earlier, it is the most common presentation of severe hypertension. Severe uncomplicated hypertension is usually found in patients with chronic essential hypertension who are undiagnosed, undertreated, or not adherent with medical therapy. It is most often discovered incidentally in an otherwise asymptomatic patient. There is no evidence of hypertensive encephalopathy or other acute end-organ dysfunction. The fundi do not show striate hemorrhages, cotton-wool spots, or papilledema. Because the potential complications of severe uncomplicated hypertension develop with a time frame of months to years, the once common practice of abrupt reduction of blood pressure with oral antihypertensive agents prior to discharge from the acute care setting is no longer accepted as the standard of care.[3–5] Instead, the goal of treatment should be the gradual reduction of blood pressure to normotensive levels over a few days in conjunction with frequent outpatient follow-up visits to modify the antihypertensive regimen and reinforce the importance of lifelong adherence with medical therapy. In the past this entity has been termed urgent hypertension. Use of the more descriptive term severe uncomplicated hypertension is preferable because there is no need for urgent reduction of blood pressure as would be required in patients with true hypertensive crises.

MALIGNANT HYPERTENSION

Etiologies of Malignant Hypertension

Hypertension of virtually any etiology can enter a malignant phase (Table 44.2). Thus, malignant hypertension is not a single disease entity but rather a syndrome in which hypertension can be either primary (essential) or secondary to

44.2 Etiologies of Malignant Hypertension

Primary (essential) malignant hypertension[a]
Secondary malignant hypertension
Chronic kidney disease
Chronic glomerulonephritis[a]
Chronic pyelonephritis[a]
Analgesic nephropathy[a]
Immunoglobulin A nephropathy[a]
Acute glomerulonephritis
Radiation nephritis
Ask-Upmark kidney
Renovascular hypertension[a]
Oral contraceptives
Renal cholesterol embolization
Scleroderma renal crisis
Antiphospholipid (anticardiolipin) antibody syndrome
Chronic lead poisoning
Endocrine hypertension
Pheochromocytoma
Aldosterone-producing adenoma
Cushing syndrome
Congenital adrenal hyperplasia

[a]Most common underlying etiologies.

one of any number of different etiologies.[10] Moreover, in the individual patient with malignant hypertension, on clinical grounds it is often difficult to distinguish whether the underlying hypertension is primary or secondary.

Malignant hypertension usually develops in patients with preexisting, poorly controlled, or undiagnosed hypertension. However, occasional patients have been described who experience an abrupt onset of so-called de novo malignant hypertension without a preceding phase of benign hypertension.[7] The presence of de novo malignant hypertension almost always indicates an underlying secondary cause of hypertension.[7]

Primary (Essential) Malignant Hypertension

In the era prior to the introduction of antihypertensive drugs, malignant hypertension evolved from underlying essential hypertension in more than 50% of patients.[8] However, more recent series found a lower incidence of primary malignant hypertension, most likely reflecting prevention of malignant hypertension through effective control of blood pressure among patients with essential hypertension.[9] In a series of patients collected between 1979 and 1985, primary malignant hypertension was found in only 20%.[10] This observation may not apply to black patients, because among blacks, essential hypertension continues to represent the most common underlying etiology of malignant hypertension.[11–13] Essential hypertension appears to be a rare cause of malignant hypertension in children. Secondary causes of hypertension such as chronic pyelonephritis, chronic glomerulonephritis, and renovascular hypertension are much more common in this younger age group.[14]

Secondary Malignant Hypertension

The most common secondary cause of malignant hypertension is primary renal parenchymal disease. Chronic glomerulonephritis was reported to underlie the development of malignant hypertension in up to 20% of patients.[10] Unless a history of an acute nephritic episode or long-standing hematuria or proteinuria is available, the underlying glomerulonephritis may be apparent only if a renal biopsy is performed. Underlying IgA nephropathy has been reported as a relatively common secondary cause of malignant hypertension.[14,15] Vesicoureteral reflux with chronic pyelonephritis may lead to malignant hypertension in children and young adults.[16] Superimposed malignant hypertension may also occur as a complication of analgesic nephropathy.[23] Malignant hypertension may develop as an early or late complication of radiation nephritis.[17] Renovascular hypertension due to either fibromuscular dysplasia or atherosclerotic renal artery stenosis is a well-recognized cause of malignant hypertension. In a series of 123 patients with malignant hypertension, renovascular hypertension was found in 43% of white patients and 7% of black patients.[18] Scleroderma renal crisis is the most acute and life-threatening manifestation of progressive systemic sclerosis. It is characterized by severe hypertension (sometimes malignant) with rapidly progressive renal failure. In one large series, scleroderma renal crisis occurred in 7% of white patients and 21% of black patients with progressive systemic sclerosis.[19] The renal histology in scleroderma renal crisis is often virtually indistinguishable from that of primary malignant nephrosclerosis.[20] However, in progressive systemic sclerosis, involvement of the renal vasculature, with proliferative endarteritis involving the interlobular arteries and fibrinoid necrosis of the afferent arterioles, may be a primary event that precedes either hypertension or renal insufficiency.[20] The renal ischemia that results from these lesions causes hypertension through activation of the renin–angiotensin system, leading to a vicious cycle of severe hypertension and renal ischemic injury. Scleroderma renal crisis was once a uniformly fatal complication of progressive systemic sclerosis. With the introduction of angiotensin-converting enzyme (ACE) inhibitors as treatment, outcomes have improved significantly, although 39% to 50% of patients with scleroderma renal crisis continue to have poor outcomes, including ESRD and death.

Epidemiology of Malignant Hypertension

Incidence

Although malignant hypertension is often a complication of preexisting hypertension, the risk of its development in hypertensive patients is difficult to estimate. In early series the incidence of malignant hypertension among hypertensive patients was 1% to 7%.[21] In the era of effective antihypertensive therapy for benign hypertension, the incidence of malignant hypertension appears to have declined to some extent. A review of death certificates in New York City between 1958 and 1974 revealed that the overall mortality due to malignant hypertension had declined by 78% from 2.25 deaths to 0.48 deaths/100,000 population/year.[22] Although some of the decreased mortality was probably due to successful treatment of patients with malignant hypertension with antihypertensive drugs and dialysis, the authors speculated that the overall incidence of malignant hypertension had declined to less than 1% due to successful treatment of benign hypertension. However, despite recent advances in the treatment of essential hypertension, malignant hypertension is clearly not a disease that has vanished. In the United States, during the period from 1983 to 1992, the number of hospital admissions with malignant hypertension or accelerated hypertension as the primary diagnosis (International Classification of Diseases, ICD-9 Code 401.0) doubled from approximately 16,000 to 32,000. Moreover, the number of admissions in which one of these conditions was listed as a diagnosis tripled from approximately 23,000 to 75,000.[23] Reported experience in a multiracial population in England indicates that malignant hypertension is still common with a small proportion of hypertensive patients presenting with malignant hypertension each year.[24] The incidence rate of malignant hypertension for the entire population was approximately one to two cases/100,000/year. Moreover, the

incidence rate did not change over the 24-year period from 1970 to 1993. Recent studies have examined the changing demography of patients with malignant hypertension over the last 40 years.[25,26] The incidence rate for malignant hypertension has remained relatively stable over time. In one study from the United Kingdom, 446 patients with malignant hypertension were included.[25] Mean age was 48 ± 12 years, 65.5% were male gender, 64.7% white European, 20.4% African-Caribbean, and 14.8% South Asian. No significant demographic differences at diagnosis were evident over the 40 years, with the exception of a significant increase in the proportion of malignant hypertension among ethnic minorities (South Asian and Afro-Caribbeans).

Age

Malignant hypertension tends to occurs more frequently in younger subjects. The mean age of patients with malignant hypertension ranges from 40 to 50 years, with 57% of patients between 30 and 50 years old.[21] No difference has been found in the age at onset in men compared to women or whites compared to blacks.[21,27] The age dependency of malignant hypertension could be related to the increased frequency of secondary, more severe forms of hypertension in the young. Alternatively, it is possible that hypertension in patients destined to enter the malignant phase may be more rapidly progressive from the onset, so that the disease would be expected to occur predominantly in younger patients. Malignant hypertension is a rare development in patients beyond the age of 65.[28] The declining incidence of malignant hypertension in patients with essential hypertension relative to age is in marked contrast to the overall incidence of benign hypertension, which reaches a peak in the eighth decade. Patients over age 60 with malignant hypertension usually have underlying renovascular hypertension or primary renal parenchymal disease.[21] In most series of patients with malignant hypertension, males predominate over females by as much as 2 to 1.[21,27]

Race

Blacks have an increased incidence of essential hypertension compared to whites. Moreover, several studies demonstrate that blacks with essential hypertension also have an increased risk of developing malignant hypertension. In a population in which 31% of all hypertensive patients were black, 46% of 200 patients with malignant hypertension were found to be black.[29] In a study of 135 pairs of black and white hypertensive patients matched for age and gender, 4.4% of the black patients had retinopathy consistent with malignant hypertension, whereas only 0.74% of the white patients had these funduscopic findings.[29] The increased frequency of malignant hypertension among blacks may be due to the fact that they presented later in the course of essential hypertension, that antihypertensive therapy in blacks was inadequate to prevent the development of malignant hypertension, or that essential hypertension may be more aggressive and likely to enter the malignant phase in blacks than whites.[30]

Preceding Duration of Benign Hypertension

Although there are occasional case reports in which the malignant phase appears to begin de novo, the majority of patients show evidence of a variable period of preceding benign hypertension before the onset of malignant hypertension. Among 77 patients with malignant hypertension, the documented duration of benign hypertension was 0 to 6 months in 4%, 6 months to 1 year in 10%, 1 to 2 years in 12%, 2 to 4 years in 23%, 4 to 6 years in 16%, 6 to 8 years in 17%, and 8 to 10 years in 4%. Only 14% had benign hypertension for more than 10 years prior to the onset of the malignant phase.[21]

Additional Risk factors for Hypertensive Crisis

A number of additional risk factors have been associated with hypertensive crisis. These include smoking[31,33] and obesity.[41] However, a major under recognized risk factor for hypertensive crisis is nonadherence to therapeutic regimens. In a recent study of 89 patients at a single center, 33 potential risk factors were assessed. Nonadherence to antihypertensive medications was the most important risk identified.[34]

Clinical Features of Malignant Hypertension

The clinical features of untreated malignant hypertension as outlined by Volhard and Fahr in 1914[35] are still valid today: (1) elevation of diastolic blood pressure, usually fixed and severe; (2) funduscopic changes of hypertensive neuroretinopathy with striate hemorrhages, cotton-wool spots, and papilledema; (3) renal insufficiency; (4) rapid progression to a fatal outcome, usually due to uremia if inadequately treated; and (5) renal histology demonstrating malignant nephrosclerosis with fibrinoid necrosis of afferent arterioles and proliferative endarteritis of interlobular arteries.

Unless hypertensive neuroretinopathy is present, malignant hypertension cannot be diagnosed regardless of the height of the arterial blood pressure.[36] However, the other clinical features need not be present initially to substantiate a diagnosis of malignant hypertension. There is no critical level of blood pressure that defines the presence of malignant hypertension. An acute increase in blood pressure in previously normotensive individuals can precipitate the malignant phase at a diastolic blood pressure as low as 100 to 110 mm Hg. Conversely, very high diastolic blood pressures may persist for many years in patients with essential hypertension without the development of malignant hypertension.[37]

With untreated malignant hypertension, severe renal impairment inevitably occurs, although there may be minimal renal involvement at the time of presentation. In this regard, in patients dying early in the course of malignant hypertension due to cerebrovascular accident or congestive heart failure, histologic features of malignant nephrosclerosis may be absent.

Some authors have distinguished accelerated hypertension (hemorrhages and cotton-wool spots) from malignant hypertension (hemorrhages, cotton-wool spots, and papilledema). However, since the finding of striate hemorrhages and cotton-wool spots has the same prognostic significance whether or not papilledema is present,[38,39] it has been recommended that accelerated hypertension and malignant hypertension be regarded as synonymous terms for a clinical syndrome in which there is widespread hypertension-induced acute arteriolar injury. In this regard, the World Health Organization has recommended that the term malignant hypertension be used to describe this disease process.[36]

Presenting Symptoms

The most common presenting complaints in patients with malignant hypertension are headache, blurred vision, and weight loss. Less common presenting symptoms include dyspnea, fatigue, malaise, gastrointestinal complaints (nausea, vomiting, epigastric pain), polyuria, nocturia, and gross hematuria. In many series, the onset of symptoms was noted to be remarkably sudden, such that it could often be dated precisely. In contrast, an "asymptomatic" presentation of malignant hypertension is not uncommon, especially in young black males who deny any prior symptoms when they present in the end-stage of the hypertensive process with florid failure of the brain, heart, and kidneys.

Weight loss is a very common symptom early in the course of malignant hypertension, and often occurs before the onset of anorexia or uremia.[21] In many patients, at least a portion of the weight loss can be attributed to volume depletion resulting from a spontaneous natriuresis with the onset of malignant hypertension.[17,21]

Level of Blood Pressure

There is apparently no absolute level of blood pressure above which malignant hypertension invariably occurs. In most series of patients with malignant hypertension, the average diastolic blood pressure is higher than 120 to 130 mm Hg.[21] However, two series found considerable overlap of blood pressure levels in patients with benign and malignant hypertension.[21,40]

Funduscopic Manifestations

Examination of the ocular fundus is of great importance in the assessment of patients with severe hypertension, especially with regard to prognosis.[41–44]

Although the original classification of hypertensive retinopathy by Keith[45] has proven useful, a number of authorities have recommended abandonment of the Keith and Wagener classification in favor of the hypertensive retinopathy classification initially proposed by Fishberg and Oppenheimer.[42] This classification draws a distinction between retinal arteriosclerosis with arteriosclerotic retinopathy, which is characteristic of benign hypertension, and hypertensive neuroretinopathy, which defines the presence of malignant hypertension (Table 44.3). In essence, two different types of retinal disease occur in patients with hypertension: one that reflects changes induced by arteriolar narrowing (retinal arteriosclerosis) and one that represents acute retinal vascular injury induced by severe hypertension (hypertensive neuroretinopathy).

Retinal arteriosclerosis with or without arteriosclerotic retinopathy is seen in patients with long-standing benign hypertension from either primary or secondary causes. Retinal arteriosclerosis (arteriolosclerosis) is characterized histologically by the accumulation of hyaline material in arterioles. Funduscopic changes reflecting retinal arteriosclerosis include irregularity of the lumen and focal narrowing, arteriovenous crossing changes, broadening of the light reflex, copper or silver wiring, perivasculitis (parallel white lines around blood column), and generalized arteriolar narrowing. Arteriosclerotic retinopathy, which results from this arteriosclerotic process, is manifested by the presence of hemorrhages and hard exudates. The hemorrhages are usually solitary, round, or oval and confined to the

44.3 Retinal Changes in Hypertension

Retinal arteriosclerosis and arteriosclerotic retinopathy
Arteriolar narrowing (diffuse)
Focal arteriolar narrowing
Arteriovenous crossing changes
Broadening of the light reflex
Copper or silver wiring
Perivasculitis
Solitary round hemorrhages
Hard exudates
Central or branch venous occlusion
Hypertensive neuroretinopathy
Generalized arteriolar narrowing
Striate (flame-shaped) hemorrhages[a]
Cotton-wool spots (soft exudates)[a]
Bilateral papilledema[a]
Macular star

[a]Features that distinguish hypertensive neuroretinopathy (characteristic of malignant hypertension) from retinal arteriosclerosis (characteristic of benign hypertension).

periphery of the fundus. They are caused by venous or arterial occlusion.[43] Hard exudates may appear as multiple small white dots that give a powdery appearance to the retina, or they may appear as large glistening spots that are sharply defined from the adjacent retina. Arteriosclerotic retinopathy can also cause localized areas of retinal edema and hemorrhage due to occlusion of small branch veins. However, the principal findings of hypertensive neuroretinopathy, namely, striate hemorrhages, cotton-wool spots, and papilledema, are absent (Table 44.3). The finding of retinal arteriosclerosis in hypertensive patients usually does not imply a poor prognosis.

The lack of clinical significance of retinal arteriosclerosis in hypertensive patients contrasts markedly with the importance and prognostic significance of the finding of hypertensive neuroretinopathy. The appearance of striate hemorrhages and cotton-wool spots with or without papilledema closely parallels the development of severe arteriolar damage (fibrinoid necrosis and proliferative endarteritis) in the circulation of other organs including the brain and kidneys. Hypertensive neuroretinopathy is the clinical sine qua non of malignant hypertension and therefore signifies a far more ominous prognosis than does the finding of retinal arteriosclerosis in benign hypertension.

The appearance of small striate (so-called flame-shaped) hemorrhages is often the first sign that malignant hypertension has developed (Fig. 44.1).

Cotton-wool spots are the most characteristic feature of malignant hypertension and are the result of ischemic infarction of nerve fiber bundles caused by arteriolar occlusion. They usually surround the optic disc and most commonly occur within three disc diameters of the optic disc (Figs. 44.2 to 44.4). Cotton-wool spots begin as grayish-white discoloration of the retina, but within 24 hours they become shiny white with fluffy margins. Red dots may be seen in the bed of the exudate (microaneurysms). Cotton-wool spots are not specific for hypertensive neuroretinopathy and can also be seen with diabetic retinopathy, retinal emboli, and central and branch retinal vein occlusion. However, differentiation of these disorders from malignant hypertension is usually not difficult.

Papilledema can occur in patients with hypertensive neuroretinopathy, but it is not invariably present. In malignant

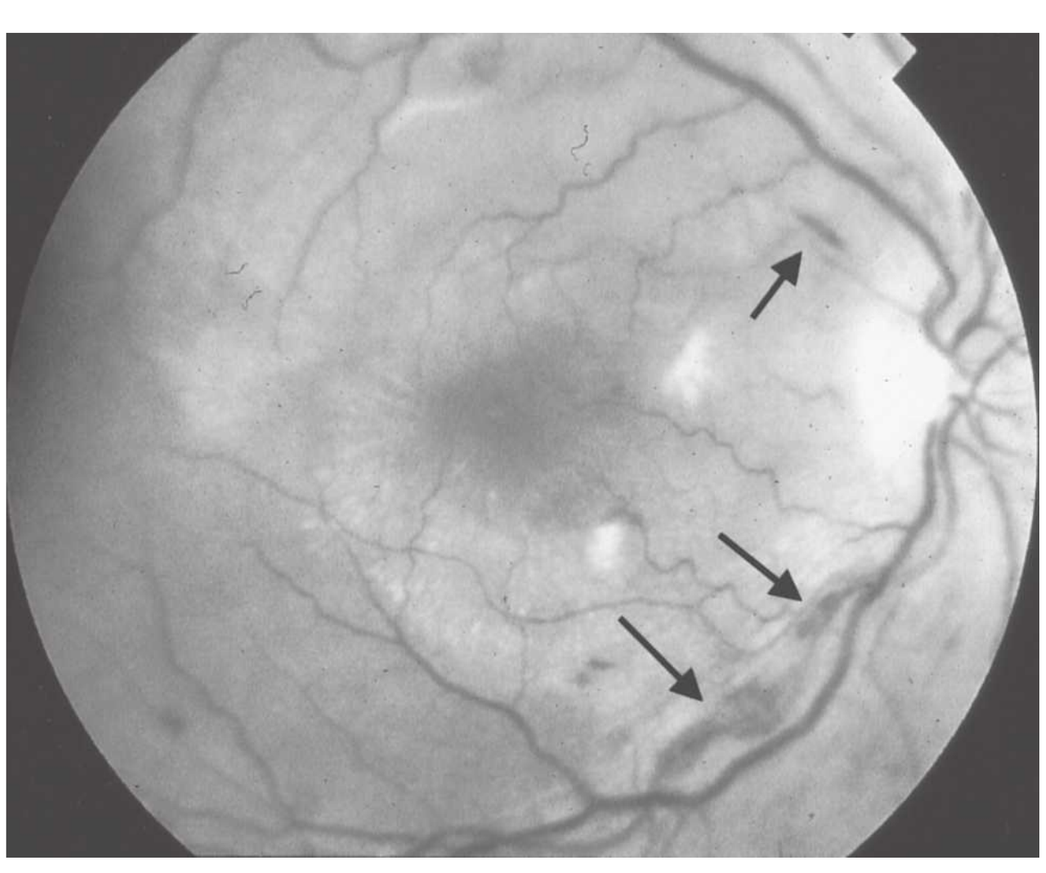


FIGURE 44.1 Striate hemorrhages (*arrows*) in the fundus of a 48-year-old white woman with secondary malignant hypertension due to underlying immunoglobulin A nephropathy.

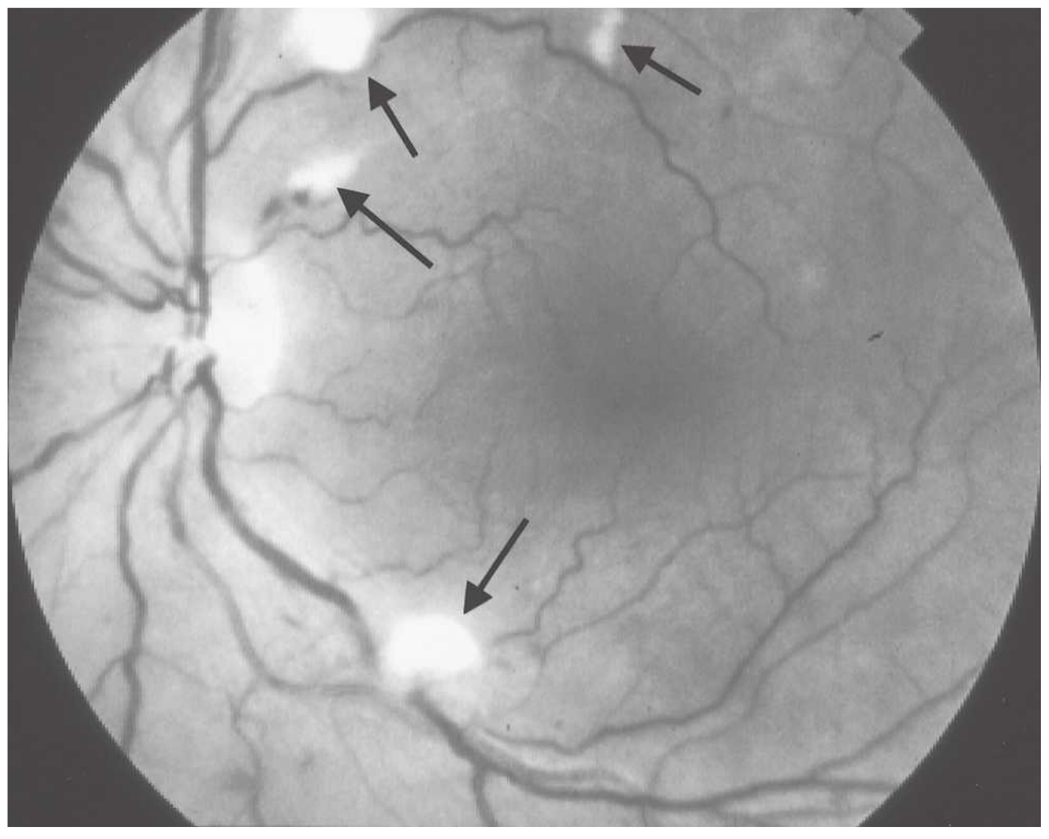


FIGURE 44.2 Cotton-wool spots (*arrows*) in the fundus of a 48-year-old white woman with secondary malignant hypertension due to underlying immunoglobulin A nephropathy. Striate hemorrhages are also seen adjacent to some of the cotton-wool spots.

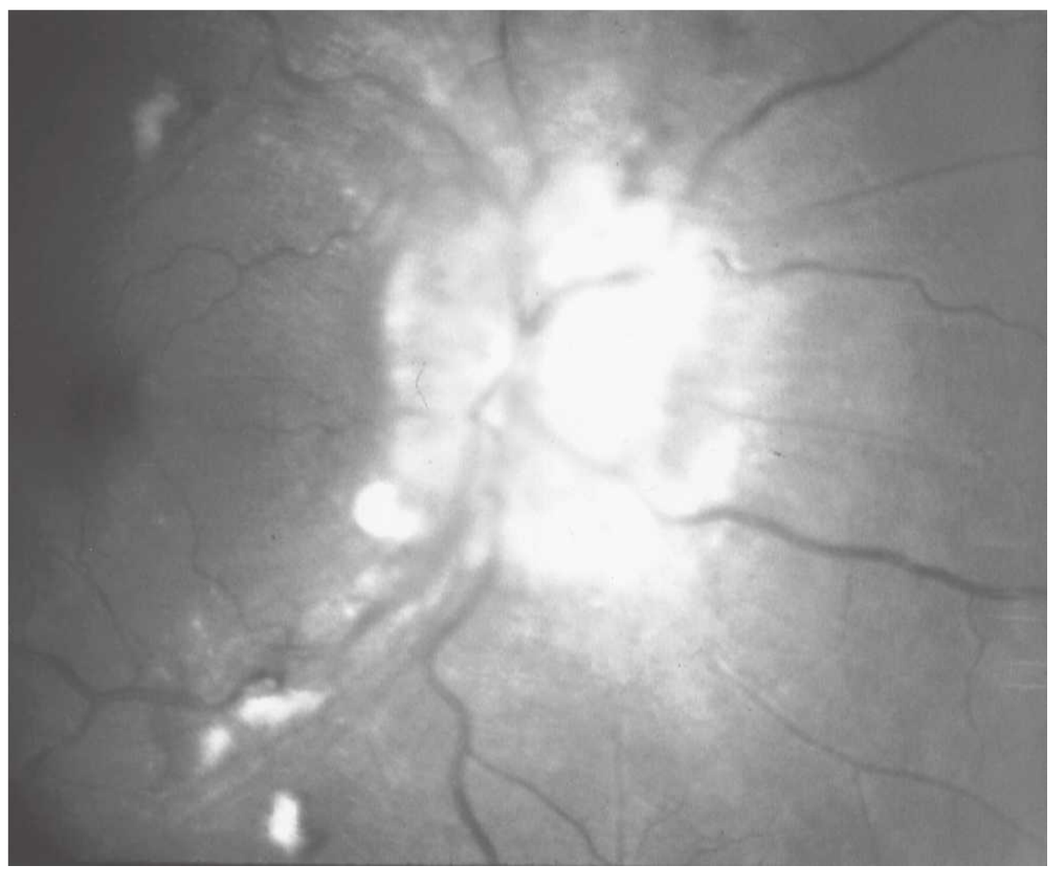


FIGURE 44.3 Papilledema in the fundus of an 18-year-old African American man with primary malignant hypertension. Cotton-wool spots are also apparent. This asymptomatic patient was incidentally noted to have severe hypertension during a routine dental examination.

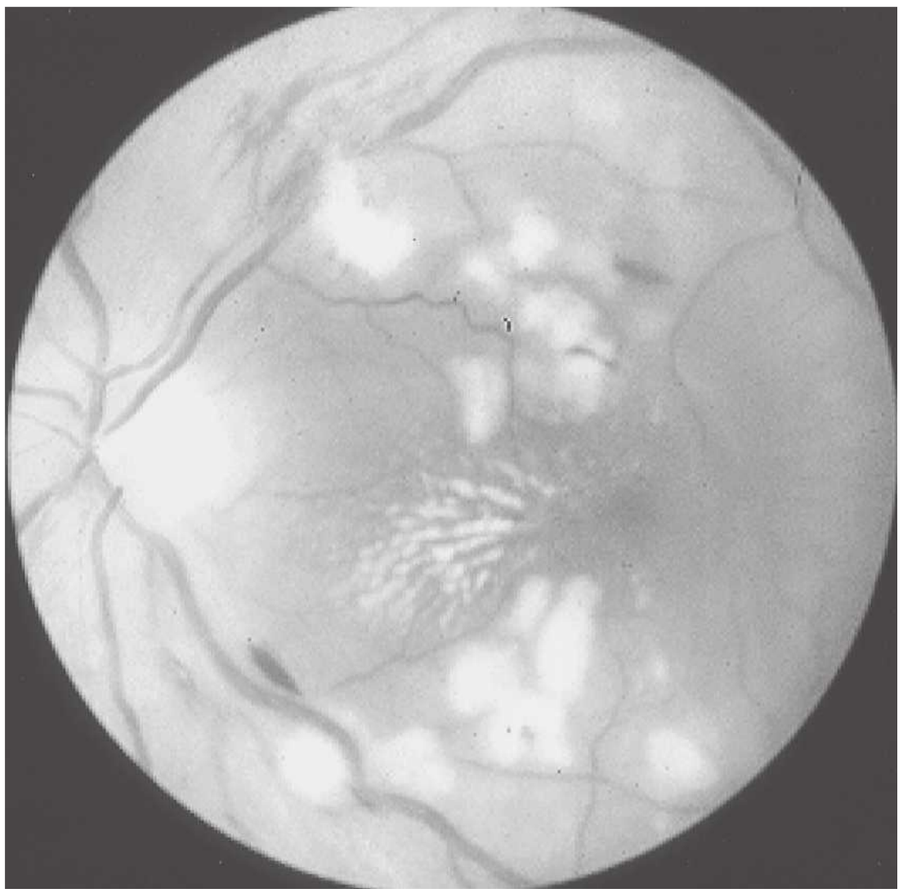


FIGURE 44.4 Full-blown hypertensive neuroretinopathy in the fundus of a 30-year-old man with malignant hypertension demonstrating linear (striate) hemorrhages, cotton-wool spots, papilledema, and a star figure at the macula. (Photograph courtesy of Daniel J. Mayer, MD.)

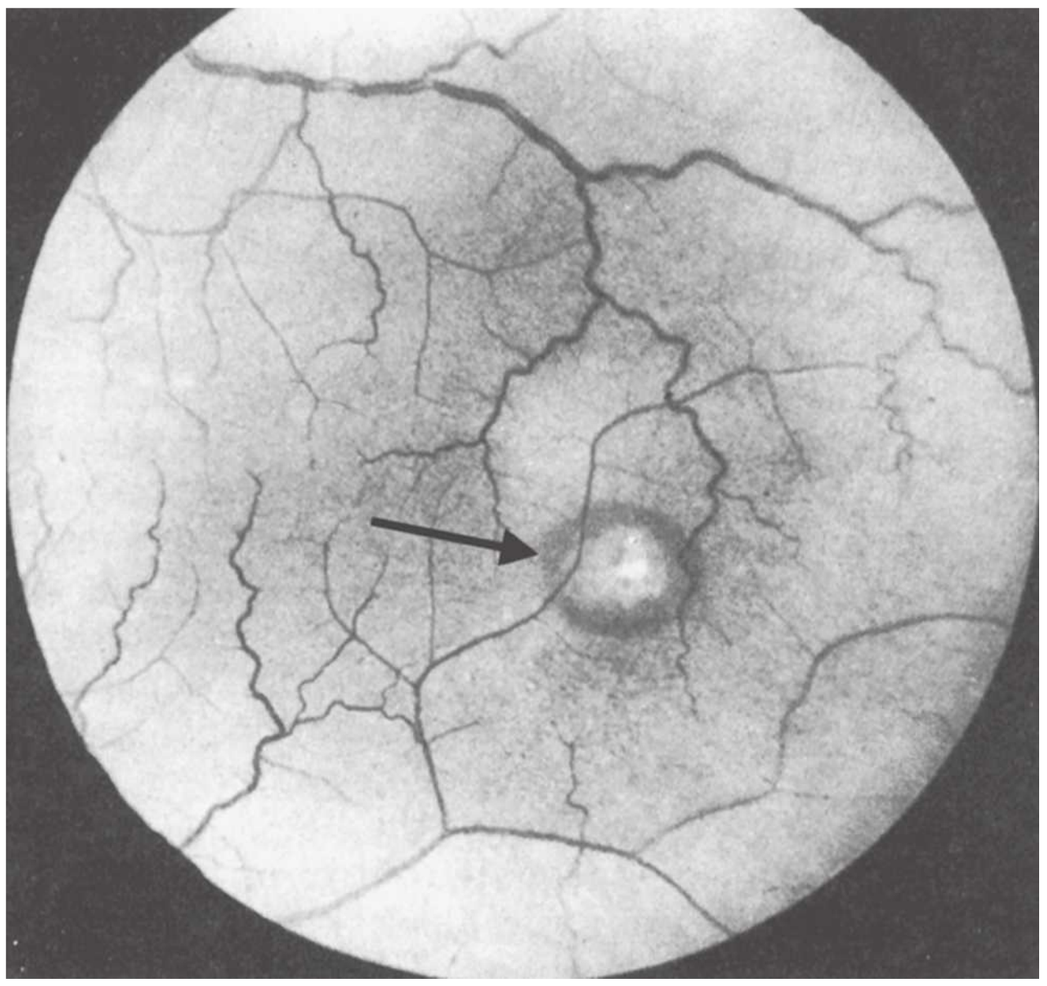


FIGURE 44.5 Hypertensive choroidopathy in malignant hypertension demonstrating focal serous detachment of the sensory retina with a whitish lesion at the level of the retinal pigment epithelium (acute Elschnig's spot). (From de Venecia G, Jampol LM. The eye in accelerated hypertension: II. Localized serous detachments of the retina in patients. *Arch Ophthalmol.* 1984;102:68, © 1984, American Medical Association, with permission.)

hypertension, papilledema is usually accompanied by striate hemorrhages and cotton-wool spots (Figs. 44.3 and 44.4). When papilledema occurs alone, the possibility of a primary intracranial process such as a tumor or cerebrovascular accident should be considered.

Hypertensive neuroretinopathy almost always precedes clinically apparent damage in other end organs but there are occasional reports of malignant nephrosclerosis appearing before the onset of hypertensive neuroretinopathy.[46] It should also be noted that the findings of striate hemorrhages, cotton-wool spots, and papilledema are not specific for malignant hypertension. Funduscopic findings that are indistinguishable from those of hypertensive neuroretinopathy can occur with severe anemia, subacute bacterial endocarditis, systemic lupus erythematosus, polyarteritis, temporal arteritis, and scleroderma. In these disorders the retinopathy may develop even in the absence of hypertension. Central retinal vein occlusion can also mimic hypertensive neuroretinopathy but is usually unilateral, whereas hypertensive neuroretinopathy is almost always bilateral.

Severe hypertension can also affect the choroidal as well as the retinal circulation. Hypertensive choroidopathy can occur with malignant hypertension and is manifested by lesions known as acute Elschnig spots, which are white areas of retinal pigment epithelial necrosis with overlying localized serous detachments of the retina (Fig. 44.5).[47] The serous retinal detachments may vary from one-third to six disc diameters. Fluorescein angiography reveals staining of the damaged pigment epithelium and leakage into the subretinal space.[47] Although most patients with this hypertensive choroidopathy also have typical changes of hypertensive neuroretinopathy with striate hemorrhages and cotton-wool spots, if the elevation of blood pressure is relatively sudden, the changes of hypertensive choroidopathy may predominate.[47]

It is important to note that papilledema should not be regarded as an essential requirement for the diagnosis of malignant hypertension. By life table analysis, the 10-year survival rate for hypertensive patients was 46% in patients with hemorrhages and exudates and 48% when papilledema was also present.[48] The lack of association between papilledema and the length of survival was confirmed using the Cox's proportional hazards model, which revealed associations between survival and age, smoking habit, initial serum creatinine concentration, and the level of blood pressure control achieved with therapy. No association was found with papilledema. When controlled for these covariates, no association was found between the presence of papilledema and survival (Fig. 44.6). There is no evidence to indicate that the apparent severity of hypertensive neuroretinopathy is predictive of a more severe hypertensive vasculopathy or more advanced end-organ destruction. Papilledema is not always present even when there is severe malignant nephrosclerosis presenting as oliguric acute renal failure. In four series with a total of 25 patients presenting with malignant hypertension and acute renal failure, only 14 patients had papilledema. The other 11 patients had hemorrhages and cotton-wool spots

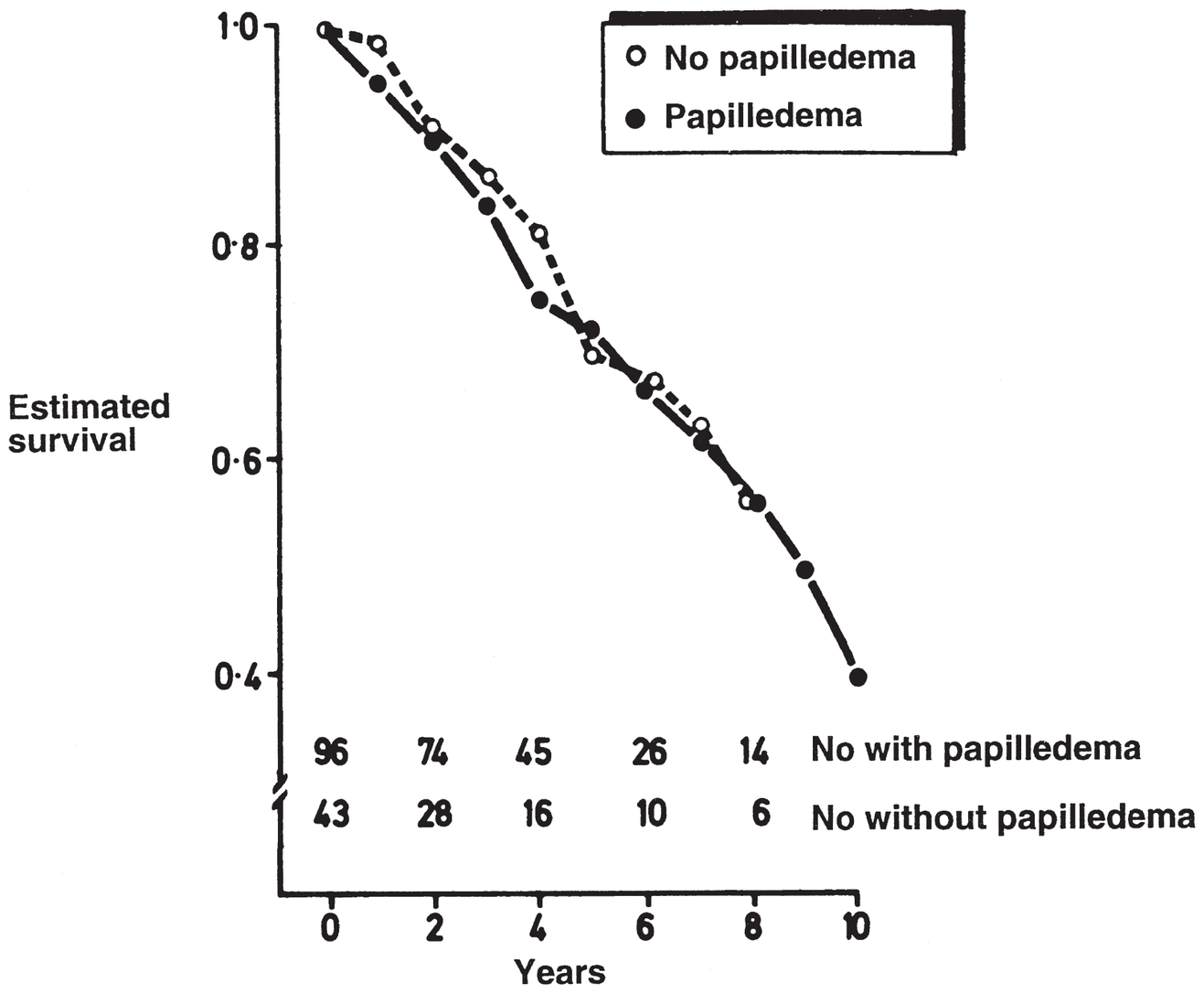


FIGURE 44.6 Relation between papilledema and survival in 139 hypertensive patients with bilateral retinal hemorrhages and exudates after controlling for age, gender, smoking habit, initial serum creatinine concentration, and initial and achieved blood pressure by multivariate analysis. Failure of papilledema to influence prognosis was confirmed by likelihood ratio test ($X = 0.89$, 1 df, $P = .34$). (From McGregor E, et al. Retinal changes in malignant hypertension. *Br Med J.* 1986;292:233, with permission.)

but no papilledema.[49–52] This lack of a difference in prognosis for patients with hypertensive neuroretinopathy whether or not it is accompanied by papilledema may be explained by the fact that cotton-wool spots and papilledema share a similar pathogenesis (see later discussion).[53,54]

Renal Manifestations

Malignant hypertension is a progressive systemic vasculopathy in which renal involvement is a secondary and relatively late development. Patients with malignant hypertension may present with a spectrum of renal involvement ranging from minimal albuminuria with normal renal function to ESRD indistinguishable from that seen in patients with primary renal parenchymal disease.[21,27]

The first sign of renal involvement in malignant hypertension is often the abrupt appearance of proteinuria. About 20% of patients also have painless gross hematuria, while 50% have microhematuria.[21] Quantitation of 24-hour protein excretion in patients with malignant hypertension has revealed less than 2 g in one third, between 2 and 4 g in one third, and more than 4 g in one third of patients.[26] The level of protein excretion is of little value in the differentiation of primary (essential) malignant hypertension from malignant hypertension due to secondary causes.[21,27]

Renal size is variable and depends on the duration of prior benign hypertension. In patients with primary (essential) malignant hypertension, the size of the kidneys may be normal to only slightly reduced. In fact, there may be little reduction in renal size even when patients develop terminal renal failure.[21]

The clinical spectrum of renal involvement in malignant hypertension is variable. Four clinical renal syndromes have been described. Progressive subacute deterioration of renal function leading to ESRD occurs in some patients. In patients presenting with malignant hypertension and initially normal renal function, in the absence of adequate treatment, it is common to observe deterioration of renal function with progression to ESRD over a period of weeks to months. The second clinical renal syndrome observed in malignant hypertension is transient deterioration of renal function following the initial control of blood pressure. This well-described entity occurs in patients presenting with mild to moderate renal impairment. In the third clinical renal syndrome, patients with malignant hypertension present with established renal failure. The close similarity between the terminal stage of primary malignant nephrosclerosis and chronic kidney disease with superimposed malignant hypertension has long been recognized. In this regard, it may not be possible to ascertain whether a patient presenting with severe hypertension, hypertensive neuroretinopathy, and renal failure has primary or secondary malignant hypertension. In the fourth clinical renal syndrome, patients with malignant hypertension present with oliguric acute renal failure. Cases of malignant hypertension have been described that were characterized by diastolic blood pressure higher than 130 mm Hg; advanced hypertensive neuroretinopathy; marked weight loss; and with an active urine sediment with proteinuria, hematuria, and red blood cell casts.[51,52] Renal size was normal. There was often evidence of microangiopathic hemolytic anemia. Although the initial blood urea nitrogen (BUN) concentration was less than 60 mg per dL, in each case oliguric renal failure occurred and necessitated the initiation of dialysis within a few days of hospitalization. Despite dialytic therapy, the blood pressure was extremely difficult to control and each patient died. Renal histology revealed malignant nephrosclerosis with fibrinoid necrosis

and proliferative endarteritis. The glomeruli were normal except for ischemic changes. Multifocal tubular necrosis was present and presumed to be secondary to ischemia. In most of these patients, the diagnosis of malignant hypertension was delayed because the patients were initially considered to have rapidly progressive glomerulonephritis or systemic vasculitis, which was treated with high-dose steroids. The diagnosis of malignant hypertension was not suspected until autopsy revealed malignant nephrosclerosis.

Neurologic Manifestations

Clarke and Murphy[55] detail the neurologic findings among 190 patients with malignant hypertension. CNS involvement was present at some time during the course in 42% of patients. Of the 65 patients for whom a cause of death could be ascertained, 33 had a fatal neurologic event. Of the total deaths, 20% were due to a neurologic cause. Intracerebral hemorrhage occurred in 23 patients. Episodes of focal brain ischemia, presumed due to cerebral thrombosis, occurred in 35 patients. Generalized seizures occurred in 11 patients and focal seizures in 8. Bell's palsy occurred in seven patients. Primary subarachnoid hemorrhage occurred in four patients. In this series, hypertensive encephalopathy was found in only 1% of patients; however, other series reported a higher incidence.[56] The clinical presentation, pathophysiology, and treatment of hypertensive encephalopathy are discussed in detail later in this chapter within Hypertensive Encephalopathy.

Gastrointestinal Manifestations

The most common gastrointestinal (GI) manifestations of malignant hypertension are nonspecific symptoms including nausea, vomiting, and epigastric pain. However, acute pancreatitis has been reported as a rare complication. In a series of 42 patients with malignant hypertension, severe acute pancreatitis that could not be attributed to gallstones or alcohol abuse developed in seven patients.[57]

Patients with malignant hypertension can present with an acute abdomen.[58] Abdominal exploration revealed necrotic bowel with involvement of the distal ileum and ascending colon. Moreover, malignant hypertension may increase the risk of subsequent development of mesenteric ischemia in patients on chronic hemodialysis.[59] GI hemorrhage can occur in patients with malignant hypertension due to hypertension-induced necrotizing mesenteric arteriolitis.[60]

Hematologic Manifestations

A variety of hematologic findings have been observed in patients with malignant hypertension. The hemoglobin concentration at the time of presentation may correlate with the etiology of the malignant phase. A hemoglobin concentration higher than 12.5 g per dL is more often associated with primary malignant hypertension, whereas a lower value is more often associated with chronic glomerulonephritis or pyelonephritis.[21]

There are numerous reports of microangiopathic hemolytic anemia in association with malignant hypertension. In one series of 24 patients with malignant hypertension, 16 were found to have evidence of microangiopathic hemolysis.[61] Other significant abnormalities reported with malignant hypertension include thrombocytopenia, increased fibrin degradation products, increased factor VIII levels, increased fibrinogen, and increased urokinase sensitivity consistent with decreased fibrinolysis.[62]

Cardiac Manifestations

Congestive heart failure can be a presenting feature of malignant hypertension. Moreover, heart failure, alone or in combination with uremia, was a common cause of death prior to the advent of effective antihypertensive drugs. Heart failure in patients with malignant hypertension is predominantly left-sided with pulmonary congestion resulting in orthopnea, paroxysmal nocturnal dyspnea, cardiac asthma, and recurrent episodes of acute pulmonary edema. Peripheral venous congestion with dependent edema or hepatic congestion may be minimal or absent even when death results from congestive heart failure.

Angina and acute myocardial infarction, although common with long-standing benign hypertension, are uncommon with malignant hypertension.[21] Aortic dissection is also rare in patients with malignant hypertension.[22]

Abnormalities of the Renin-Angiotensin-Aldosterone Axis

Evidence of activation of the renin–angiotensin–aldosterone axis is present in many, but not all, patients with malignant hypertension.[63] Among 53 patients with malignant hypertension not secondary to renal artery stenosis, 55% had increased plasma renin activity (PRA).[64] Among 25 patients with malignant hypertension secondary to renal artery stenosis, PRA was consistently elevated.[64]

Aldosterone secretion rate has been studied in patients with malignant hypertension.[65] There was a marked increase in secretion rate in seven of eight patients with malignant hypertension (papilledema present), and in five of eight patients with accelerated hypertension (retinal hemorrhages without papilledema). The aldosterone secretion rate in these patients was often higher than that seen in patients with aldosterone-producing adenoma.

Electrolyte Abnormalities

Hypokalemic metabolic alkalosis was found in up to 50% of patients with malignant hypertension, presumably reflecting a state of hyperreninemia and secondary hyperaldosteronism.[65] After effective therapy, aldosterone hypersecretion can persist long after volume depletion is corrected and renin levels have returned to normal. Thus, the findings of hypokalemia, increased urinary potassium losses, and aldosterone hypersecretion with suppressed PRA may mimic the findings of primary hyperaldosteronism.[63]

Hyponatremia is not uncommon in patients with malignant hypertension, particularly when sodium restriction is instituted. Patients with malignant hypertension due to renal artery stenosis occasionally present with the striking hyponatremic hypertensive syndrome.[66,67] The characteristic features of this syndrome include severe hypertension, hypertensive neuroretinopathy, polyuria, polydipsia, weight loss, and salt craving. Biochemical changes include hyponatremia, hypokalemia, and low total exchangeable sodium and potassium, with markedly elevated PRA, angiotensin II, aldosterone, and arginine vasopressin (AVP) levels. This syndrome may result from a vicious cycle of volume depletion with further activation of the renin–angiotensin–aldosterone axis as a result of a pressure-induced natriuresis from the contralateral kidney.

Pathologic Findings

Renal Pathology

With malignant nephrosclerosis, small pinpoint petechial hemorrhages may be present on the cortical surface, giving the kidney a peculiar flea-bitten appearance. The renal size varies depending on the duration of preexisting benign hypertension or the presence of underlying primary renal parenchymal disease. When terminal renal failure occurs in patients with primary malignant hypertension, the kidneys may be normal in size.[37] However, when secondary malignant hypertension is superimposed on primary renal disease, the kidneys may be small.

Fibrinoid necrosis of the afferent arterioles has traditionally been regarded as the hallmark of malignant nephrosclerosis (Fig. 44.7).[21] The characteristic finding is the deposition in the arteriolar wall of a granular material that appears bright pink with hematoxylin and eosin stain. On trichrome staining, this granular material is deep red. This fibrinoid material is usually found in the media, but it may also be present in the intima. Histochemical and immunofluorescent techniques have identified this material as fibrin. Within the media, muscle fibers cannot be identified and cell nuclei are lost or fragmented. Whole or fragmented erythrocytes may be extravasated into the arteriolar wall. The hemorrhages that occur may account for the petechiae observed on the cortical surface. The arteriolar lumen may be reduced in size as a result of wall thickening and intraluminal fibrin thrombi. Infrequently, polymorphonuclear leukocytes and monocytes may infiltrate the arterioles, giving the appearance of necrotizing arteriolitis.

The interlobular arteries reveal characteristic lesions variously referred to as proliferative endarteritis, productive endarteritis, endarteritis *fi*brosa, or the onionskin lesion. The typical finding is intimal thickening that causes moderate to severe luminal narrowing. In severely affected vessels, the luminal diameter may be reduced to the size of a single red blood cell. Occasionally, there is complete obliteration of the lumen by a fibrin thrombus.

Traditionally, three patterns of intimal thickening in malignant nephrosclerosis have been described.[68] The onionskin pattern consists of pale layers of elongated, concentrically arranged, myointimal cells. Delicate connective tissue fibrils give rise to a lamellated appearance (Fig. 44.8). The media often appears as an attenuated layer stretched around the expanded intima. Mucinous intimal thickening consists of a scarcely cellular lesion containing a lucent, faintly basophilic-staining

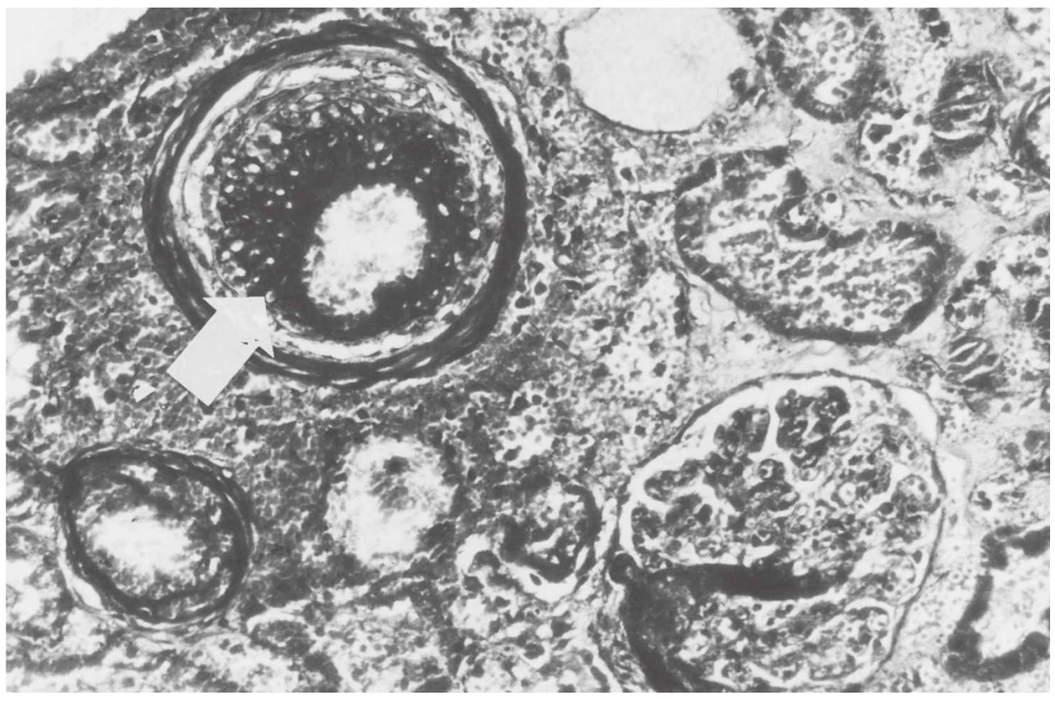


FIGURE 44.7 Fibrinoid necrosis in a large arteriole (*arrow*). Intimal onionskin formation is also present. (Trichrome stain.) (Photograph courtesy of Steve Guggenheim, MD.)

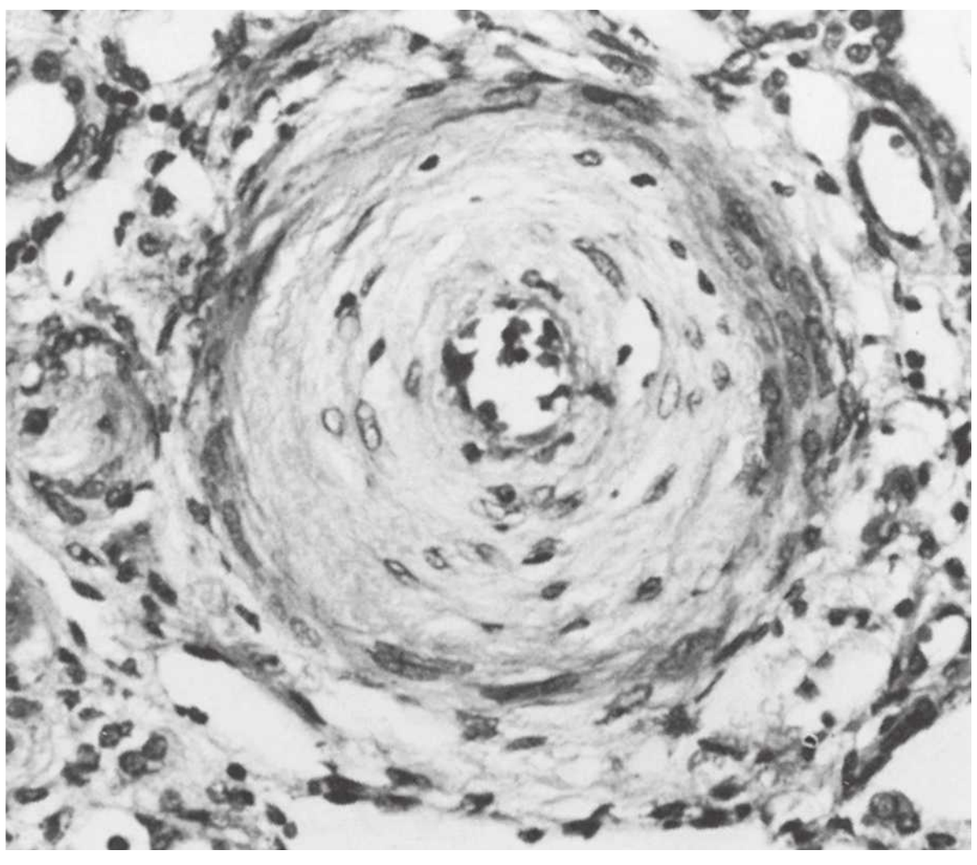


FIGURE 44.8 Onionskin lesion consisting of pale layers of elongated, concentrically arranged myointimal cells and delicate connective tissue fibrils that produce a lamellated appearance. The media is attenuated and stretched around the thickened intima. (Hematoxylin and eosin stain ×350.) (From Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol*. 1976;7:565, with permission.)

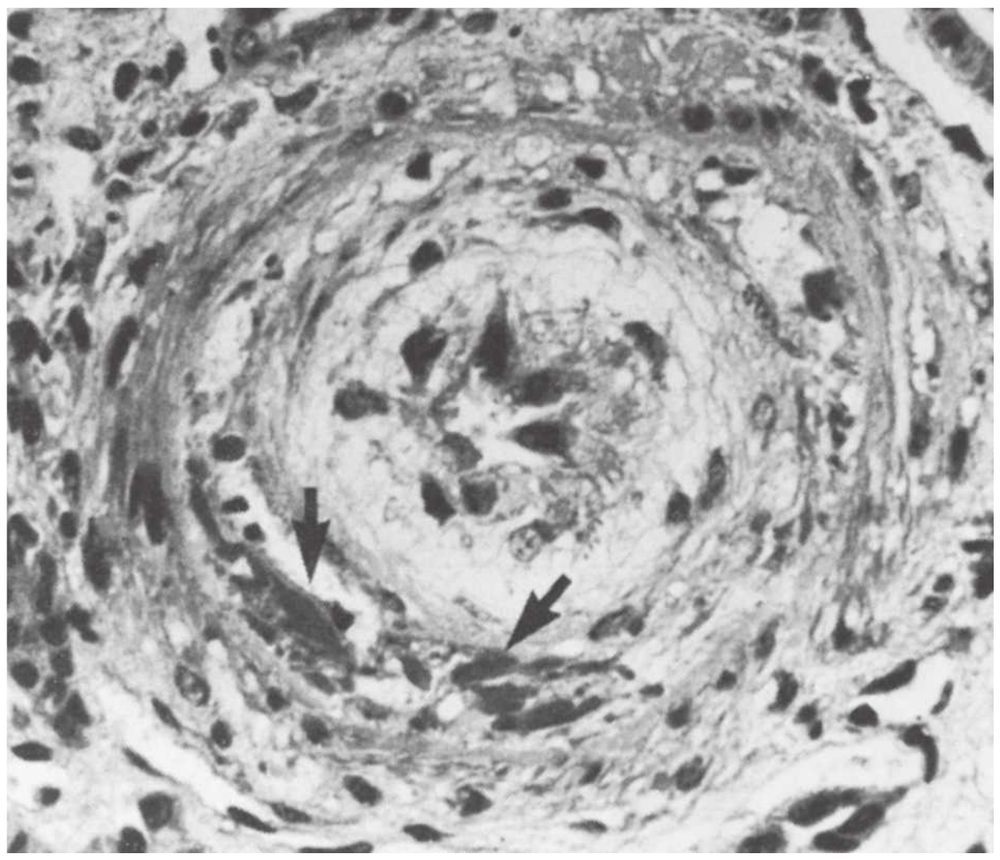


FIGURE 44.9 Mucinous intimal thickening. The lesion is sparsely cellular and consists mainly of a lucent, faintly basophilic-staining amorphous material. There are small foci of fibrinoid necrosis (*arrows*) deep within the intima. (Hematoxylin and eosin stain ×350.) (From Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol.* 1976;7:565, with permission.)

amorphous material (Fig. 44.9). In *f*ibrous intimal thickening, there are hyaline deposits, reduplicated bands of elastica, and coarse layers of pale connective tissue with the staining properties of collagen (Fig. 44.10). In rare cases, fibrinoid necrosis may also be apparent in the interlobular arteries.[68]

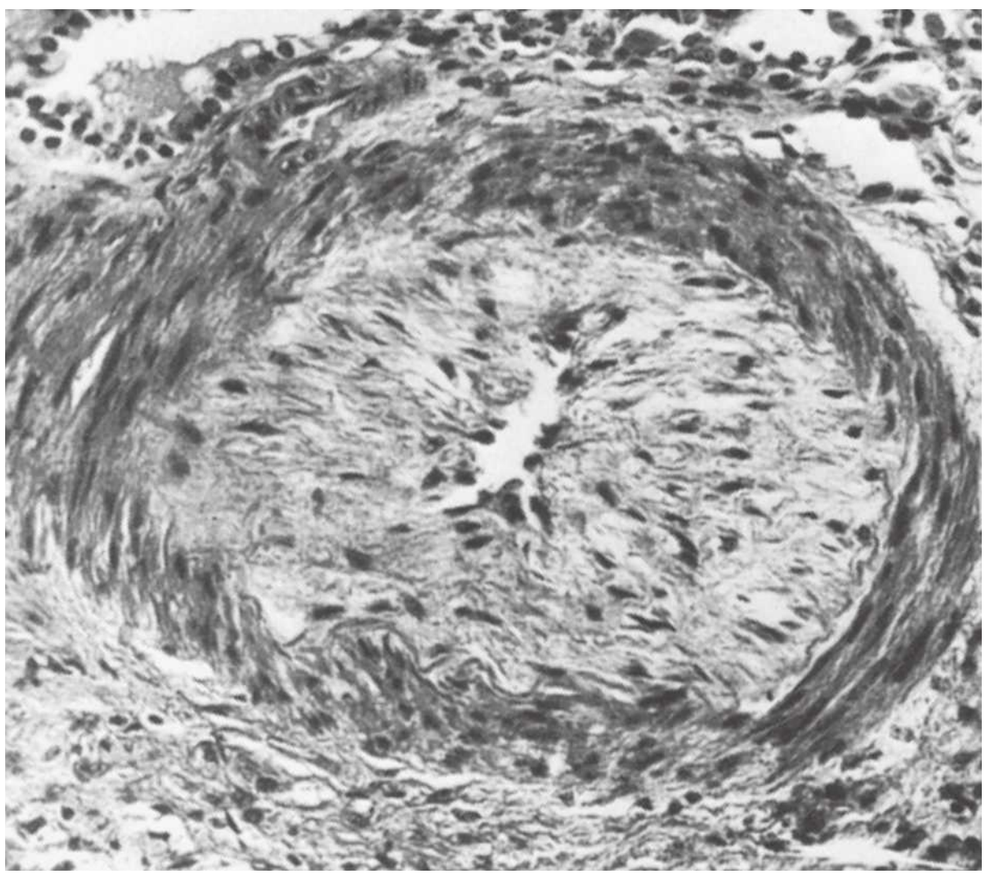


FIGURE 44.10 Fibrous intimal thickening. The lesion consists of a thick layer of connective tissue, which stains for collagen and elastin. (Hematoxylin and eosin stain ×300.) (From Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol.* 1976;7:565, with permission.)

The renal histology in blacks with malignant hypertension may be somewhat different.[69,70] Although fibrinoid necrosis of the afferent arterioles is not found, there is instead a marked degree of arteriolar hyalinization. In addition, there is a prominent and characteristic finding in the larger arterioles and interlobular arteries known as musculomucoid intimal hyperplasia (Fig. 44.11).[69–71] The arterial walls are thickened due to the presence of hyperplastic smooth muscle cells. A small amount of myxoid material, which stains light blue with hematoxylin and eosin, is observed between the cells. With periodic acid-Schiff staining this material resembles basement membrane. Staining for acid mucopolysaccharide suggests the presence of chondroitin sulfate and possibly hyaluronic acid.

By electron microscopy, in each of the above-mentioned types of intimal thickening, the most abundant cellular element is a modified smooth muscle cell called a myointimal cell. In these cells there are smooth musclelike ultrastructural features including cytoplasmic myofilaments and abundant rough endoplasmic reticulum.[68,72] In the pure onionskin variant, the intercellular space is occupied by multiple strands of nonperiodic fibrils with the ultrastructural features of basement membrane.[68] In the mucinous variant, broad electron-lucent zones with scattered finely granular material are found in the intercellular space.[72] With the fibrous variant, numerous bundles of collagen, recognizable by characteristic banding, are dispersed between the myointimal cells.[72]

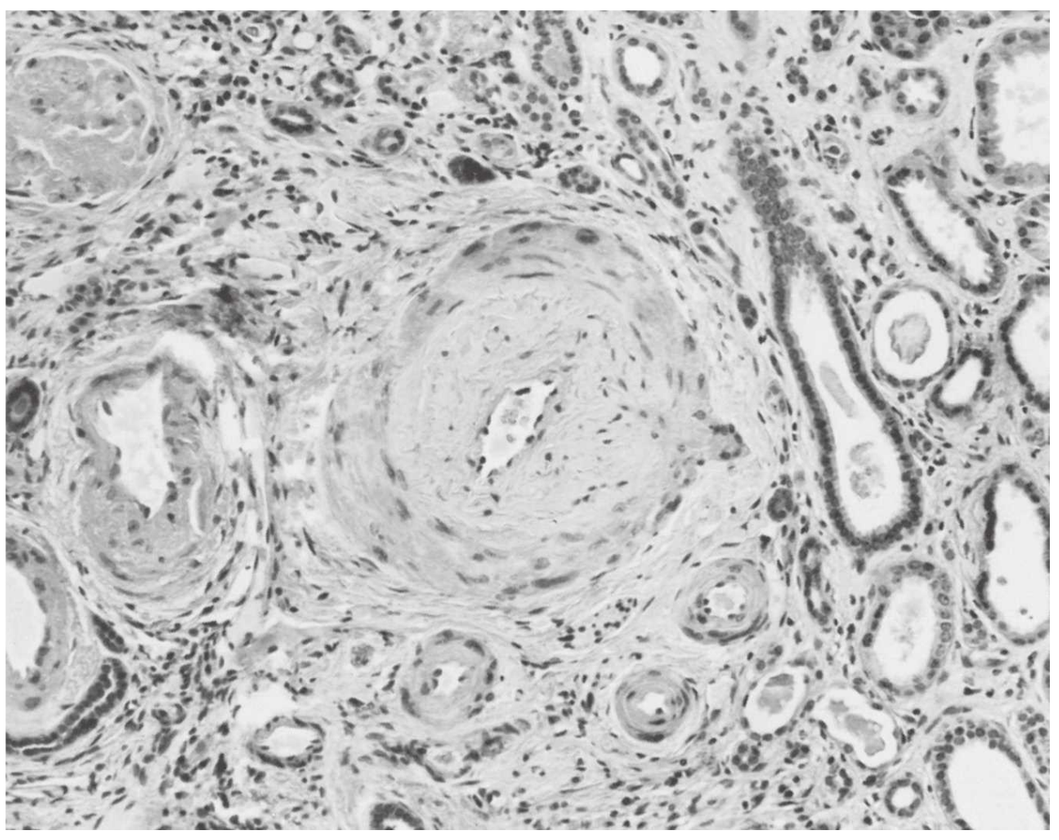


FIGURE 44.11 Musculomucoid intimal hyperplasia of an interlobular artery. The arterial walls are thickened by hyperplastic smooth muscle cells. A small amount of myxoid material is seen between the smooth muscle cells. (Hematoxylin and eosin stain ×170.) (From Pitcock JA, Johnson JG, Hatch FE, et al. Malignant hypertension in blacks: malignant intrarenal arterial disease as observed by light and electron microscopy. *Hum Pathol.* 1976;7:333, with permission.)

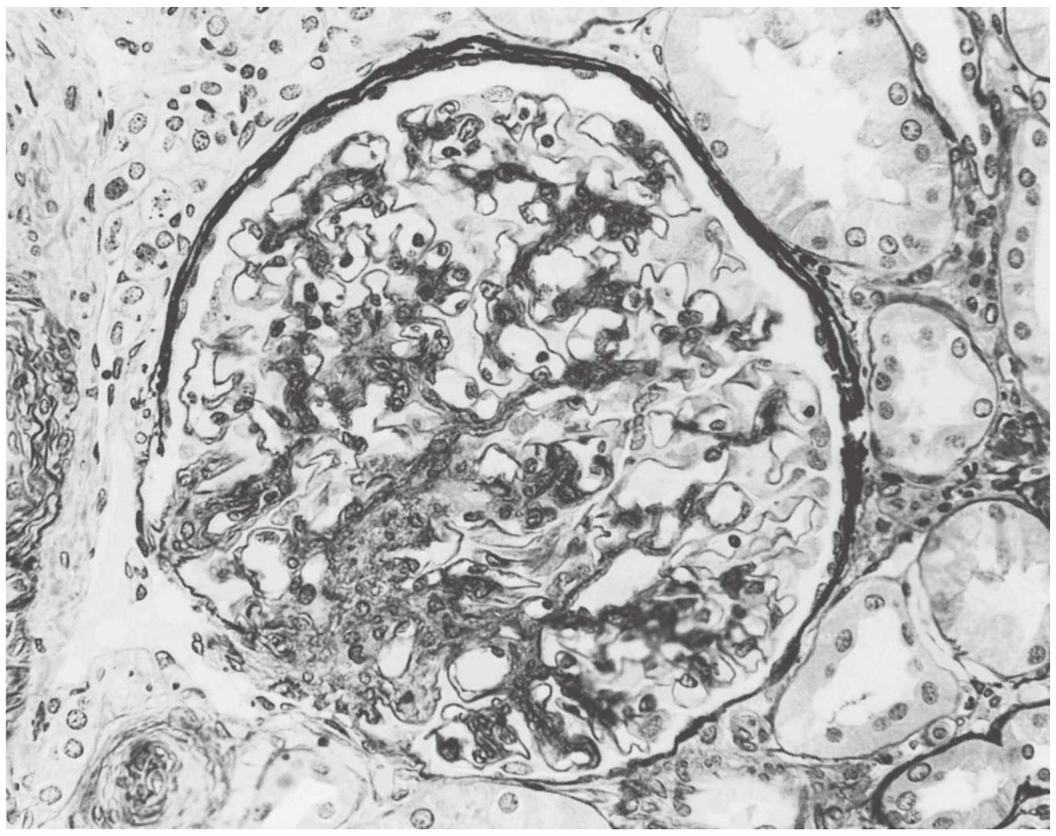


FIGURE 44.12 The earliest ischemic glomerular change in malignant hypertension consists of some basement membrane wrinkling, particularly in areas adjacent to the mesangium, with a slight increase in mesangial matrix. (Periodic acid–silver methenamine stain ×250.) (From Pitcock JA, Johnson JG, Hatch FE, et al. Malignant hypertension in blacks: malignant intrarenal arterial disease as observed by light and electron microscopy. *Hum Pathol.* 1976; 7:333, with permission.)

In patients who have received antihypertensive therapy, as well as blacks with treated or untreated malignant hypertension, the most characteristic glomerular lesion in malignant nephrosclerosis is accelerated glomerular obsolescence secondary to the intense ischemia produced by the obliterative arterial lesions.[70,73] The earliest glomerular changes consist of thickening and wrinkling of the basement membrane (Fig. 44.12).[75,78] Later, there is shrinkage of the tuft such that it does not fill Bowman's space. There is laminar reduplication of Bowman's capsule around the shrunken glomerulus.[68] The end stage is the obsolescent glomerulus, which is an avascular, wrinkled glomerular tuft surrounded by a collagenous scar that fills Bowman's space (Fig. 44.13). Focal segmental glomerulosclerosis may occur in primary malignant hypertension either as the result of glomerular hyperfiltration or fibrinoid necrosis, and may contribute to renal dysfunction. In an autopsy series of 38 black South Africans with primary malignant hypertension, mucoid intimal hyperplasia was present in all sections whereas fibrinoid necrosis was seen in 76%. Glomerulosclerosis was present in 38 cases, and was axially distributed in 18%, segmental in 58%, and global in 24% of sections. Cases with segmental sclerosis tended to have the highest proteinuria, whereas those with global glomerulosclerosis had the highest serum creatinine levels.[74]

By electron microscopy, the lamina densa of the glomerular capillary basement membrane is thickened and wrinkled (Fig. 44.14).[73] Eventually, the entire basement membrane becomes thickened. These glomerular

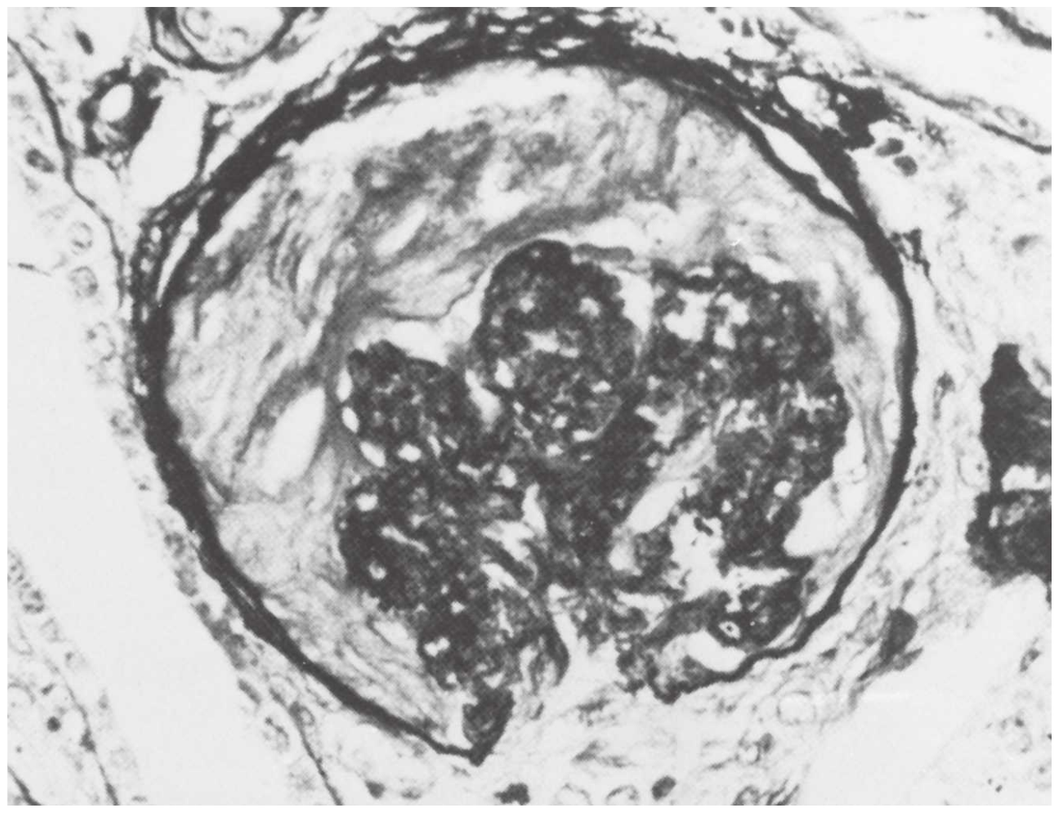


FIGURE 44.13 Glomerular obsolescence in malignant hypertension. The collapsed, avascular glomerular tuft consists predominantly of markedly convoluted basement membranes. The sclerosed tuft is partially enclosed within a collar of hyaline material filling Bowman's space. (Periodic acid–silver methenamine stain ×485.) (From Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol.* 1976; 7:565, with permission.)

changes are not specific for malignant nephrosclerosis as they also can occur in scleroderma renal crisis, hemolytic–uremic syndrome, and even severe benign nephrosclerosis. However, the glomerular changes in malignant nephrosclerosis differ from the simple ischemic

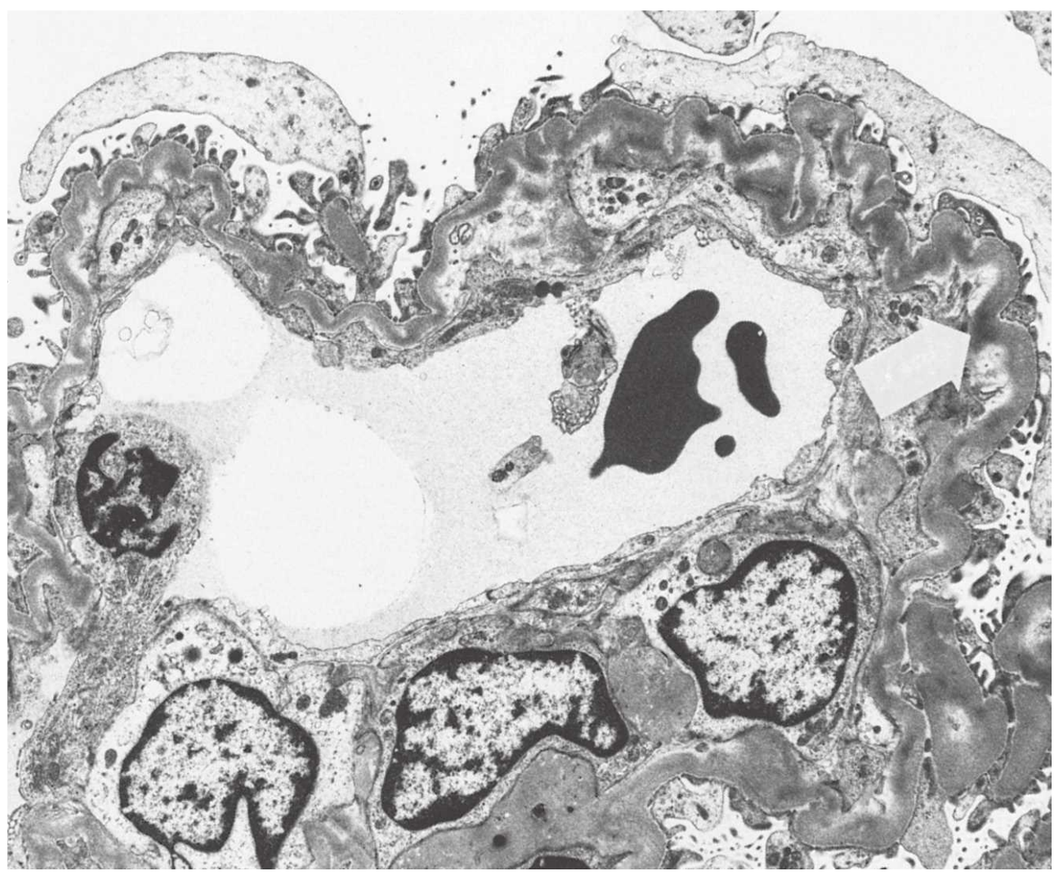


FIGURE 44.14 Accelerated glomerular obsolescence in malignant hypertension. The glomerular capillaries show striking basement membrane wrinkling (*arrow*) and some reduplication of the inner basement membrane. (Uranyl acetate and lead citrate ×4,250.) (From Jones DB. Arterial and glomerular lesions associated with severe hypertension: light and electron microscopic studies. *Lab Invest.* 1974; 31:303, with permission.)

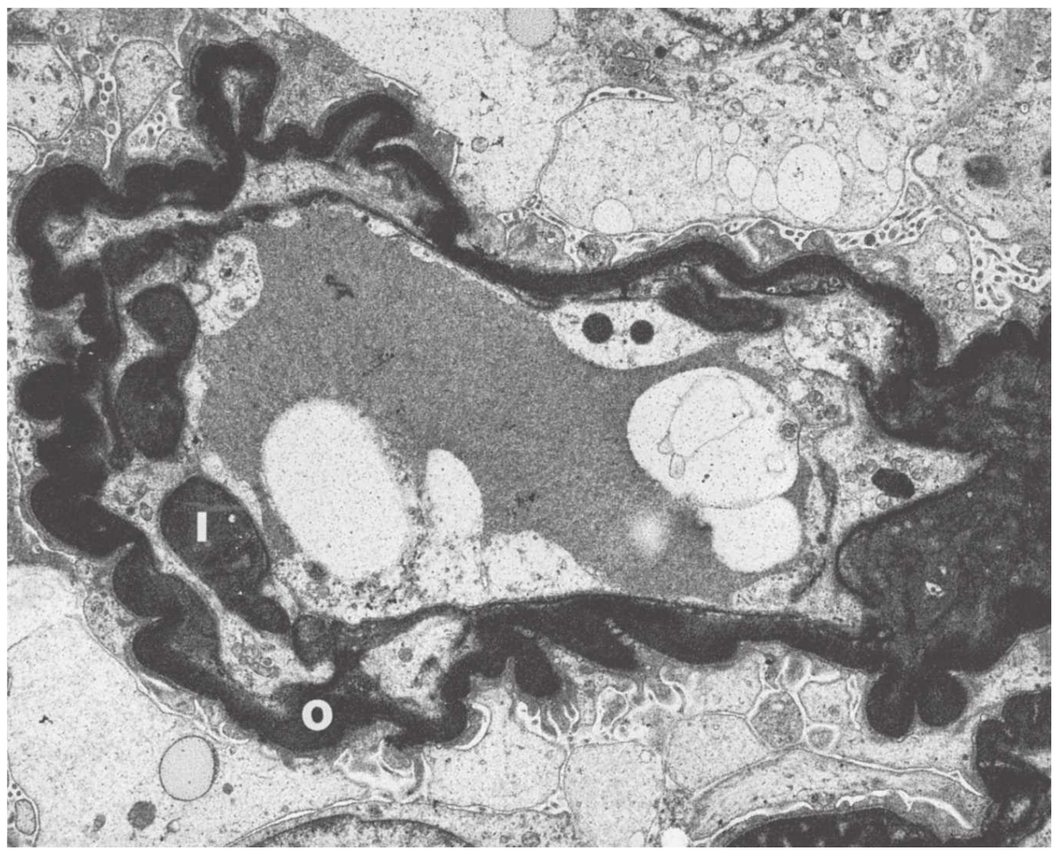


FIGURE 44.15 Accelerated glomerular obsolescence in malignant hypertension. The outer basement membrane (*O*) is thickened and wrinkled. There is a reduplicated inner basement membrane (*I*) with the capillary lumen still patent. (Uranyl acetate and lead citrate stain ×4,250.) (From Jones DB. Arterial and glomerular lesions associated with severe hypertension: light and electron microscopic studies. *Lab Invest.* 1974;31:303, with permission.)

obsolescence observed in benign hypertension. In addition to the wrinkled basement membrane observed in benign nephrosclerosis, there is constriction of the glomerular vascular bed in malignant nephrosclerosis due to the deposition of a new subendothelial layer of basement membrane material inside the original basement membrane (Fig. 44.15).[73] The new capillary lumen formed by this process is smaller, resulting in decreased blood volume in the ischemic glomerulus.

In malignant nephrosclerosis, the tubules may be atrophied and focally destroyed in areas supplied by severely narrowed arteries. Occasional tubules may be dilated and filled with eosinophilic cast material.[75] In the interstitium in these areas, there may be a fine reticular fibrosis and chronic inflammatory cells. In malignant hypertension, as in primary renal parenchymal diseases, renal insufficiency appears to correlate best with the degree of tubular atrophy.[70]

Immunofluorescence microscopy in patients with malignant nephrosclerosis has demonstrated deposition of gamma globulin, fibrinogen, albumin, and sometimes complement components in the walls of arterioles demonstrating fibrinoid necrosis by light microscopy.[81] Some of the glomeruli, especially those with focal necrosis, may contain immunoglobulin, albumin, and complement. Fibrinogen may be found diffusely along capillary basement membranes. Fibrinogen may also be found in the intima of interlobular arteries that by light microscopy show cellular or mucinous thickening.[76]

Striking juxtaglomerular hyperplasia has been reported in patients with malignant hypertension.[77] This ultrastructural finding is consistent with the hyperreninemic state often noted clinically.

Effective antihypertensive therapy may alter the pathology of malignant nephrosclerosis.[78–80] Within days, there may be resolution of fibrinoid necrosis, which leaves behind residual hyaline deposits in the arteriolar wall. In contrast to benign nephrosclerosis in which arteriolar hyaline change is often subendothelial, in treated malignant hypertension the hyaline material may be present throughout the entire vessel wall. Fibrosis of the arterioles with collagen replacement of the arteriolar muscle and elastica may also occur. Within several weeks after initiation of therapy, segmental fibrinoid necrosis in the glomeruli may also resolve, leaving behind an area of hyaline deposition that can mimic focal segmental glomerulosclerosis (FSGS). Furthermore, with treatment, in the intima of the interlobular arteries there may be an evolution from cellular hyperplasia to a more fibrous form of intimal thickening. A newly formed internal elastic lamina often separates this new collagen from the narrowed lumen. Heptinstall has postulated that the cellular lesion is an early finding implying active disease, whereas the acellular fibrotic lesion is a later process often reflecting a response to treatment.[75] These modifications in the interlobular arteries that occur following treatment may not be accompanied by any increase in the caliber of the lumen. Severely narrowed interlobular arteries often do not improve and the renal parenchyma distal to these arteries undergoes severe ischemic atrophy and scarring.[79] However, the nephrons supplied by interlobular arteries of normal caliber may undergo substantial hypertrophy following treatment of malignant hypertension. These histologic changes may explain the improvement in renal function that sometimes occurs in some patients following institution of antihypertensive therapy with resolution of malignant hypertension.

In summary, although fibrinoid necrosis was the hallmark of malignant nephrosclerosis in untreated patients at autopsy, it is now rarely observed. In treated patients with malignant hypertension or blacks with untreated malignant hypertension, closed renal biopsy most often reveals marked intimal hyperplasia of the interlobular arteries in association with accelerated glomerular obsolescence.[70,73]

Pathophysiology

Pathophysiology of Malignant Hypertension

The exact pathophysiologic mechanism underlying the transition from benign to malignant hypertension is not fully understood. The postulated pathogenesis of malignant hypertension is outlined in Figure 44.16. According to the pressure hypothesis, the development of fibrinoid necrosis and proliferative endarteritis is a direct consequence of the mechanical stress placed on vessel walls

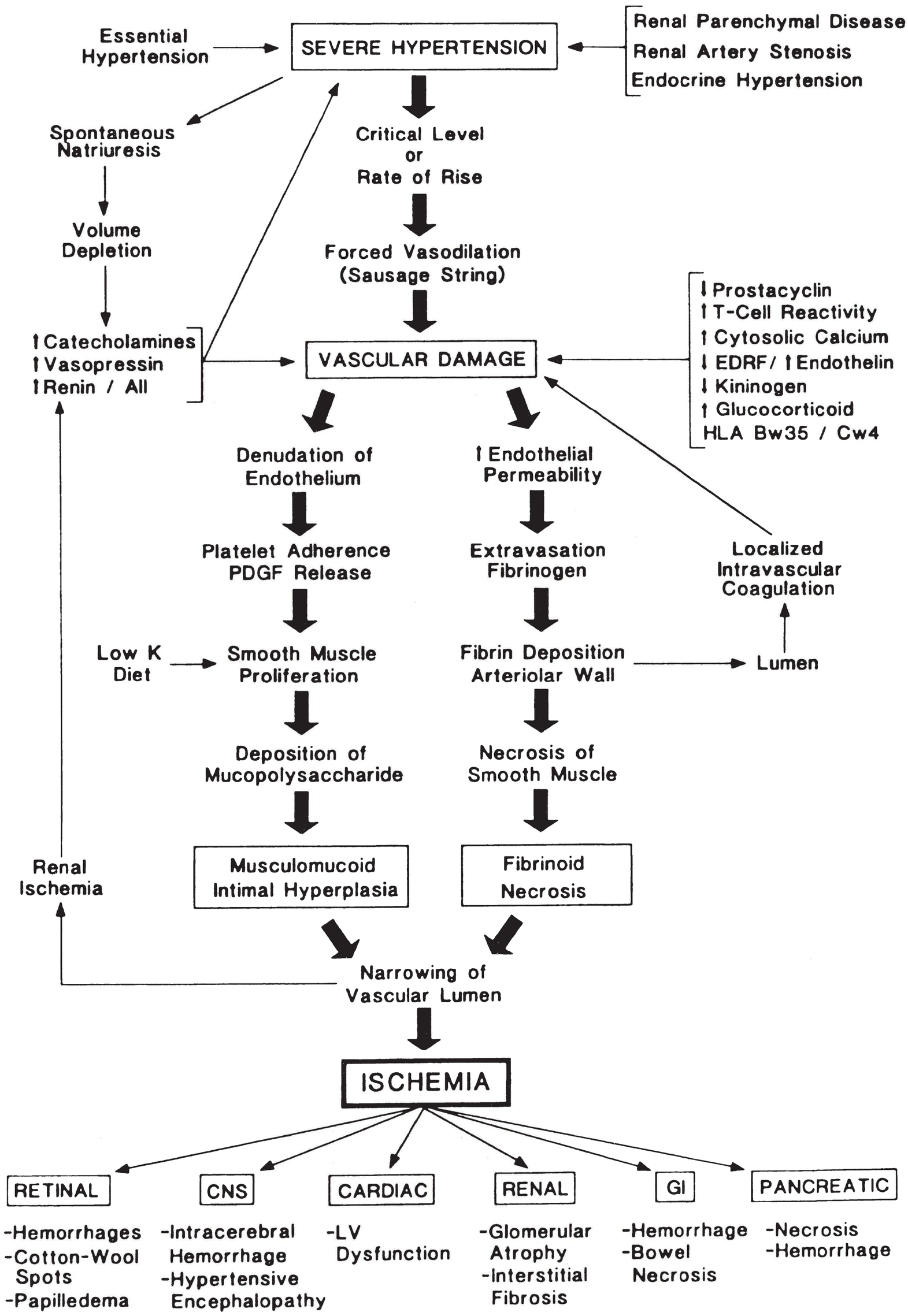


FIGURE 44.16 Pathophysiology of malignant hypertension. *AII,* angiotensin II; *EDRF,* endothelium-derived relaxing factor; *PDGF,* platelet-derived growth factor; *LV,* left ventricular.

by severe hypertension.[81] Undoubtedly, a marked increase in blood pressure is pivotal. Severe hypertension is the common element in malignant hypertension in humans and in each of the animal models of malignant hypertension. Moreover, reduction of the blood pressure leads to a resolution of the malignant phase regardless of the underlying etiology. Thus, a significant elevation of the blood pressure is necessary for the development and progression of malignant hypertension. The major issue is whether the mechanical stress of severe hypertension alone is sufficient to cause the transition from benign to malignant hypertension. Because there is considerable overlap in the levels of blood pressure seen in patients with benign and those with malignant hypertension, it is likely that severe hypertension alone is not sufficient to cause the malignant hypertension in all patients and that additional factor(s) probably participate. The vasculotoxic theory proposes that humoral factors interact with the hypertension-induced hemodynamic stress to cause the vascular damage observed in malignant hypertension.

These cofactors are not necessarily the same in every case. For example, activation of the renin–angiotensin axis may be important in some patients but not in others. In some patients, perhaps catecholamines, vasopressin, endothelin, or activation of the clotting cascade interact with hemodynamic stress to induce malignant hypertension. In several experimental models, spontaneous natriuresis appears to be the initiating event in the transition from benign to malignant hypertension.[82–84] In patients with malignant hypertension, an abrupt onset of weight loss early in the course of the disease has been reported. In the series of Kincaid-Smith et al.,[21] the onset of malignant hypertension often appeared suddenly. Despite minor increases in blood pressure, the patients became suddenly ill with weakness, wasting, and profound weight loss. The rapidity of the weight loss could only be explained by natriuresis-induced volume depletion and may be the human counterpart of the rat two-kidney, one-clip model of malignant hypertension in which spontaneous natriuresis is the inciting event.[82,83]

Pathophysiology of Hypertensive Neuroretinopathy

Retinal arteriolar vasculopathy in malignant hypertension leads to obliteration or rupture of vessels, resulting in striate hemorrhages, cotton-wool spots, and papilledema. Hypertensive neuroretinopathy is not simply the result of renal failure as hypertensive neuroretinopathy can clearly occur in malignant hypertension prior to the onset of clinically significant renal disease.[42] It also appears that hypertensive neuroretinopathy often occurs in the absence of increased intracranial pressure.[42]

The retinal circulation is under autoregulatory control and does not have a sympathetic nerve supply. As the systemic blood pressure increases, if autoregulation is intact, the retinal arterioles constrict to keep the retinal blood flow constant. The appearance of hypertensive neuroretinopathy implies that autoregulation has failed.[41]

Striate hemorrhages result from bleeding from superficial capillaries in the nerve fiber bundles near the optic disc. These capillaries originate from arterioles, so that when autoregulation fails, the high systemic pressure is transmitted directly to the capillaries. This leads to breaks in the continuity of the capillary endothelium with subsequent hemorrhage.[41]

Cotton-wool spots result from ischemic infarction of nerve fiber bundles due to arteriolar occlusion.[41] Fluorescein angiography demonstrates that cotton-wool spots are areas of retinal nonperfusion.[85] Embolization of pig retina with glass beads produces immediate intracellular edema followed by accumulation of mitochondria and other subcellular organelles in the ischemic nerve fibers.[87] It has been postulated that the normal axoplasmic flow of subcellular organelles is disrupted by ischemia such that accumulation of organelles in ischemic nerve fiber bundles results in a visible white patch.[53,54] Cotton-wool spots tend to distribute around the optic disc because the nerve fiber bundles are most dense in this region.

The pathogenesis of papilledema in malignant hypertension has been controversial. Papilledema may result from increased intracranial pressure. However, intracranial pressure is not always increased in malignant hypertension.[42] Papilledema has been produced in rhesus monkeys by occlusion of the long posterior ciliary artery, which supplies the optic disc.[88] Thus papilledema, like cotton-wool spots, most likely results from ischemia of nerve fibers in the optic disc.[87,88]

Treatment

Malignant hypertension must be treated expeditiously in order to prevent complications such as hypertensive encephalopathy, intracerebral hemorrhage, acute pulmonary edema, and renal failure. The hypertensive patient with hypertensive neuroretinopathy (hemorrhages, cotton-wool spots with or without papilledema) should be hospitalized for intensive medical therapy. Initiation of appropriate therapy should not be delayed pending extensive laboratory and roentgenographic examinations aimed at defining a potential underlying etiology. The workup for secondary causes should be deferred until the blood pressure has been controlled and the patient stabilized.

The traditional approach to patients with malignant hypertension has been the initiation of therapy with rapid-acting parenteral hypotensive agents such as sodium nitroprusside.[89] Table 44.4 lists the settings in which the use of

44.4 Indications for Parenteral Therapy in Malignant Hypertension

Patients unable to tolerate oral therapy due to intractable vomiting
Hypertensive encephalopathy
Rapidly failing vision
Intracerebral hemorrhage
Acute pulmonary edema
Acute myocardial infarction
Rapid deterioration of renal function
Acute pancreatitis
Gastrointestinal hemorrhage
Acute abdomen secondary to mesenteric vasculitis

parenteral antihypertensive agents is recommended for the initial management of malignant hypertension. In general, parenteral therapy should be utilized in patients who have evidence of acute end-organ damage or who are unable to tolerate oral medications.

The drug of choice for the management of patients with malignant hypertension requiring parenteral therapy is sodium nitroprusside.[90] Nicardipine and labetalol administered by continuous infusion are useful alternative agents.[90,91] The dopamine receptor (DA_1 selective) agonist fenoldopam may also be useful for parenteral treatment of malignant hypertension.[91,92] There are no absolute guidelines for the blood pressure goal during parenteral therapy. The theoretic risks of rapid reduction of blood pressure are discussed later in the section on the controversy over gradual versus rapid reduction of blood pressure. As a general rule, it is safe to initially reduce the mean arterial pressure by 20% or to a level of 160 to 170 mm Hg systolic and 100 to 110 mm Hg diastolic.[93] During the reduction of blood pressure with parenteral antihypertensives, the patient should be monitored closely for evidence of cerebral or myocardial hypoperfusion. The use of a short-acting agent such as sodium nitroprusside or fenoldopam has obvious advantages because the blood pressure can be stabilized quickly at a higher level if complications develop during rapid blood pressure reduction. If there is no evidence of vital organ hypoperfusion following this initial reduction of blood pressure, the diastolic blood pressure can gradually be lowered to 90 mm Hg over a period of 12 to 36 hours.

Oral antihypertensive agents should be initiated as soon as possible so that the duration of parenteral therapy can be minimized. However, a common error in the management of patients with malignant hypertension is the abrupt discontinuation of parenteral therapy immediately after oral therapy has been initiated. With this approach, severe rebound hypertension often develops before the oral antihypertensive regimen becomes effective. Ideally, oral antihypertensives should be initiated as soon as the patient has been stabilized and is able to tolerate medications by mouth. The nitroprusside infusion should be continued until the oral agents have taken effect and have been titrated to an effective dose. The nitroprusside or fenoldopam infusion can then be weaned as the oral regimen is gradually increased.

Although other agents may be effective in the long-term management of patients with malignant hypertension, the cornerstone of initial oral therapy should be an arteriolar vasodilator such as hydralazine, dihydropyridine calcium channel blocker, or minoxidil. Vasodilators may reflexively activate the adrenergic system and cause tachycardia with an increase in cardiac output, which may blunt the hypotensive response. Therefore, treatment with β-adrenergic blockers is usually also required. Direct-acting vasodilators also cause renal salt and water retention, fluid overload, and the development of pseudotolerance to the hypotensive effect of the drug. Thus, although diuretics may not be required for the initial management of patients with malignant hypertension (see later), they are usually required as a part of the long-term maintenance antihypertensive regimen. The regimen that follows has proved to be generally effective in the conversion from parenteral to oral therapy. After the blood pressure has been controlled with sodium nitroprusside and while the infusion is continued, hydralazine (50 mg) and beta-blocker are administered orally. As the oral agents become effective and the blood pressure declines, the nitroprusside infusion is tapered. Brief interruption of the infusion can be used to assess the hypotensive response to oral agents. If after 6 to 8 hours the diastolic blood pressure remains higher than 100 mm Hg, a second dose of hydralazine (100 mg) should be given. The beta blocker dose is increased as needed to maintain the heart rate in the 60 to 80 beats per minute range. If the blood pressure is not controlled with hydralazine at a dose of 100 mg twice daily, minoxidil should be substituted for hydralazine. The starting dose of minoxidil (2.5 mg) is increased by 2.5 to 5.0 mg every 6 to 8 hours until the blood pressure is adequately controlled. The usual effective dose is 5 to 10 mg twice daily. Treatment with a beta-blocker is recommended as for hydralazine. As the blood pressure is brought under control with oral agents, the sodium nitroprusside infusion is gradually weaned. When the convalescing patient is mobilized, upright blood pressure should be carefully monitored to avoid significant orthostatic hypotension. A diuretic, usually furosemide at a starting dose of 40 mg twice daily, is added to either the hydralazine or the minoxidil regimen when it becomes evident that salt and water retention is beginning to occur.

Volume Status and the Role of Diuretics

Routine parenteral diuretic therapy during the acute phase of treatment for malignant hypertension may actually be deleterious. Overdiuresis may result in deterioration of renal function due to superimposed prerenal azotemia. Moreover, volume depletion may activate the renin–angiotensin axis and other pressor hormone systems.

Even patients with malignant hypertension and pulmonary edema may not have an increase in total body salt and water content. Pulmonary congestion in this setting may result from an increase in left ventricular filling pressure due to a decrease in the compliance of the left ventricle (diastolic dysfunction) rather than an increase in left ventricular volume per se. With severe hypertension, the ventricle may become noncompliant due to the excessive workload imposed by the elevated systemic vascular resistance. As a result, left ventricular end-diastolic pressure (LVEDP) increases dramatically even though left ventricular end-diastolic volume may be near normal. With vasodilator therapy, the systemic vascular resistance decreases, left ventricular compliance improves, LVEDP decreases, and left ventricular end-diastolic volume may actually increase.[94] Despite the increase in left ventricular

end-diastolic volume, pulmonary congestion improves because of the reduction in pulmonary capillary pressure. Thus, even in patients with malignant hypertension complicated by pulmonary edema, afterload reduction rather than vigorous diuretic therapy should be the mainstay of initial therapy.

Some patients with malignant hypertension may actually benefit from a cautious trial of volume expansion. Intravascular volume depletion in patients with malignant hypertension should be considered in patients with exquisite sensitivity to vasodilator therapy manifest by a precipitous drop in blood pressure at relatively low infusion rates. Patients with malignant hypertension due to analgesic nephropathy are particularly prone to be severely volume-depleted at presentation due to the presence of chronic interstitial damage with a salt-wasting nephropathy.[17]

In summary, the need for diuretic therapy during the initial phase of treatment for malignant hypertension depends on an assessment of volume status. Unless obvious fluid overload is present, diuretics should not be given initially.

Management of Malignant Hypertension Complicated by Renal Insufficiency

All patients with malignant hypertension should receive aggressive antihypertensive therapy to prevent further renal damage, regardless of the degree of renal impairment. Control of blood pressure in patients with malignant hypertension and renal insufficiency occasionally precipitates oliguric acute renal failure, especially when the initial glomerular filtration rate is less than 20 mL per minute. However, this is not a contraindication to aggressive antihypertensive therapy aimed at normalization of the blood pressure. Control of hypertension protects other vital organs such as the brain and heart whose function cannot be replaced. Moreover, with tight blood pressure control, even patients who appear to have ESRD due to malignant nephrosclerosis have recovered renal function.[95–100]

In patients in whom aggressive control of hypertension precipitates the need for dialysis, dialysis is utilized to control serum chemistry values, treat uremia, and correct fluid overload. However, since dialysis alone rarely results in adequate control of blood pressure in patients with malignant hypertension, concomitant antihypertensive drug therapy is almost always required. A regimen with minoxidil and beta-blocker has proved to be particularly efficacious in this setting.[98,99]

Initial Oral Therapy

Although many patients with malignant hypertension require prompt treatment with parenteral antihypertensive agents, some patients may not yet have evidence of cerebral or cardiac complications, or rapidly deteriorating renal function and therefore do not require instantaneous control of the blood pressure.[89,101,102] These patients may be safely managed with an intensive oral regimen designed to bring the blood pressure under control over a period of 12 to 24 hours.

In patients with malignant hypertension, a multidrug oral regimen is often required to achieve adequate blood pressure control. The most useful combinations include a diuretic, a β-adrenergic blocker, and an arteriolar vasodilator. Minoxidil appears to be particularly well suited for the initial management of malignant hypertension that requires prompt but not immediate blood pressure reduction.[103–105] Alpert and Bauer[105] describe the use of a triple regimen of furosemide, beta-blocker, and minoxidil in nine patients with a diastolic blood pressure higher than 120 mm Hg. Seven of these patients had malignant hypertension. Furosemide (40 mg) and propranolol (40 mg) were given initially by mouth. Two hours later, if the diastolic pressure was higher than 120 mm Hg, a loading dose of minoxidil (20 mg) was administered. If the diastolic pressure was still over 100 mm Hg 4 hours after the loading dose, a booster dose of minoxidil was given. The amount of the booster dose was estimated based on the magnitude of the response to the loading dose. Maintenance therapy with minoxidil was begun with one half the sum of the loading and booster doses given twice daily, with adjustment of beta-blocker and diuretic doses as necessary for control of heart rate and fluid balance. Following the booster dose of minoxidil, a sustained decrease in blood pressure was seen in all patients. No overshoot hypotension or other adverse effects were encountered. During long-term therapy, the physicians were able to substitute hydralazine for minoxidil in five patients. However, the remaining four patients required chronic minoxidil therapy for adequate blood pressure control.[101]

Initial oral therapy with the dihydropyridine calcium channel blocker nifedipine has been shown to be effective in the management of malignant hypertension in black patients who did not require parenteral therapy for hypertensive encephalopathy or acute pulmonary edema.[102] No precipitous decreases in blood pressure or neurologic complications were encountered. However, despite adequate control of blood pressure during the first 24 hours with sustained-released nifedipine, all patients eventually required one or more additional drugs for long-term blood pressure control. Oral loading regimens with clonidine have been advocated in severe uncomplicated (urgent) hypertension.[106] However, there is limited information on the use of oral clonidine loading in the initial management of malignant hypertension. Clonidine loading can cause significant sedation, which may interfere with the assessment of potential neurologic complications during acute blood pressure reduction. Moreover, common side effects such as sedation and dry mouth can have a negative impact on compliance in patients treated with clonidine for the long term. Thus, oral clonidine loading regimens are not indicated for the initial management of malignant hypertension.

Long-Term Management

After the immediate crisis has resolved and the blood pressure has been brought under control with parenteral therapy, oral therapy, or both, lifelong surveillance of the blood pressure is essential. Close follow-up and aggressive treatment are mandatory because noncompliance or inadequate therapy may have devastating consequences. If blood pressure control becomes inadequate, malignant hypertension may recur even after years of successful antihypertensive therapy. In a study of the quality of care provided to patients with a history of malignant hypertension who subsequently died, only 27% of patients had an average treated diastolic blood pressure of less than 110 mm Hg.[107] Thus, meticulous long-term treatment of hypertension is imperative in patients with a history of malignant hypertension. Triple therapy with a diuretic, a beta-blocker, and a vasodilator is often required to achieve satisfactory blood pressure control.

Response to Therapy

In the absence of adequate blood pressure control, malignant hypertension has a uniformly poor prognosis. Without treatment, the 1-year mortality rate approaches 80% to 90%, and uremia is the most common cause of death.[21] However, since the introduction of potent antihypertensive agents, studies have shown that with control of blood pressure, dialysis-free survival can be substantially prolonged. A recent study of survival trends for patients with malignant hypertension (n = 446) over the last 40 years found that there was a significant improvement in 5-year survival from 32% prior to 1977 to 91% for patients diagnosed between 1997 and 2006.[25] Multivariate analysis revealed that age, decade of diagnosis of malignant hypertension, baseline creatinine, and follow-up systolic blood pressure were independent predictors of survival. In a another single-center retrospective analysis of 197 patients with malignant hypertension diagnosed in the period 1974 to 2007, renal damage at presentation was common (63%) but renal function improved or remained stable after diagnosis in the majority of patients.[26] The probability of renal survival was 84% and 72% after 5 and 10 years, respectively. The number of patients with malignant hypertension who improved or stabilized their renal function significantly increased in the second and third periods of the study (1987–2007). Diagnosis during the early study period (1974–1985), baseline renal function, proteinuria, and the presence of microhematuria were associated with an unfavorable outcome. However, by multivariate analysis, mean proteinuria during follow-up remained as the only significant risk factor (OR 2.72; 95% CI, 1.59–4.64). Renal survival for patients with mean protein excretion less than 0.5 g per 24 hours was 100% and 95% at 5 and 10 years, respectively.

The severity of renal impairment at the time of presentation with severe hypertension may also have prognostic significance.[108] Chronic kidney disease and acute kidney injury are common in patients hospitalized with severe hypertension. In the ongoing STAT trial, a U.S.-based, retrospective observational study of management practices and outcomes of patients with severe hypertension, both chronic kidney disease (CKD) and acute kidney injury (AKI) were common. AKI was a strong predictor of greater morbidity and cardiovascular mortality.[109]

Reversal of Hypertensive Neuroretinopathy

The funduscopic changes associated with hypertensive neuroretinopathy are reversible with control of blood pressure.[110] Striate hemorrhages cease to form as soon as the blood pressure is controlled. Clearance of existing hemorrhages takes 2 to 8 weeks. Cotton-wool spots may continue to form for several days after the blood pressure is controlled. The cellular (axonal) debris that comprises the cotton-wool spots is cleared away within 2 to 12 weeks. Hard exudates clear more slowly. A macular star may require more than a year to resolve completely. Papilledema often continues to increase during the first few days of treatment. However, in the majority of patients, it resolves slowly over several weeks. In contrast, the changes reflecting retinal arteriolosclerosis such as arteriolar narrowing, arteriovenous crossing defects, and changes in the light reflexes usually persist despite adequate blood pressure control.[110]

Evaluation for Secondary Causes

The various secondary causes of malignant hypertension were discussed previously in the section on etiologies of malignant hypertension. Whereas less than 5% of patients with benign hypertension have an underlying secondary cause of hypertension, malignant hypertension may be associated with a secondary cause in up to 50% of patients. For example, among patients with benign hypertension, the incidence of renovascular hypertension was less than 0.5%.[111] In contrast, there is a substantial incidence of renovascular hypertension (43% in whites, 7% in blacks) among patients with malignant hypertension.[18] Thus, after malignant hypertension has been treated successfully, the possibility of underlying renovascular hypertension should be investigated. Noninvasive screening tests such as radionuclide renal scans are of little value because of the high frequency of false-positive and false-negative results.[111] Renal arteriography is the procedure of choice to exclude the possibility of anatomic renal artery stenosis. The diagnosis and treatment of renovascular hypertension is discussed in detail in Chapter 42.

Pheochromocytoma is a rare cause of malignant hypertension. However, given the likelihood of surgical cure or amelioration of hypertension, pheochromocytoma should be considered if symptoms consistent with catecholamine excess persist following control of blood pressure. The approach to the diagnosis of pheochromocytoma is discussed in Chapter 43.

The role of renal biopsy in the diagnosis of possible underlying primary renal parenchymal disease in patients with malignant hypertension is controversial. In patients

presenting with malignant hypertension and renal failure, it may not be possible on clinical grounds to distinguish primary malignant hypertension from chronic glomerulonephritis or chronic interstitial nephritis with superimposed malignant nephrosclerosis. A renal biopsy may be required to make this distinction. When the kidneys appear small by ultrasonography, a biopsy is not indicated because it is unlikely that the results of the biopsy will alter therapy. In contrast, when the kidneys are normal in size, a renal biopsy may provide useful information. If primary malignant nephrosclerosis with ischemic but viable glomeruli is found, then intensive antihypertensive therapy may be associated with the eventual recovery of renal function, even after months of maintenance dialysis. Conversely, the finding of chronic glomerulonephritis or chronic interstitial nephritis with superimposed malignant nephrosclerosis suggests a less favorable long-term outcome.

Malignant hypertension can mimic acute glomerulonephritis or vasculitis. Patients can present with severe hypertension and oliguric acute renal failure with nephritic urinary sediment. In this setting, diagnostic renal biopsy is essential since acute glomerulonephritis or vasculitis may require specific therapy in addition to antihypertensive treatment.

Because uremia and severe hypertension predispose to serious hemorrhagic complications after renal biopsy, it is prudent to manage the patient with dialysis and blood pressure control for 1 to 3 weeks prior to performance of a percutaneous renal biopsy. Unfortunately, this delay in obtaining tissue may make the diagnosis of malignant nephrosclerosis more difficult because the lesions of fibrinoid necrosis may heal rapidly with the institution of antihypertensive treatment, leaving a residual hyaline or fibrous scar.[80] Moreover, given the sampling error inherent in closed renal biopsy, the patchy lesions of malignant nephrosclerosis might be missed. Thus, the diagnosis of malignant nephrosclerosis is often made on the basis of the findings of accelerated glomerular obsolescence and marked intimal hyperplasia of the arterioles.[73]

Benign Versus Malignant Hypertension

Since the original description by Volhard and Fahr,[35] two forms of essential hypertension have been recognized: benign and malignant. It is worth emphasizing that these two forms of hypertension should be conceptualized as distinct clinical and pathologic entities. In benign hypertension there is usually a long asymptomatic phase, with death resulting from complications of cerebrovascular disease, atherosclerotic disease, or congestive heart failure, rather than renal disease. In benign essential hypertension (i.e., without underlying primary renal disease or superimposition of malignant hypertension), ESRD seldom occurs.[112–115] In contrast, malignant hypertension left untreated uniformly progresses to ESRD.

There is much controversy in the field of hypertension regarding the frequency with which benign hypertension (benign arteriolar nephrosclerosis), in the absence of occult primary renal disease or superimposed malignant hypertension, causes ESRD. Recent reviews suggest that the number of patients reaching ESRD attributable to benign nephrosclerosis might have been significantly overestimated.[114,115] Goldring and Chasis[46] extensively evaluated renal function in a large group of patients with essential hypertension in the preantihypertensive treatment era. Most patients with long-standing essential hypertension had anatomic lesions in their kidneys consistent with hyaline arteriolar nephrosclerosis. Moreover, the majority had demonstrable renal abnormalities including abnormal urinalysis with hyaline and granular casts, low-grade proteinuria (less than 1 g per day), decreased tubular maximum for para-aminohippurate, decreased renal blood flow, normal to slightly decreased glomerular filtration rate, and increased filtration fraction. However, they found that ESRD rarely occurred in patients with benign hypertension. Among 150 hypertensive patients with ESRD, only one was found to have benign nephrosclerosis as the sole underlying etiology.[46] These authors concluded that in patients with benign hypertension, functional failure occurred earlier in the heart and brain than in the kidney and that death from renal failure without superimposed malignant hypertension was a rare event.

In contrast to these early reports, which were based principally on renal histologic findings at autopsy, in more recent series, "hypertensive nephrosclerosis" is listed as a common cause of ESRD, especially among African American patients. For example, blacks have a four- to eightfold elevation in the risk of hypertension-induced ESRD compared to whites.[116,117] The studies suggest that much of the excess risk of ESRD among blacks can be explained by an extraordinarily high rate of renal failure from hypertensive nephrosclerosis. On a national scale, an estimated 29% of blacks with ESRD have hypertension as the primary cause.[116] However, in these recent studies, classification of the causes of ESRD was based on clinical rather than histologic evidence. Furthermore, in these studies it was not clear whether the term hypertensive nephrosclerosis refers to benign or malignant nephrosclerosis. In the few available studies detailing the pathologic findings in blacks with ESRD due to hypertension, the characteristic findings have been those of malignant nephrosclerosis, namely, musculomucoid intimal hyperplasia of the interlobular arteries and accelerated glomerular obsolescence.[13] Moreover, there appears to be a racial bias with regard to the diagnosis of hypertensive nephrosclerosis. When nephrologists were asked to review identical case histories of patients with ESRD in which only the race of the patient was randomly assigned as either black or white, it was found that black patients were twice as likely as white patients to be labeled as having ESRD secondary to hypertensive nephrosclerosis.[118]

The relationship between essential hypertension and ESRD remains circumstantial despite the fact that these syndromes have long been associated in the medical literature.[115] Nephrologists credit essential hypertension as the cause of ESRD in 25% of patients initiating Medicare-supported renal

replacement therapy. Surprisingly, the widely held notion that benign hypertension with benign nephrosclerosis is a common cause of ESRD is difficult to support.[114,115,119] In contrast to the large body of literature relating mild to moderate benign hypertension to excessive cardiovascular morbidity, there is a dearth of information available regarding the corresponding risk of significant renal disease.[119] In available studies, serum creatinine levels infrequently increase in patients with long-standing mild to moderate hypertension. An analysis of the data from three large clinical trials in patients with essential hypertension revealed that advanced renal failure developed in less than 1% of 10,000 patients during the 4 to 6 years of follow-up.[120–122] Moreover, a very low incidence of clinically significant deterioration of renal function was also noted in the Hypertension Detection and Follow-up Program.[123] Another study of untreated patients with mild to moderate essential hypertension found only minor declines in glomerular filtration rate (1.6% per year) and renal blood flow (2.1% per year), which did not differ from the renal function decline associated with aging in normotensive individuals.[124] Even severe untreated hypertension (diastolic blood pressure, 120 to 150 mm Hg), in the absence of a malignant hypertension (hypertensive neuroretinopathy), caused only a minor decrement in glomerular filtration rate (1.7% per year).[124] Thus, hypertensive nephrosclerosis is commonly reported to Medicare as the cause of ESRD despite the fact that the risk of progressive renal dysfunction in clinical studies of patients with essential hypertension appears to be very low. This paradox could possibly be explained by the fact that the number of patients with essential hypertension is so large that even the small percentage at risk constitutes a relatively large number of patients who eventually develop ESRD. Long-term follow-up data from the Multiple Risk Factor Intervention Trial (MRFIT), in which over 322,000 men were screened for possible entry, support this hypothesis.[125] A direct correlation was found between the initial blood pressure and the risk of development of ESRD from any cause at 16-year follow-up. Nonetheless, the age-adjusted rate of ESRD in this group was only 0.34% at 16 years.

Patients classified as having hypertensive ESRD typically present with advanced disease, making the processes that initiated the renal disease difficult to discern. It has been proposed that many patients classified as having hypertensive nephrosclerosis actually have intrinsic renal parenchymal disease (often immunoglobulin A [IgA] nephropathy), unrecognized renal artery stenosis with ischemic nephropathy, unrecognized episodes of malignant hypertension, or primary renal microvascular disease.[114,115] At least among white patients with hypertension and renal impairment, if renal artery stenosis and malignant hypertension have been excluded, the most likely diagnosis is underlying primary renal parenchymal disease rather than benign nephrosclerosis.[126]

In contrast to these studies, a provocative study found that mild to moderate benign hypertension did cause renal insufficiency that progressed despite adequate blood pressure control.[127] However, since renal biopsies were not performed, the data do not exclude the possibility of occult primary renal parenchymal disease in patients demonstrating progressive renal insufficiency.[128]

In summary, although it is clear that malignant hypertension is a frequent cause of ESRD, especially among blacks, there remains considerable controversy regarding the commonly held belief that benign hypertension per se commonly causes ESRD. The critical issue that has yet to be resolved is why blacks constitute a disproportionately high percentage of patients with ESRD in the United States.[116] Epidemiologic studies suggest that essential hypertension occurs more frequently in blacks and is associated with more severe cardiovascular end-organ damage for any given level of blood pressure.[129] In angiographic studies of patients with mild to moderate essential hypertension and normal renal function, blacks tended to have more severe angiographic evidence of nephrosclerosis than did whites.[130] There are several other plausible explanations for the high frequency with which hypertensive nephrosclerosis is reported as a cause of ESRD in the black population. Since most of the available data are based on clinical diagnoses, there may be a tendency on the part of physicians to identify hypertension as the cause of ESRD given the known high prevalence of hypertension in blacks, even when a primary renal parenchymal disease cannot be excluded on clinical grounds.[119] Another possibility is that blacks with essential hypertension tend to develop more severe benign nephrosclerosis, which, unlike benign nephrosclerosis in whites, more often results in progressive renal insufficiency and ESRD.[115] Results from the African American Study of Kidney Disease (AASK) Trial indicate that benign nephrosclerosis can be accurately diagnosed in black patients with hypertension and renal insufficiency. A renal biopsy was performed in 39 nondiabetic black patients with chronic renal failure who did not have marked proteinuria (urine protein to creatinine ratio less than 2.0). Changes compatible with benign nephrosclerosis were seen in 38 patients. The remaining patient most likely had primary focal segmental glomerulosclerosis.[131] It is possible that genetic factors may increase the susceptibility of blacks to renal damage induced by benign hypertension. On the other hand, the AASK trial demonstrated that strict blood pressure control, including use of ACE inhibitors, failed to halt the progression of renal disease in these black patients with biopsy-proven hypertensive nephrosclerosis.[132] This finding suggests that the focal global glomerulosclerosis lesion identified in AASK trial participants may represent a form of primary renal disease rather than a primary consequence of hypertension-induced renal injury per se. Finally, it is possible that recurrent bouts of unrecognized or inadequately treated malignant hypertension are an underestimated cause of the high incidence of ESRD in minority populations. In this regard, a study of 100 patients admitted to an inner city hospital with a diagnosis of hypertensive emergency showed that two thirds had malignant hypertension based on funduscopic findings.[133] These patients were predominantly

young, male, black, or Hispanic individuals of lower socioeconomic status. At least 93% of these patients had been previously diagnosed as hypertensive, and at least 83% were aware of their diagnosis of hypertension. At least 87% were known to have received prior pharmacologic treatment for hypertension. However, no source of regular health care could be documented in 60% of patients. More than 50% were noted to have stopped their antihypertensive medications more than 30 days prior to admission and only 24% had taken any medication on the day of admission. If the overrepresentation of young blacks with ESRD is due to undiagnosed or inadequately treated malignant hypertension, this would have tremendous public health implications given that malignant hypertension is clearly preventable, and even significant renal dysfunction is potentially reversible with tight control of blood pressure.

HYPERTENSIVE ENCEPHALOPATHY

Most of the deleterious effects of hypertension on the brain are the result of long-standing mild to moderate elevations of blood pressure, including atherothrombotic infarction, lacunar infarction, and intracerebral hemorrhage. Occasionally, severe acute hypertension can produce dramatic and life-threatening cerebral complications. Hypertensive encephalopathy is an acute cerebral syndrome that develops in association with a sudden, sustained elevation of blood pressure.[56] It can occur with malignant hypertension or severe "benign" hypertension that is not accompanied by hypertensive neuroretinopathy. Hypertensive encephalopathy is a medical emergency that demands prompt diagnosis and rapid control of blood pressure to prevent irreversible brain damage or death. The clinical sine qua non of hypertensive encephalopathy is the prompt resolution of symptoms when the blood pressure is brought under control.

Clinical Presentation

The diagnosis of hypertensive encephalopathy is usually made on clinical grounds. The appearance of cerebral symptoms usually follows the sudden onset of hypertension in previously normotensive individuals or an abrupt increase in blood pressure in patients with chronic hypertension. The abrupt blood pressure elevation usually occurs 12 to 48 hours before the onset of symptoms, although this is often difficult to document. Symptoms may appear at lower levels of blood pressure in previously normotensive individuals compared to those with chronic hypertension. For example, in children with acute glomerulonephritis or pregnant women with eclampsia, hypertensive encephalopathy may occur when the blood pressure is no higher than 160/100 mm Hg.[134] However, the syndrome rarely occurs in chronically hypertensive individuals at pressures less than 200/120 mm Hg and may not occur until the blood pressure is more than 250/150 mm Hg.

The initial symptom of hypertensive encephalopathy is usually a severe, generalized headache that increases steadily in severity.[135] Unfortunately, headache is a nonspecific symptom, and even among patients with malignant hypertension, it does not necessarily imply CNS damage. Weakness, nausea, and vomiting (sometimes projectile) are often present. Neck stiffness is an occasional finding. Loss of vision is another common feature. Visual loss may be caused by the retinal edema and hemorrhages that accompany hypertensive neuroretinopathy or as the result of cortical (occipital) blindness.[136] Denial of visual loss or loss of vision in the presence of a normal light reflex suggests cortical blindness.

Altered mental status is a prominent clinical feature of hypertensive encephalopathy. Apathy, somnolence, and confusion are the initial manifestations that usually appear several hours to days after the onset of headache. If treatment is not instituted, coma and death can occur. Recurrent seizures are common, and they can be either focal or generalized.

There are numerous reports of transient focal neurologic disturbances in patients with hypertensive encephalopathy including fleeting paresthesias and numbness in the extremities, transient paralysis, and aphasia.[56,136] Thus, the presence of focal neurologic deficit in a patient with severe hypertension does not necessarily exclude the diagnosis of hypertensive encephalopathy.

Hypertensive neuroretinopathy (striate hemorrhages, cotton-wool spots, and papilledema) is present when hypertensive encephalopathy occurs in patients with malignant hypertension. However, it may be absent when hypertensive encephalopathy develops in the setting of acute glomerulonephritis, eclampsia, monoamine oxidase inhibitor–tyramine interactions, antihypertensive drug withdrawal syndromes, or pheochromocytoma.[134,136,137]

Many authors have cautioned that lumbar puncture should be avoided in patients with suspected hypertensive encephalopathy because of the risk of cerebellar herniation.[138] When performed, lumbar puncture has revealed elevated cerebrospinal fluid (CSF) pressure in most patients ranging from 230 to 560 mm of water.[136] CSF protein concentration is usually moderately elevated (48 to 90 mg per dL) but may be normal. The cell count is usually normal,[136] but neutrophilic pleocytosis has also been reported in hypertensive encephalopathy.[138]

Computed tomography (CT) and magnetic resonance imaging (MRI) reveal characteristic findings in hypertensive encephalopathy.[139–143] Abnormalities on imaging include areas of low white matter attenuation on CT scans and T1-weighted hypointense and T2-weighted hyperintense areas on MRI. These changes probably represent cerebral edema with increased water in the white matter. The most common location of the white matter abnormalities on neuroimaging is the posterior regions of the cerebral hemispheres. The multifocal abnormalities include both hemispheres and tend to be symmetric. Commonly involved areas in descending order of frequency include the occipital lobes, the posterior parietal lobes, and the posterior temporal lobes. The pons, the thalamus, and the cerebellum are occasionally involved. The term reversible posterior leukoencephalopathy syndrome

has been coined to describe patients with these typical radiographic findings and a reversible syndrome of headache, altered mental status, seizures, and loss of vision.[140] A reversible hypertensive brainstem encephalopathy with predominant involvement of the brainstem and relative sparing of supratentorial regions has also been reported.[143]

Etiologies

Although hypertensive encephalopathy can complicate malignant hypertension, not all patients with hypertensive encephalopathy have malignant hypertension. In fact, it most commonly occurs in previously normotensive individuals who experience sudden, severe hypertension (Table 44.5). The reported causes of hypertensive encephalopathy include acute glomerulonephritis,[134,135] eclampsia,[144,145] renovascular hypertension,[134] postcoronary artery bypass hypertension, clonidine withdrawal,[146] monoamine oxidase inhibitor–tyramine interactions,[147] pheochromocytoma,[148] phencyclidine (PCP) poisoning,[149] licorice ingestion,[150] phenylpropanolamine overdose,[151,152] acute renal artery occlusion,[136] acute lead poisoning,[136] immunosuppressive therapy with cyclosporine or tacrolimus for kidney, liver, or bone marrow transplantation,[153,154] chemotherapy for acute leukemia in children,[155] transplant renal artery stenosis or acute rejection,[156,157] and femoral lengthening procedures in children.[158] The preeclampsia–eclampsia syndrome has been hypothesized to reflect a subtype of hypertensive encephalopathy accompanied by impaired cerebral autoregulation and endothelial dysfunction.[137,141,144,145] The clinical and radiographic findings in patients with cyclosporine-induced neurotoxicity have been found to be identical to those seen in hypertensive encephalopathy.[154] The only major factor found to be associated with the neurotoxic effect of cyclosporine in all patients was hypertension. Subcortical edema, affecting the posterior regions of the brain, tends to resolve following reduction in blood pressure, with or without concomitant reduction in cyclosporine dose. Hypertensive encephalopathy may also occur in patients with acute or chronic spinal cord injuries if there is autonomic hyperreflexia due to bowel or bladder distention.[159,160] Acute elevation of blood pressure during recombinant human erythropoietin therapy occasionally results in hypertensive encephalopathy and seizures.[161] This complication is unrelated to the extent or rate of increase in hematocrit, but is associated with a rapid increase in blood pressure and may occur in previously normotensive patients. Scorpion envenomization results in stimulation of the autonomic nervous system and adrenals and in children can lead to severe hypertension and a clinical picture consistent with hypertensive encephalopathy.[162] Cocaine use can also induce a sudden increase in blood pressure accompanied by hypertensive encephalopathy.[163]

44.5 Etiologies of Hypertensive Encephalopathy

Malignant hypertension of any etiology
Acute glomerulonephritis
Eclampsia
Renovascular hypertension
Postcoronary artery bypass hypertension
Abrupt withdrawal of antihypertensive therapy
Monoamine oxidase inhibitor-tyramine interactions
Pheochromocytoma
Phencyclidine (PCP) poisoning
Phenylpropanolamine overdose
Recombinant erythropoietin therapy in dialysis patients
Scorpion envenomation, especially in children
Cocaine hydrochloride or alkaloidal (crack) cocaine
Acute renal artery occlusion
Acute lead poisoning in children
Cyclosporine-induced or tacrolimus-induced hypertension
Transplant renal artery stenosis or acute rejection
Femoral lengthening procedures
Acute or chronic spinal cord injuries

Pathogenesis

The breakthrough theory of autoregulation originally proposed by Lassen and Angoli[164] is the generally accepted view of the pathogenesis of hypertensive encephalopathy (Fig. 44.17). Under normal circumstances, there is autoregulation of the cerebral microcirculation such that, over a wide range of perfusion pressures, cerebral blood flow remains constant. It has been proposed that in the setting of a sudden, severe increase in blood pressure, autoregulatory vasoconstriction fails and there is forced vasodilation. The dilation is initially segmental (sausage-string pattern), but eventually becomes diffuse. The endothelium in the dilated segments becomes abnormally permeable, and there is extravasation of plasma components with the development of cerebral edema. This theory may explain the clinical observation that hypertensive encephalopathy develops at a much lower blood pressure in previously normotensive individuals than it does in those with chronic hypertension. With long-standing hypertension, structural changes and remodeling

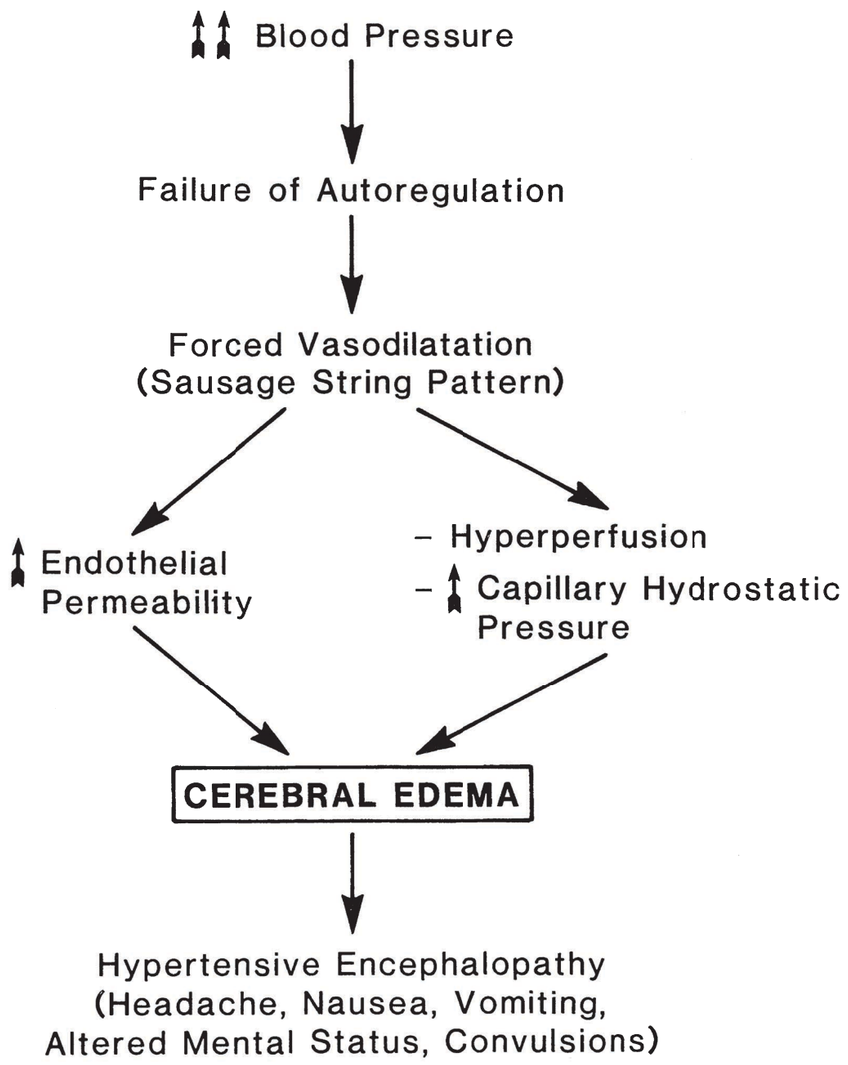


FIGURE 44.17 The breakthrough theory of hypertensive encephalopathy.

of the cerebral arterioles may lead to a shift in the autoregulatory curve such that much higher perfusion pressures can be tolerated before forced vasodilation and breakthrough of autoregulation occur.[165,166]

Treatment

The treatment of choice for hypertensive encephalopathy is prompt reduction of blood pressure. When the diagnosis of hypertensive encephalopathy seems likely, antihypertensive therapy should be initiated prior to obtaining the results of time-consuming laboratory and radiologic examinations. The goal of therapy, especially in the previously normotensive patient with acute hypertension, should be the reduction of blood pressure to normal or near-normal levels as quickly as possible.[134] Although cerebral blood flow could theoretically be jeopardized by failure of autoregulation during rapid reduction of blood pressure in patients with chronic hypertension, clinical experience has shown that the prompt reduction of blood pressure with the avoidance of frank hypotension is beneficial in patients with hypertensive encephalopathy.[134] Of the conditions in the differential diagnosis of hypertension with acute cerebral dysfunction, only cerebral infarction might be adversely affected by the abrupt reduction of blood pressure. Pharmacologic agents that have a rapid onset and short duration of action such as sodium nitroprusside, continuous infusion labetalol, or possibly fenoldopam should be utilized so that the blood pressure can be carefully titrated with close monitoring of the patient's neurologic status. There is some evidence that compared to labetalol, nitroprusside reduces systemic vascular resistance more than cerebral vascular resistance and could, therefore, result in shunting of blood from the brain.[167] The clinical sine qua non of hypertensive encephalopathy is a prompt clinical response to blood pressure reduction. Conversely, when antihypertensive therapy is associated with the development of new or progressive neurologic deficits, other diagnoses should be considered, and the blood pressure should be stabilized at a higher level.

In women with eclampsia, convulsions and other neurologic manifestations occur and are indistinguishable from those observed in nonpregnant individuals with hypertensive encephalopathy, except that in eclampsia they occur at a lower level of blood pressure.[145] Eclampsia is associated with extreme risk to both the mother and the fetus. Although delivery of the fetus is the definitive cure in most cases, rapid control of the blood pressure and encephalopathic manifestations is essential before the induction of labor or performance of a cesarean section.[137,144]

ACUTE HYPERTENSION COMPLICATING CEREBROVASCULAR ACCIDENT

The importance of hypertension as a risk factor for cerebrovascular accident is well established. The Framingham Study shows that regardless of gender or age, hypertension is associated with an increased incidence of ischemic and hemorrhagic stroke.[168] Several prospective, randomized clinical trials demonstrate that long-term antihypertensive drug therapy results in a significant reduction in morbidity and mortality from cerebrovascular accident.[169] Despite the proven benefits of blood pressure control in the prevention of stroke, the role of treatment of hypertension in the acute phase of stroke remains controversial. Whether antihypertensive therapy is indicated depends not only on the magnitude of the blood pressure elevation, but also on the type of cerebrovascular accident. It should be emphasized that the management of hypertension accompanying cerebral infarction is different from that for hypertension complicating either intracerebral hemorrhage or subarachnoid hemorrhage.

Cerebral Infarction

In the cerebral circulation, the sites of predilection for atherosclerosis are the bifurcations of the common carotid arteries, the carotid siphons, the origins of the vertebral and basilar arteries, the circle of Willis, and the proximal parts of the cerebral arteries.[170] Cerebral infarction can result from partial or complete occlusion of an artery by a plaque or embolization of atherothrombotic debris from a plaque. The atherothromboembolic infarcts produced by one of these mechanisms typically involve the cerebral or cerebellar cortex or the pons.[170] In contrast, hypertension-induced lipohyalinosis of the small penetrating cerebral end arteries is the principal cause of the small, deep lacunar infarcts that occur in the basal ganglia, pons, thalamus, cerebellum, and deep hemispheric white matter.[170]

Hypertension is common in the setting of acute cerebral infarction. In a series of 334 consecutive patients admitted for acute stroke, the blood pressure was elevated in 84% of the patients on the day of admission. Even without specific antihypertensive treatment, the blood pressure decreased spontaneously by an average of 20 mm Hg systolic and 10 mm Hg diastolic in the 10 days following the acute event.[171] This early elevation in blood pressure most likely represents a physiologic response to brain ischemia. Decreases in blood pressure accompany recovery of brain function.

Because of the known benefits of antihypertensive therapy with regard to stroke prevention, it has been assumed that reduction in blood pressure would benefit patients with acute cerebral infarction. Unfortunately, because treatment of hypertension in this setting has never been evaluated in a prospective, randomized trial, there are no good data to guide management. Moreover, there is no evidence to suggest that rapid reduction of blood pressure is beneficial. In fact, several cases have been reported in which worsening of the patient's neurologic status was apparently precipitated by emergency treatment of hypertension.[172,173] The rationale for not treating hypertension in acute ischemic strokes is based on concerns regarding impairment in autoregulation of cerebral blood flow in this setting.[173,174] In normal individuals, cerebral blood flow is maintained constant at mean arterial pressures ranging between 60 and 120 mm Hg. However, in patients with chronic hypertension as well as older adult patients, the curve is shifted such that the lower limit of autoregulation occurs at a higher mean arterial pressure. Furthermore, there is evidence that local autoregulation of cerebral blood flow is disturbed in the so-called ischemic penumbra that surrounds an area of acute infarction.[173,174] Without intact autoregulation, the regional blood flow becomes critically dependent on the perfusion pressure. Thus, to some extent, the presence of hypertension may be beneficial in the setting of acute cerebral infarction, whereas reduction of blood pressure may cause a regional decrease in blood flow with extension of the infarct.

The recently published SCAST trial examined whether careful blood pressure reduction with the angiotensin-receptor blocker candesartan is beneficial in the treatment of patients with acute stroke accompanied by elevated blood pressure.[175] Participants were recruited from 146 centers in nine northern European countries. Patients older than 18 years with acute stroke (ischemic or hemorrhagic) and systolic blood pressure of 140 mm Hg or higher were initiated on study drug or placebo therapy within 30 hours of onset of symptoms. Patients were randomly allocated to candesartan ($n = 1017$) or placebo treatment ($n = 1012$) for 7 days, with doses increasing from 4 mg on day 1 to 16 mg on days 3 to 7. There were two coprimary outcome variables: the composite endpoint of vascular death, myocardial infarction, or stroke within the first 6 months; and the functional outcome at 6 months. During the 7-day treatment period, blood pressures were significantly lower in the candesartan group (147/82 mm Hg, standard deviation [SD] 23/14 mm Hg) compared to the placebo group (152/84 mm Hg, SD 22/14 mm Hg; $P < .0001$). During the 6 months of follow-up, the risk of the composite vascular endpoint did not differ between treatment groups (candesartan, 120 events, versus placebo, 111 events; adjusted hazard ratio 1.09, 95% CI 0.84–1.41; $P = .52$). Analysis of functional outcomes suggested a higher risk of poor outcomes in the candesartan group (adjusted common OR 1.17, 95% CI 1.00–1.38; $P = .048$, not significant at $P \leq .025$ level). The observed effects were similar for all prespecified secondary endpoints including death from any cause, vascular death, ischemic stroke, hemorrhagic stroke, stroke progression, myocardial infarction, symptomatic hypotension, and renal failure. The authors conclude that there was no indication that careful blood pressure reduction with candesartan in the setting of acute stroke is beneficial; if anything, the evidence suggested a harmful effect of blood pressure reduction.

A recent metaregression analysis of the differences in on-treatment blood pressure and odds ratio for outcomes after acute stroke has been performed.[176] In an analysis of 37 trials involving 9008 patients, a U or J shaped relationship was identified amongst on-treatment blood pressure differences and early or 90-day death rates. Although large increases in blood pressure were associated with worse outcomes, modest reductions in blood pressure were beneficial in this large observational study.

Because there is no evidence that mild to moderate hypertension has a deleterious effect on the outcome of cerebral infarction during the acute stage, it is probably wise to allow the blood pressure to seek its own level during the first few days to weeks after the event. In most cases, the hypertension tends to resolve spontaneously over the first week without specific therapy.[171] On the other hand, if hypertension persists for more than 3 weeks in a patient with a completed stroke, gradual reduction of blood pressure into the normal range can be accomplished safely. The goal of long-term antihypertensive treatment in hypertensive stroke survivors is the prevention of stroke recurrence. The benefits of antihypertensive therapy in secondary stroke prevention are uncertain, but large clinical trials are in progress that should provide helpful guidelines for clinical practice.

Although benign neglect of mild to moderate hypertension is prudent in the setting of acute cerebral infarction, there may be certain indications for active treatment of hypertension. When the diastolic blood pressure is sustained at more than 130 mm Hg, many authorities recommend cautious reduction of the systolic blood pressure to 160 to 170 mm Hg and diastolic to 100 to 110 mm Hg with a short-acting parenteral agent such as sodium nitroprusside.[173,177–179] Stroke accompanied by other hypertensive crises such as acute myocardial ischemia or left ventricular dysfunction with acute pulmonary edema is also an indication for cautious blood pressure reduction.[173,178] Stroke due to carotid occlusion caused by aortic dissection mandates aggressive blood pressure reduction to prevent propagation of the dissection.[173,178] In some patients with severe hypertension,

it may be impossible to distinguish between hypertensive encephalopathy and cerebral infarction on clinical grounds. Because rapid lowering of the blood pressure may be life-saving in the patient with hypertensive encephalopathy, a cautious diagnostic trial of blood pressure reduction with a short-acting parenteral antihypertensive agent, such as sodium nitroprusside, may be indicated.[178] In patients who have suffered a stroke and require anticoagulation therapy, moderate control of severe hypertension into the 160 to 170 mm Hg systolic and 100 to 110 mm Hg diastolic range may also be prudent. In the severely hypertensive patient with progressing stroke in whom continued deterioration is believed to be secondary to concomitant cerebral edema, cautious blood pressure reduction may be warranted. Appropriate management of such patients may require continuous intracranial as well as intra-arterial pressure monitoring so that cerebral perfusion pressure can be optimized.[178]

It has been demonstrated that sodium nitroprusside, given at a dose that reduced mean arterial pressure by 10 mm Hg, significantly inhibited platelet aggregation and adhesion molecule expression and improved regional cerebral blood flow in patients with acute ischemic stroke.[180] These findings were attributed to beneficial effects of nitric oxide on platelet function and local vasodilation in the area of the ischemic penumbra.

In the setting of acute cerebral infarction, hypertension tends to be very labile and exquisitely sensitive to hypotensive therapy. Even modest doses of oral antihypertensive agents may cause profound and devastating overshoot hypotension.[172] Antihypertensive treatment, when indicated, should be initiated with extreme caution using small doses of short-acting agents such as sodium nitroprusside. Use of oral or sublingual nifedipine may be associated with overshoot hypotension resulting in extension of the infarct and is contraindicated for the treatment of hypertension accompanying acute cerebral infarction. Oral clonidine loading is also contraindicated because it may induce overshoot hypotension or lead to sedation, which will interfere with assessment of mental status. It had been proposed that there the calcium channel blocker nimodipine, which is a cerebral vasodilator, might theoretically minimize arterial spasm and therefore improve cerebral ischemia. However, a large controlled clinical trial demonstrated no improvement in outcome in patients with thrombotic stroke treated with nimodipine when compared to placebo treatment.[181]

Intracerebral Hemorrhage

Hypertension is a major risk factor for intracerebral hemorrhage. The small-diameter, penetrating cerebral end arteries are especially vulnerable to the deleterious effects of hypertension because they arise directly from the main arterial trunks.[170] The most common sites of hypertension-associated hemorrhage include the basal ganglia, pons, thalamus, cerebellum, and deep hemispheric white matter.[182] Lacunar infarcts arise from the same vessels and are similarly distributed.

Hypertensive hemorrhage most often occurs in patients older than 50 years of age. Intracerebral hemorrhage characteristically begins abruptly with headache and vomiting followed by steadily increasing focal neurologic deficits and alteration of consciousness.[182] More than 90% of hemorrhages rupture through brain parenchyma into the ventricles, producing a bloody CSF.[182] Patients presenting with acute intracerebral hemorrhage invariably have elevated blood pressure. In fact, the finding of a normal or low blood pressure makes the diagnosis of intracerebral hemorrhage unlikely.[182] In contrast to cerebral infarction, the blood pressure does not tend to decrease spontaneously during the first week after the event.[171] Once the hemorrhage has occurred, the patient's condition worsens steadily over a period of minutes to days until either the neurologic deficit stabilizes, or the patient dies. When death occurs, it is most often due to herniation caused by the expanding hematoma and surrounding edema.

Small hemorrhages, which may be clinically indistinguishable from cerebral infarction, probably require no specific therapy.[170] The issue of treatment of hypertension in the setting of a large (greater than 3 cm) intracerebral hemorrhage is controversial. There is almost always a rise in intracranial pressure accompanied by a reflex increase in systemic blood pressure.[171] Because cerebral perfusion pressure is a function of the difference between systemic arterial pressure and intracranial pressure, reduction of blood pressure may compromise cerebral perfusion. Furthermore, the hematoma impairs the local autoregulatory response in the surrounding area of marginal ischemia.[173] Because there is no good evidence that persistent hypertension promotes further bleeding, some authorities strongly advise against treating hypertension in patients with intracerebral hemorrhage.[172] On the other hand, cerebral vasogenic edema may develop as a consequence of an abrupt, severe increase in blood pressure,[170] and treatment of hypertension may be beneficial by virtue of a reduction in cerebral edema and intracranial pressure. Thus, in deciding to treat hypertension, a precarious balance must be struck between prevention of cerebral edema on the one hand, and deleterious reduction of cerebral blood flow on the other. In a study of eight patients with intracerebral hemorrhage treated with trimethaphan, cerebral blood flow measurements revealed that the cerebral autoregulation curve was intact but shifted such that the lower limit of autoregulation was at 80% of the initial level of blood pressure.[183] Thus, a 20% decrease in mean arterial pressure should be considered the maximal reduction of blood pressure during the acute stage. Active treatment of the blood pressure should only be undertaken in the intensive care environment where intracranial pressure and intra-arterial pressure can be closely monitored.[170,184]

Although current specific therapeutic guidelines for blood pressure treatment in intracerebral hemorrhage are not based on strong evidence, a large ongoing therapeutic trial has reported on the value of intensive blood pressure reduction in acute cerebral hemorrhage—the INTERACT Trial.[184] Patients with acute hemorrhage were randomized

within 6 hours to targeted systolic blood pressure of 140 mm Hg (intensive group) or to targeted systolic blood pressure of 180 mm Hg (guideline group). The primary endpoint was hematoma growth at 24 hours. At 24 hours the achieved blood pressure was 146 mm Hg in the intensive group and 157 mm Hg in the guideline group. Hematoma growth was reduced in the intensive group with an absolute risk reduction of 8% ($P < .05$). There were no differences in adverse events between the two groups. The authors concluded that early blood pressure lowering in hemorrhagic stroke is feasible, safe, and reduces hematoma growth. It will be important to determine if these results yield better functional outcomes in further larger trials.

The drug of choice for the management of hypertension in the setting of intracerebral hemorrhage is a matter of debate. Sodium nitroprusside has traditionally been regarded as the best agent because its brief duration of action allows for rapid titration with avoidance of the catastrophic consequence of sustained overshoot hypotension.[179] However, concern has been expressed that because sodium nitroprusside causes an increase in venous capacitance as well as cerebral arterial vasodilation, the resulting increase in cerebral blood volume may cause a further elevation of intracranial pressure.[185,186] Other cerebral vasodilators such as intravenous nitroglycerin, hydralazine, or calcium channel blockers also can cause potentially deleterious elevations of intracranial pressure in patients with compromised intracranial compliance due to intracranial disease.[186] Because labetalol and urapidil (a postsynaptic α-receptor blocker) may not alter intracranial pressure, they have been recommended for treatment of hypertension in patients undergoing neurosurgery.[186] Unfortunately, these agents have the potential to cause overshoot hypotension, which may be difficult to quickly reverse. Thus, despite the theoretic risk of elevation of intracranial pressure, sodium nitroprusside remains the treatment of choice when severe hypertension must be treated in the patient with intracerebral hemorrhage because its brief duration of action allows for cautious, graded blood pressure reduction, which can be quickly reversed if the patient's neurologic status deteriorates or a further increase in intracranial pressure occurs. Of interest, some patients with cerebral infarction or hemorrhage have extreme elevations of catecholamine levels that may render hypertension refractory to sodium nitroprusside in the absence of concomitant beta-blocker therapy.[187]

A recent prospective randomized trial compared safety and efficacy of intravenous nicardipine and sodium nitroprusside drip for control of hypertension in the neurosurgical intensive care unit in patients with subarachnoid hemorrhage or intracerebral hemorrhage.[188] One hundred and sixty-three patients were randomized including 89 in the sodium nitroprusside group and 74 in the nicardipine group. Both drugs proved safe and effective for control of hypertension. However, patients randomized to nicardipine had fewer dose adjustments per day: 5.7 versus 8.8 in the nitroprusside group ($P = .0012$). There were fewer additional medications per day to maintain blood pressure control in the nicardipine group: 1.4 versus 1.9 ($P = .043$). Moreover, blood pressure control was similar in the two groups: 66% versus 69% of the time within study-defined parameters for nicardipine versus nifedipine respectively ($P =$ not significant).

Another recent study compared the clinical outcomes between patients with intracerebral hemorrhage treated with different antihypertensive medications during the first 24 hours after admission.[189] Analysis of the Premier database, a nationally representative hospital discharge database, was used to compare discharge outcomes, length of stay, and cost of hospitalization between groups of patients with intracerebral hemorrhage who were treated with either intravenous nicardipine or nitroprusside infusion. Logistic and linear regression analyses were performed to adjust for baseline risk of mortality between the two groups. A total of 12,767 admissions with primary diagnosis of intracerebral hemorrhage were identified. Nicardipine was administered in 926 patients (7.3%) and nitroprusside was administered in 530 (4.3%). There were no differences in baseline disease severity or risk of mortality between the two groups. After adjustment for baseline risk of mortality, the risk of in-hospital mortality was higher among patients treated with nitroprusside compared with nicardipine (OR 1.7, 95% CI 1.3–2.2). There was no difference in length of stay or total hospital costs in the multivariate analysis. The authors conclude that nicardipine compared with nitroprusside during the first 24 hours after intracerebral hemorrhage was associated with a significantly reduced risk of in-hospital mortality.

Cerebellar hemorrhage represents a neurosurgical emergency requiring prompt diagnosis and treatment.[170] Typically, patients complain of the sudden onset of dizziness, nausea, vomiting, headache, and difficulty walking. Truncal ataxia, nystagmus, and ipsilateral sixth nerve paresis may be present. If the process continues unchecked, brainstem compression or herniation produces progressive stupor and coma. The untreated mortality is extremely high. The diagnosis can usually be confirmed by computerized tomography. Treatment consists of emergency suboccipital craniotomy with evacuation of the hematoma.[170]

Subarachnoid Hemorrhage

Subarachnoid hemorrhage (SAH) accounts for less than 10% of all cerebrovascular accidents. Rupture of a congenital aneurysm is the most common cause. Rupture is heralded by the sudden onset of a profound headache and is often followed by brief syncope. If the mass of the hemorrhage is large, patients rapidly become comatose. As the hemorrhage diffuses throughout the subarachnoid space, the patient may awaken and experience headache, nausea, vomiting, and seizures. Within 24 hours, nuchal rigidity and other meningeal signs develop. Initially, neurologic findings are nonfocal. CT can be used to confirm the diagnosis.

Recurrent hemorrhage is a potential complication associated with high mortality. Whether treatment of hypertension after SAH reduces the risk of recurrent bleeding or

improves prognosis is uncertain. In the setting of elevated intracranial pressure or cerebral arterial vasospasm, hypertension may actually be protective because it helps to maintain cerebral perfusion pressure. Thus, reduction of the blood pressure could conceivably result in aggravation of cerebral vasospasm and ischemia.

Early surgical repair of the aneurysm has reduced the incidence of rebleeding in patients with SAH. In fact, delayed cerebral ischemia due to cerebral arterial vasospasm has been found to be the most important cause of morbidity and mortality in patients who survive the initial hemorrhage.[190] Vasospasm, which is probably caused by the irritating effects of blood in the subarachnoid space closely opposed to the large arteries, usually develops 4 to 12 days after the acute hemorrhage. Symptoms include a gradual deterioration of the level of consciousness, accompanied by focal neurologic deficits.

Surgical clipping of the aneurysm is usually undertaken as soon as possible to prevent rebleeding.[190,191] There is conflicting evidence as to whether or not postoperative treatment with intravascular volume expansion, in conjunction with deliberate induction of arterial hypertension using dopamine or dobutamine, may be an effective means of reversing the ischemic neurologic deficits caused by cerebral vasospasm.[192,193]

Nimodipine, a 1,4-dihydropyridine calcium channel blocker, has been approved for the prevention and treatment of delayed cerebral ischemia caused by subarachnoid hemorrhage from ruptured congenital aneurysms. Nimodipine is highly lipid-soluble and readily crosses the blood–brain barrier.[194] It dilates cerebral blood vessels at concentrations lower than those required for dilation of the peripheral vasculature.[194] Thus, it may dilate intracerebral vessels at doses that do not result in a significant reduction in mean arterial pressure. Furthermore, inhibition of calcium uptake by neurons may also protect against ischemic injury at the cellular level, independent of an effect on cerebral blood flow.[194] Nimodipine has been shown, in randomized, placebo-controlled trials, to reduce the severity of neurologic deficits resulting from vasospasm in patients who have had a recent SAH.[194–196] The recommended dosage is 60 mg orally every 4 hours for 21 consecutive days beginning within 96 hours of the SAH. The liquid content of the capsules can be given through a nasogastric tube in unconscious patients. The optimal timing of surgery in nimodipine-treated patients has not yet been defined.

CATECHOLAMINE-RELATED HYPERTENSIVE CRISES

Hypertensive Crises with Pheochromocytoma

The diagnosis and treatment of pheochromocytoma are discussed in detail in Chapter 43. The comments here are restricted to treatment of hypertensive crises in patients with pheochromocytoma, with emphasis on the perioperative management of hypertension. In the majority of patients, pheochromocytoma causes sustained hypertension that occasionally enters the malignant phase. In roughly 30% of patients, paroxysmal hypertension is present. Paroxysms usually occur spontaneously and consist of severe hypertension, headache, profuse diaphoresis, pallor of the face, coldness of the hands and feet, palpitations, and abdominal discomfort. Marked elevation of blood pressure can lead to intracerebral hemorrhage, hypertensive encephalopathy, or acute pulmonary edema.[198] Prompt reduction of blood pressure is mandatory to prevent these life-threatening complications. Although the nonselective α-adrenergic receptor blocker phentolamine is often cited as the treatment of choice for pheochromocytoma-related hypertensive crises, sodium nitroprusside is equally effective.[198,199] Phentolamine is given in 5- to 10-mg intravenous boluses every 5 minutes as necessary to control blood pressure. Given its short duration of action, a continuous infusion of phentolamine can also be utilized. After the blood pressure has been controlled with sodium nitroprusside or phentolamine, intravenous β-adrenergic receptor blockers such as esmolol and metoprolol can be used to control tachycardia or arrhythmias. After resolution of the hypertensive crisis, oral antihypertensive agents should be instituted as the parenteral agents are weaned.

Skillful preoperative management of blood pressure and volume status is clearly a prerequisite to successful surgical intervention.[198–200]Usually, the nonselective α-blocker phenoxybenzamine is administered for 1 to 2 weeks prior to elective surgery. The initial dose of 10 mg twice daily is increased every other day until normotension, accompanied by moderate (15 mm Hg) asymptomatic orthostatic hypertension, has been attained and paroxysms are well controlled.[199,200] The last dose of phenoxybenzamine is usually administered at 10 PM on the evening before surgery. After adequate α-blockade has been achieved, oral beta-blocker therapy can be initiated if needed to control tachycardia. Oral or intravenous beta-blockers should never be administered before adequate α-adrenergic blockade has been achieved. Administration of a beta-blocker to patients with catecholamine-secreting tumors can lead to severe hypertension with acute pulmonary edema as the result of intense α-adrenergic-mediated vasoconstriction that is no longer opposed by β-adrenergic vasodilatory stimuli. Prazosin, a selective α_1-antagonist, has been used for preoperative management of hypertension.[201] However, hypertensive crises responsive to low-dose phenoxybenzamine have been observed in patients receiving apparently adequate α-blockade with prazosin.[202] Labetalol has also been advocated for the preoperative management of hypertension in patients with pheochromocytoma.[203] However, hypertensive crises precipitated by the use of labetalol have been reported.[204] The paradoxical increase in blood pressure is due to the fact that labetalol exhibits more potent β-blockade than α-blockade.

Careful attention to volume status is imperative in the preoperative period.[199,200] Alleviation of the chronic state of catecholamine-induced vasoconstriction by α-blockade results in increases in both arterial and venous capacitance. Preoperative volume expansion guided by measurements of central venous pressure or pulmonary capillary wedge pressure has been advocated to reduce the severity of intraoperative hypotension.[200] However, other authors maintain that a high-salt diet or infusions of crystalloid are usually not necessary in the majority of patients during the preoperative period because treatment with α-adrenergic blockade for 1 to 2 weeks alleviates the chronic state of vasoconstriction and allows for spontaneous restoration of normal plasma volume.[198] Moreover, caution has been advised if intravenous fluids are administered during the preoperative period because pulmonary edema can occur if an underlying catecholamine-induced cardiomyopathy is present.[198]

Cardiac status should be evaluated carefully in the preoperative period. Approximately 25% of patients with catecholamine-secreting tumors have some degree of cardiomyopathy with biventricular dysfunction caused either by a direct toxic effect of catecholamines on the myocardium or indirectly by chronic hypertension.[206] This catecholamine-induced cardiomyopathy is associated with an increased risk of sudden death from arrhythmias, as well as increased surgical risk. Thus, preoperative evaluation should include echocardiography to assess ventricular function. The cardiomyopathy is usually reversible with adequate preoperative chronic adrenergic blockade. Surgical intervention should generally be deferred until serial echocardiograms confirm that ventricular function has improved in response to treatment with adrenergic blocking drugs.

During surgery, rapid and wide fluctuation in blood pressure should be anticipated.[199] Adequate premedication should be used to minimize the risk of sympathetic activation during endotracheal intubation and induction of anesthesia. Diazepam and short-acting barbiturates are the agents of choice for premedication.[199] Droperidol, phenothiazines, and morphine are contraindicated because they can cause catecholamine release. Atropine should be avoided because its vagolytic effect results in tachycardia in the setting of high-circulating catecholamine levels.

Careful intraoperative monitoring of intraarterial blood pressure, cardiac output, pulmonary capillary wedge pressure, and systemic vascular resistance is required to manage rapid swings in blood pressure.[199] Despite adequate preoperative α-blockade with phenoxybenzamine, severe hypertension can occur during intubation or intraoperatively due to catecholamine release during tumor manipulation. Although intermittent bolus phentolamine has been advocated in this setting, prolonged α-blockade may predispose to significant hypotension following tumor devascularization.[199] Therefore, sodium nitroprusside, with its immediate onset and short duration of action, is the agent of choice for controlling acute hypertension during pheochromocytoma surgery.[199] Infusions of esmolol can be used for short-term control of arrhythmias.[198,199]

At the opposite end of the spectrum, severe intraoperative hypotension can occur. Hypotension or even frank shock can supervene following isolation of tumor venous drainage from the circulation, with a resultant abrupt decrease in circulating catecholamine levels. This hypotension is caused by a precipitous reduction in vascular tone, which can be aggravated further by operative blood loss, downregulation of adrenergic receptors in response to chronic increases in catecholamines, α-adrenergic blockade, or impaired heart rate response resulting from β-adrenergic blocking drugs.[199] Volume expansion with crystalloid, colloid, or blood as needed is the recommended treatment for intraoperative hypotension. Volume repletion should be guided by measurements of pulmonary capillary wedge pressure and cardiac output. Pressors should only be employed when hypotension is unresponsive to adequate volume repletion.[199] The risk of hypotension due to hypovolemia extends into the postoperative period during which close monitoring of volume status is essential. In the postoperative period, required volume replacement not uncommonly exceeds measured fluid losses.[199]

Hypertensive Crises Secondary to Withdrawal of Antihypertensive Therapy

Abrupt discontinuation of high doses of centrally acting antihypertensive agents such as clonidine,[205] methyldopa,[206] and guanabenz[205,207] can produce a withdrawal syndrome characterized by sympathetic overactivity.[208] Symptoms consisting of headache, nausea, restlessness, agitation, insomnia, and tremor usually begin 12 to 72 hours after discontinuation of the drug. Occasionally, this withdrawal syndrome is accompanied by a rapid increase in blood pressure to above pretreatment levels (overshoot hypertension).[209] The abrupt rise in blood pressure can precipitate a hypertensive crisis with hypertensive encephalopathy or acute pulmonary edema.

The symptoms that develop following cessation of centrally acting α-receptor agonists are suggestive of sympathetic overactivity. It has been postulated that the syndrome may be related to excessive circulating catecholamine levels.[208] Because the antihypertensive action of central α-agonists is due to a reduction in catecholamine release from nerve terminals, abrupt discontinuation may provoke a sudden catecholamine surge. Increased plasma and urine catecholamine levels have been found after abrupt discontinuation of high-dose clonidine. The renin–angiotensin system may also be involved in withdrawal phenomenon. As clonidine and methyldopa suppress PRA, it is possible that a rebound increase in PRA and angiotensin II could mediate the hypertensive overshoot following drug withdrawal.[210]

In general, withdrawal symptoms or rebound hypertension occur only after cessation of large doses of drugs. Withdrawal symptoms rarely appear after discontinuation of clonidine in doses less than 1.2 mg per day.[211] The average dose of guanabenz in the reported cases of withdrawal syndrome was 48 mg per day.[207] However, the withdrawal syndrome can occasionally be precipitated by cessation of lower

doses of drugs. This is especially apt to occur in patients with underlying renal insufficiency or renovascular hypertension.[210] Patients treated with beta-blockers may be predisposed to develop severe hypertension during withdrawal of centrally acting α-agonists.[212] Beta-adrenergic receptor blockade inhibits the vasodilatory effect of β_2-receptors on the peripheral vasculature, leaving vasoconstrictor α_1-receptors unopposed.

Treatment of antihypertensive drug withdrawal syndromes should be individualized. In patients with generalized symptoms of sympathetic overactivity but without excessive blood pressure elevation, reinstitution of the previously administered drug is usually all that is required.[210] However, if the withdrawal syndrome is associated with severe hypertension, hypertensive encephalopathy, or acute pulmonary edema, rapid control of blood pressure with parenteral antihypertensive agents is imperative. Sodium nitroprusside or phentolamine should be used for the management of these hypertensive crises. After the blood pressure is controlled with parenteral agents, oral clonidine, guanabenz, or methyldopa should be restarted. The offending drug should then be gradually withdrawn with close monitoring for withdrawal symptoms and rebound hypertension. Another oral antihypertensive regimen, preferably without a beta-blocker, should be initiated simultaneously.

THE CONTROVERSY OVER GRADUAL VERSUS RAPID REDUCTION OF BLOOD PRESSURE

Some authors have cautioned against rapid lowering of blood pressure in patients with hypertensive crises and have recommended a more gradual reduction of blood pressure.[213,214] The case for gradual reduction of blood pressure is based largely on the finding of altered autoregulation of cerebral blood flow in hypertensive patients and scattered case reports of serious neurologic sequelae resulting from overly aggressive reduction of blood pressure in patients with severe hypertension or hypertensive crises.[215–221]

In both hypertensive and normotensive individuals, cerebral blood flow is maintained constant, at approximately 50 mL/minute/100 g of brain tissue, over a wide range of perfusion pressures, by virtue of various intrinsic and neurohumoral autoregulatory mechanisms. The lower limit of cerebral blood flow autoregulation is the blood pressure below which autoregulatory vasodilation becomes maximal and cerebral blood flow decreases. In normotensive subjects, the lower limit of autoregulation is a mean arterial pressure of 60 to 70 mm Hg. In chronically hypertensive patients, the lower limit of autoregulation is shifted so that autoregulation fails and cerebral blood flow decreases at a higher blood pressure than in normotensive individuals.[222,223] This effect may be the result of hypertension-induced changes in the cerebral arterioles. In animal models, chronic hypertension causes hypertrophy of the walls of cerebral vessels with a reduction in internal diameter. Moreover, during chronic hypertension, cerebral arterioles undergo structural remodeling, which results in a smaller external diameter and encroachment on the vascular lumen.[224]

On the one hand, these structural changes are protective in that the thickened cerebral arterioles are able to maintain constant cerebral blood flow at a higher perfusion pressure than would be tolerated by normotensive individuals. In this regard, in chronically hypertensive individuals, the mean arterial pressure at which autoregulatory vasoconstriction gives way to pressure-induced forced vasodilation and hyperperfusion—that is, the upper limit of cerebral blood flow autoregulation—is shifted to a higher level compared to the upper limit in normotensive individuals (see discussion of breakthrough theory in the above section on hypertensive encephalopathy). However, as a consequence of these structural changes, the arterioles are not able to dilate fully at low mean arterial pressures, which could predispose hypertensive patients to cerebral ischemia if the blood pressure is lowered excessively.

Fortunately, with long-term control of blood pressure these changes in cerebral arterioles appear to be at least partially reversible given the observation that patients with previously severe but adequately treated hypertension have a lower limit of autoregulation, which is shifted toward the range for normotensive subjects (Table 44.6).[222]

The upward shift in the autoregulatory curve in patients with chronic hypertension is one of the major arguments put forward by those who favor gradual reduction of blood pressure in patients with hypertensive crises.[222] However, the clinical importance and therapeutic implications of this shift in the autoregulatory curve may have been overemphasized. The demonstration of hypertensive adaptation of cerebral autoregulation should not be interpreted to mean that acute reduction of blood pressure in hypertensive crises is unwise. In the various hypertensive crises in which rapid reduction of blood pressure is indicated (see later), the proven benefits of acute reduction of blood pressure (i.e., decreased risk of intracerebral hemorrhage, hypertensive encephalopathy, or acute pulmonary edema) clearly outweigh the theoretic risk of blood pressure reduction (i.e., possible cerebral ischemia).

In practice, moderate, controlled reduction of blood pressure in hypertensive crises rarely causes cerebral ischemia. This clinical observation may be explained by the fact that even though the autoregulatory curve is shifted toward a higher blood pressure in chronically hypertensive patients, there is still a considerable difference between the presenting blood pressure and the lower limit of autoregulation (Table 44.6). Strandgaard[223] has studied the autoregulation of cerebral blood flow during controlled hypotension produced with trimethaphan and a 25-degree head-up tilt in 13 patients with untreated or ineffectively treated hypertension. At least eight of these patients had grade III or grade IV changes on funduscopy consistent with the diagnosis of malignant hypertension. The control groups included nine patients who had been severely hypertensive in the past but whose blood pressure was effectively controlled at the time of the study, and 10 normotensive subjects. Baseline

44.6 Autoregulation of Cerebral Blood Flow During Trimethaphan-Induced Hypotension[a]

Group	MAP (mm Hg)		Percent of Resting MAP %	
	Resting Level	Autoregulation	Tolerated MAP	Autoregulation	Tolerated
Uncontrolled severe hypertensives (n = 13)	145 ± 17	113 ± 17[b,c]	65 ± 10[b]	79 ± 10	45 ± 6
Controlled hypertensives (n = 9)	116 ± 18	96 ± 17	53 ± 18	72 ± 29	46 ± 16
Normotensives (n = 10)	98 ± 10	73 ± 9	43 ± 8	74 ± 12	45 ± 12

[a]Values given as mean ± SD.
[b]$P < .01$ for difference between normotensives and uncontrolled hypertensives.
[c]$P < .01$ for difference between controlled and uncontrolled hypertensives.
MAP, mean arterial pressure.
Adapted from Gifford RW Jr. Effect of reducing elevated blood pressure on cerebral circulation. Hypertension. 1983;5[Suppl III]:III-17, with permission.)

mean arterial pressures in the three groups were 145 ± 17, 116 ± 18, and 98 ± 10 mm Hg, respectively (Table 44.6). The lower limit of mean arterial pressure at which autoregulation of cerebral blood flow failed was 113 ± 17 mm Hg in uncontrolled hypertensives, 96 ± 17 mm Hg in controlled hypertensives, and 73 ± 9 mm Hg in normotensive individuals. Although the absolute level at which autoregulation failed differed substantially in the three groups, the percentage reduction of mean arterial pressure at which autoregulation failed was similar. The mean arterial pressure at the lower limit of autoregulation was 79 ± 10% of the resting mean arterial pressure in the uncontrolled hypertensives, 72 ± 29% in the controlled hypertensive group, and 74 ± 12% in the normotensive group. Thus, a reduction in mean arterial pressure of approximately 20% to 25% from the baseline level was required in each group to reach the lower limit of autoregulation. Therefore, even in uncontrolled hypertensive patients, there was a considerable safety margin before the limit of autoregulation was reached. Another important observation from this study was that symptoms of cerebral hypoperfusion did not occur until the blood pressure was reduced substantially below the lower limit of autoregulation.[223] Studies have shown that with normal cerebral blood flow, oxygen extraction is not maximal because oxygen saturation in the jugular venous blood at rest is normally 60% to 70%. Thus, even when the mean arterial pressure is reduced below the lower limit of autoregulation, cerebral metabolism can be maintained and ischemia prevented by increasing oxygen extraction from the blood. The lowest tolerated blood pressure, which was defined as the level at which mild symptoms of brain hypoperfusion were encountered (yawning, nausea, and hyperventilation with hypocapnia), was 65 ± 10 mm Hg in patients with uncontrolled hypertension, 53 ± 18 mm Hg in patients with controlled hypertension, and 43 ± 8 mm Hg in normotensive subjects. These values were 45 ± 6%, 46 ± 16%, and 45 ± 12% of the resting baseline mean arterial pressures, respectively. Thus, symptoms of cerebral hypoperfusion did not occur until the mean arterial pressure was reduced by an average of 55% from the resting level (Table 44.6).

In summary, with regard to the shift in cerebral autoregulation in chronically hypertensive patients, there is a therapeutic threshold above which the blood pressure can be reduced safely in patients with hypertensive crises who require immediate control of hypertension. Strandgaard concludes that the upward shift in cerebral autoregulation should not be taken as a warning against aggressive antihypertensive therapy in hypertensive crises. It merely implies that the initial treatment should be aimed at partial reduction but not complete normalization of blood pressure.[222,223]

The second argument used to support the recommendation for gradual reduction of blood pressure is based on case reports of the occurrence of acute neurologic sequelae during rapid blood pressure reduction in the treatment of severe hypertension or hypertensive crises.[215–221]

Franklin reviews 19 reported cases of neurologic complications following aggressive antihypertensive therapy.[93] The average age of the patients was 36 years. All had evidence of severe antecedent hypertension with an average mean arterial pressure of 188 ± 19 mm Hg. Malignant hypertension, based on the finding of hypertensive neuroretinopathy, was present in 79% and hypertensive encephalopathy was present in 53% of these patients. Aggressive antihypertensive treatment resulted in a reduction of mean arterial pressure to 84 ± 18 mm Hg. This represented a 56% decrease from the baseline blood pressure level, a level clearly below the predicted autoregulatory range for hypertensive patients. The time course of blood pressure reduction was

within minutes in 26% and over hours in 74% of patients. However, the most critical factor in the development of neurologic sequelae was the long duration of drug-induced overshoot hypotension, which varied from a period of hours to days. Neurologic complications consisted of permanent blindness in 47%, coma in 32%, pyramidal tract signs in 32%, residual neurologic deficits after therapy in 58%, and death in three patients. The majority of these patients (80%) had received a large intravenous bolus of diazoxide. Three patients received no parenteral agents but had sustained hypotension induced with multiple oral agents. Franklin concludes that rather than the rapidity with which blood pressure was reduced, the duration of excessive hypotension was the factor that correlated best with the development of neurologic complications.

In summary, the data suggest that in the treatment of patients with hypertensive crises who require prompt control of blood pressure, potent parenteral agents can be used safely if excessive lowering of blood pressure is avoided. The studies of Strandgaard suggest that autoregulation of cerebral blood flow can be maintained in hypertensive patients as long as the mean arterial pressure is not reduced below 120 mm Hg.[222,223] This value is two standard deviations above the average mean arterial pressure at which patients in the reported series developed neurologic sequelae.

In general, an initial blood pressure reduction to 160 to 170 mm Hg systolic and 100 to 110 mm Hg diastolic or to a mean arterial pressure of 120 to 130 mm Hg can be safely accomplished in patients who require immediate control of blood pressure in the setting of hypertensive crises.[93] Alternatively, the initial antihypertensive therapy can be individualized based on the pretreatment level of blood pressure. In the individual patient, reduction of the mean arterial pressure by 20% should be the initial therapeutic goal. At this level, the blood pressure should still be above the predicted autoregulatory lower limit. Once this goal is obtained, the patient should be carefully evaluated for evidence of cerebral hypoperfusion. Further reduction of blood pressure can then be undertaken if necessary in a controlled fashion based on the overall status of the patient. In previously normotensive individuals in whom acute hypertensive crises develop, such as patients with acute glomerulonephritis complicated by hypertensive encephalopathy, eclampsia, and autonomic hyperreflexia, the autoregulatory curve may not yet be shifted and the initial goal of therapy will often be normalization of the blood pressure.

The use of potent parenteral agents with a rapid onset and short duration of action, such as sodium nitroprusside, has obvious advantages. If overshoot hypotension or neurologic sequelae develop, they can be quickly reversed by allowing the blood pressure to stabilize at a higher level. Agents with a long duration of action all have an inherent disadvantage in that excessive reduction of blood pressure cannot be easily reversed. Thus, diazoxide, labetalol, minoxidil, hydralazine, converting enzyme inhibitors, calcium channel blockers, and central α-agonists should be used with extreme caution in patients requiring rapid blood pressure reduction in order to avoid prolonged overshoot hypotension.

Although in the great majority of hypertensive patients, cautious blood pressure reduction can be undertaken without a significant risk of causing cerebral hypoperfusion, it should be noted that there is one clinical setting in which there is a significant risk of causing cerebral ischemia even with moderate blood pressure reduction. In patients with acute cerebral infarction, because of failure of autoregulation in the surrounding marginally ischemic zone, even moderate blood pressure reduction can be detrimental. Therefore, in acute cerebral infarction, the aforementioned considerations regarding the general safety of acute blood pressure reduction do not apply. The management of hypertension complicating acute cerebral infarction is outlined in the section entitled Hypertension Complicating Cerebrovascular Accident.

PHARMACOLOGY OF DRUGS USEFUL IN THE TREATMENT OF HYPERTENSIVE CRISES

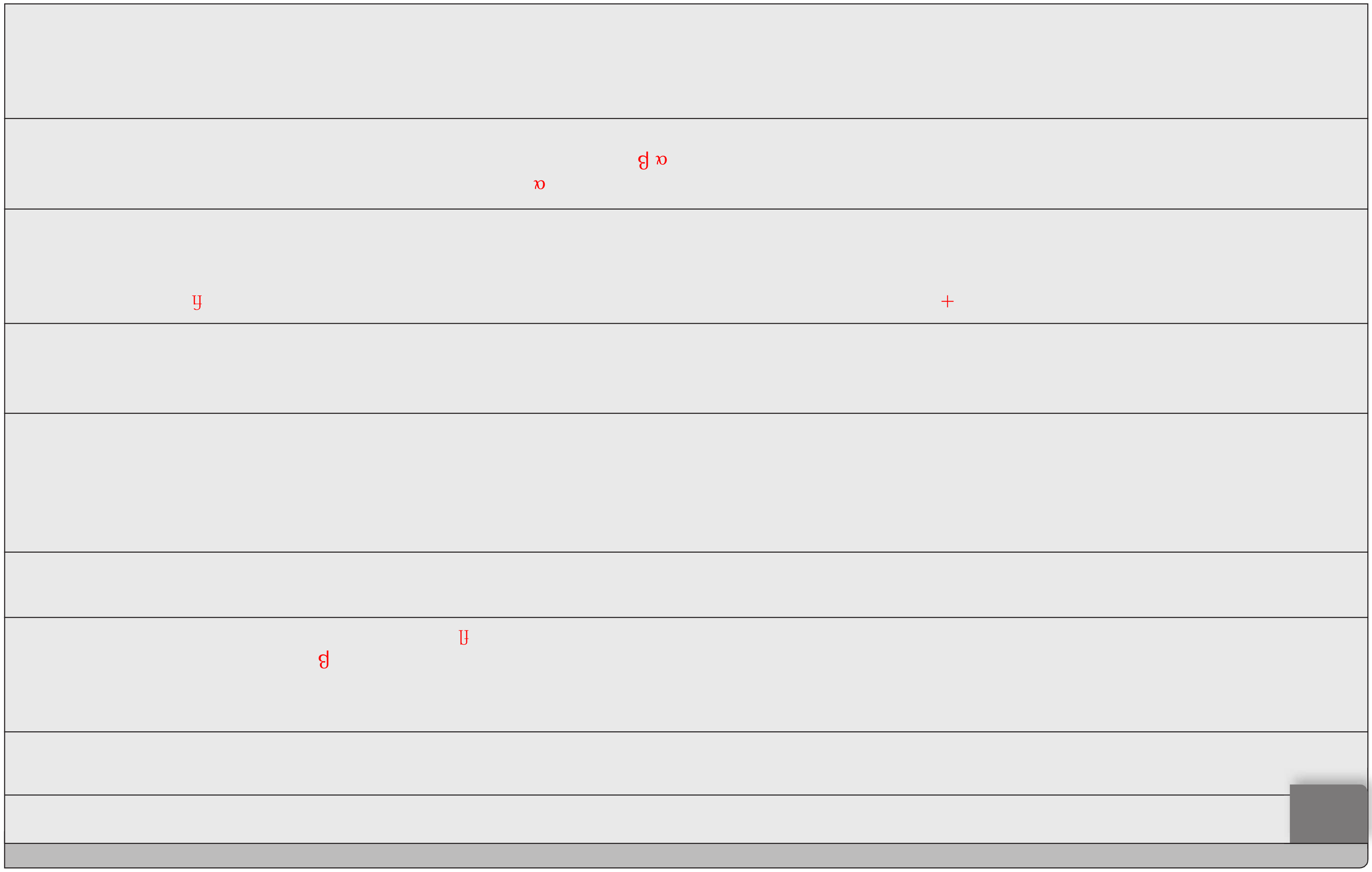
The pharmacopeia of parenteral vasodilators and adrenergic blockers available for the treatment of hypertensive crises is outlined in Table 44.7. Preferred parenteral agents for the treatment of selected hypertensive crises are detailed in Table 44.8.

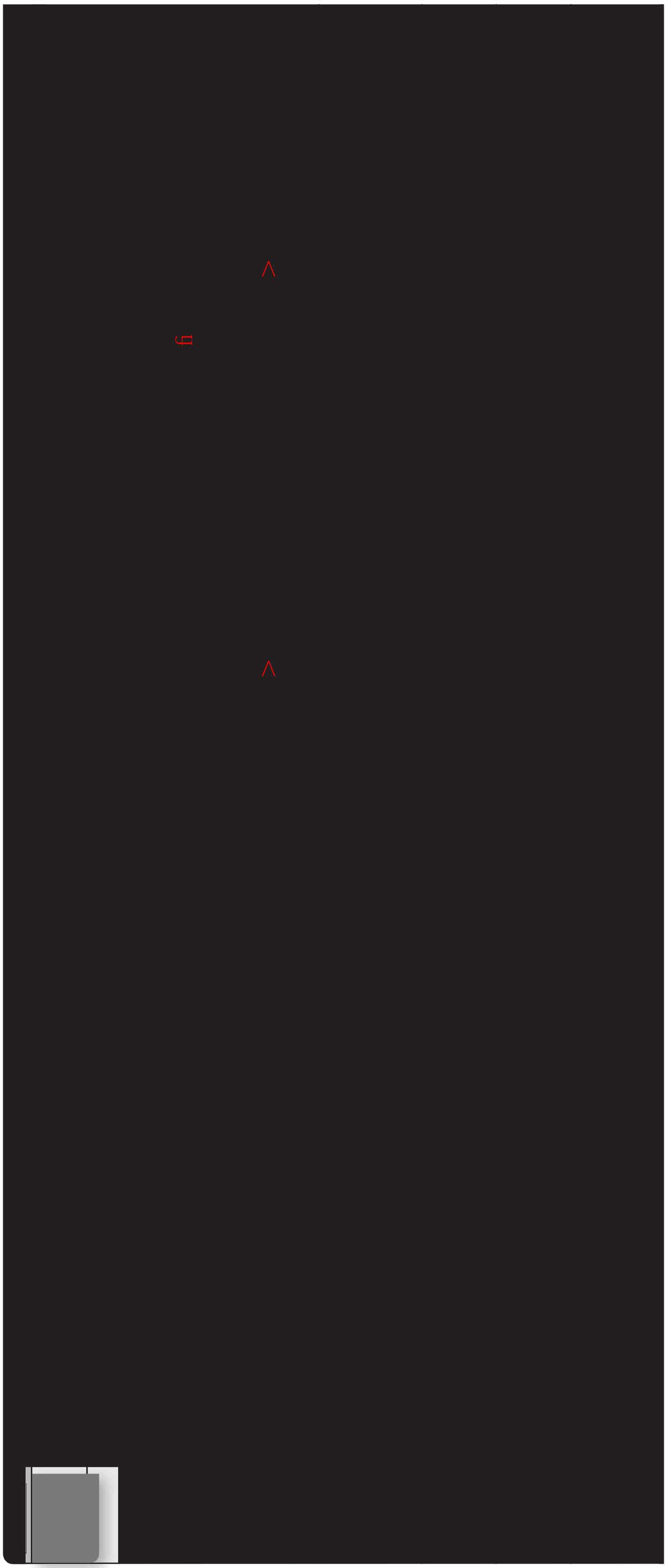
Sodium Nitroprusside

In 1929, intravenous administration of the color indicator sodium nitroprusside was reported to lower blood pressure.[225] Nonetheless, concern that the hypotensive action of the drug was related to the release of cyanide led to a delay in the introduction of the drug. In 1955, intravenous infusion of sodium nitroprusside was shown to be a safe and effective method for achieving short-term blood pressure control.[226] However, it was not until 1974 that sodium nitroprusside (Nipride) was approved for clinical use. Over the ensuing decades, it has remained the drug of choice for the management of virtually all hypertensive crises. Sodium nitroprusside is useful for the management of hypertensive crises due to malignant hypertension, pheochromocytoma, and other catecholamine-related hypertensive crises, hypertensive encephalopathy, acute pulmonary edema, intracerebral hemorrhage, aortic dissection (in combination with a beta-blocker), and perioperative hypertension.[227]

Mechanism of Action

Sodium nitroprusside is a potent intravenous hypotensive agent with an immediate onset and brief duration of action. The site of action is the vascular smooth muscle. It has no direct effect on the myocardium, although it may indirectly affect cardiac performance through alterations in systemic hemodynamics. In therapeutic doses it has no effect on





duodenal or uterine smooth muscle.[226] It has no direct CNS effect. Sodium nitroprusside causes vasodilation of both arteriolar resistance vessels and venous capacitance vessels. Its hypotensive action is the result of a decrease in systemic vascular resistance. Venodilation results in a decrease in venous return; hence, preload is reduced. The combined decrease in preload and afterload reduces left ventricular wall tension and myocardial oxygen demand.

The net effect of sodium nitroprusside on cardiac output and heart rate depends on the intrinsic state of the myocardium.[227] In the absence of congestive heart failure, venodilation and preload reduction can result in a small decrease in cardiac output with a reflex increase in sympathetic tone and heart rate.In contrast, in patients with left ventricular dysfunction and elevated left ventricular end-diastolic volume or pressure, sodium nitroprusside causes an increase in stroke volume and cardiac output as the result of a reduction in afterload and impedance to left ventricular ejection. There is usually a reduction in heart rate as the result of improved cardiac performance.[228–230]

The cellular mechanism of action of nitroprusside has been well defined.[231,232] Nitroprusside is an iron coordination complex with five cyanide moieties and a nitroso group. The action of sodium nitroprusside, as well as that of other nitrogen oxide-containing vasodilators, is mediated by a reaction with cysteine to form nitrosocysteine and other short-acting S-nitrosothiols. Nitrosocysteine, a potent activator of guanylate cyclase, causes cyclic guanosine monophosphate accumulation and relaxation of vascular smooth muscle.

Pharmacokinetics

The hypotensive effect of sodium nitroprusside appears within seconds and is immediately reversible when the infusion is stopped. It is rapidly metabolized, with a reported half-life of 3 to 4 minutes. Cyanide is formed, as a short-lived intermediate product, by direct combination of sodium nitroprusside with sulfhydryl groups in red cells and tissues.[226] The cyanide groups are rapidly converted to thiocyanate by the liver in a reaction in which thiosulfate acts as a sulfur donor. Thiocyanate is excreted unchanged by the kidney with a half-life of 1 week in patients with normal renal function.[227]

Dosage and Administration

The contents of a 50-mg sodium nitroprusside vial should be dissolved in 2 mL of dextrose in water. No other diluent should be used. The stock solution is diluted in 250 mL of dextrose in water to yield a concentration of 200 μg/mL. The container is immediately wrapped in aluminum foil to prevent decomposition on exposure to light. A small portion of the tubing can be left uncovered to observe the solution for color changes during administration. The freshly prepared solution has a faint brownish tint. The nitroprusside molecule reacts with a wide variety of organic and inorganic substances to yield highly colored reaction products. Therefore, the infusion fluid should not be used as a vehicle for the delivery of other drugs. If a color change occurs, the solution should be replaced. Regardless, the solution should be changed every 24 hours.

In patients who are not taking other antihypertensive agents, the average effective dose is 3.0 μg/kg/minute (range, 0.5 to 10.0 μg/kg/minute). The initial infusion rate should be 0.5 μg/kg/minute. The flow rate should be increased in increments of 1 μg/kg/minute every 2 to 3 minutes until the desired hypotensive response is obtained. The solution should be administered by infusion pump or microdrip regulator to allow for precise measurement of flow rate. The blood pressure should be monitored every 30 to 60 seconds during the initial titration and every 15 minutes thereafter. To avoid excessive accumulation of thiocyanate and the risk of cyanide toxicity, the infusion rate should not be increased above 10 μg/kg/minute. Sodium nitroprusside failures are extremely rare, and tachyphylaxis does not occur. Concomitant oral antihypertensive agents should be initiated as soon as possible and the sodium nitroprusside infusion weaned as it becomes effective.

Adequate facilities, equipment, and personnel must be available for close monitoring of blood pressure during sodium nitroprusside administration. Auscultatory or oscillometric pressure is usually adequate, so that intra-arterial pressure monitoring is not routinely required. However, in hypertensive patients with acute myocardial infarction or acute pulmonary edema, hemodynamic monitoring may be required for assessment of left ventricular filling pressure and cardiac output.[227]

Adverse Effects

Nitroprusside is the most effective parenteral agent for the management of hypertensive crises. When properly administered in an intensive care unit setting, it is very safe and clinically significant adverse reactions are uncommon. Overshoot hypotension can result from accidental bolus infusion, faulty infusion equipment, or failure to frequently monitor the blood pressure. However, the hypotensive action is evanescent and hypotension can be reversed easily by slowing or discontinuing the infusion.

The most frequent side effects include anorexia, nausea, vomiting, abdominal cramps, diaphoresis, headache, apprehension, restlessness, and palpitations. Most of these adverse reactions result from rapid blood pressure reduction per se and they usually disappear if the infusion is slowed.

Thiocyanate accumulation and toxicity can occur when a high-dose or prolonged infusion is required, especially in the setting of renal insufficiency. When these factors are present, thiocyanate levels should be monitored and the infusion reduced or discontinued if the plasma level exceeds 10 mg per dL. Thiocyanate toxicity is rare in patients with normal renal function requiring less than 3 μg/kg/minute for less than 72 hours. Symptoms of thiocyanate toxicity include fatigue, anorexia, weakness, tinnitus, blurred vision, and disorientation, which may progress to frank organic

psychosis with hallucinations. Seizures have also been reported. Treatment consists of discontinuing the infusion. Thiocyanate is also efficiently removed by both peritoneal dialysis and hemodialysis.[227]

Cyanide poisoning is a very rare complication of sodium nitroprusside use. Since hepatic clearance of cyanide may be deficient in patients with severe liver disease and in rare conditions such as Leber's optic atrophy or tobacco amblyopia,[233] the use of sodium nitroprusside is contraindicated in these settings. Most of the reported deaths from cyanide poisoning occurred when very high doses of nitroprusside (20 μg/kg/minute) were required for the control of refractory hypertension or in normotensive patients in whom very large doses were used to induce deliberate surgical hypotension.[234,235] The cyanide ion combines with cytochrome c and inhibits aerobic metabolism so that lactic acidosis results. Cyanide toxicity most often occurs within the first 6 to 8 hours of therapy. Cyanide toxicity should be considered if there appears to be increased tolerance to the drug. Tachyphylaxis and an increased anion gap metabolic acidosis are the most reliable early signs of cyanide toxicity. Other signs include the smell of bitter almonds on the breath, anxiety, headache, stiffness of the lower jaw, dyspnea, and widely dilated pupils. Coma, seizures, and death may follow. Occult cyanide toxicity has been reported in patients who are treated with prolonged low-dose infusion of sodium nitroprusside following cardiac surgery. Treatment of cyanide toxicity consists of amyl nitrite inhalation, and sodium nitrite, thiosulfate, and hydroxocobalamin infusions.[236]

The safe use of sodium nitroprusside during pregnancy has not been established. In animals, nitroprusside readily crosses the placenta. In a study of eight normotensive gravid ewes, five required high doses of nitroprusside (mean, 25 μg/kg/minute) to reduce blood pressure by 20% for 1 hour.[237] Among these five animals, a marked accumulation of maternal cyanide occurred. Fetal blood levels of cyanide were even higher and all of these fetuses died. However, in the other three ewes, hypotension was achieved with low doses of sodium nitroprusside (less than 1 μg/kg/minute). In this group, all of the fetuses survived and umbilical cord blood cyanide levels were low.

When sodium nitroprusside was used to achieve normotension for 50 minutes in ewes with norepinephrine-induced hypertension, the mean infusion rate required to control blood pressure was only 2.3 μg/kg/minute, and no fetal or maternal deaths occurred.[238] Neither maternal nor fetal blood samples contained more than 50 μg per L of cyanide (toxic levels in humans, 5,000 μg per L).

There are some reports on the safe use of sodium nitroprusside for hypertensive crises in pregnant women.[239,241] It has been recommended that the use of sodium nitroprusside for hypertensive crises during pregnancy be restricted to patients who are unresponsive to intravenous hydralazine or diazoxide.[242] When nitroprusside is required, it should only be used briefly to manage the acute crisis, and delivery should be performed as quickly as possible.

In summary, sodium nitroprusside has several characteristics that make it nearly the ideal drug for the short-term management of hypertensive crises. These include rapid onset of action, immediate reversibility, specific effects on resistance and capacitance vessels with no direct effect on the myocardium or CNS, lack of tachyphylaxis, and high potency. It is also a very safe drug when used appropriately. It is the most useful and consistently effective drug available for parenteral use in the treatment of hypertensive crises.

Fenoldopam

Fenoldopam is a selective dopamine receptor (DA_1) agonist. Recent studies have shown that intravenous fenoldopam, when used in the setting of hypertensive crises or perioperative hypertension, can safely lower blood pressure while maintaining or improving renal function.[243] Fenoldopam, a benazepine derivative of dopamine, was initially developed as an oral agent for the treatment of hypertension, renal insufficiency, and congestive heart failure. However, it was eventually withdrawn from development because of poor oral bioavailability. When subsequent studies demonstrated that intravenous fenoldopam exhibited a short-half life and predictable pharmacokinetics and dose-response characteristics, it was subsequently evaluated as a potential alternative to sodium nitroprusside for parenteral treatment of hypertension. Intravenous fenoldopam mesylate (Corlopam) was approved by the U.S. Food and Drug Administration (FDA) in 1997 for use in hypertension when oral therapy is not feasible or possible and for use in patients with severe hypertension, with or without target-organ damage.

Pharmacology and Pharmacokinetics

Fenoldopam selectively binds to DA_1 receptors and functions as a dopamine agonist. It does not bind to DA_2 receptors or β-adrenergic receptors. Fenoldopam is also an α-adrenergic receptor antagonist with greater activity at α_2 than α_1 receptors. However, this activity is observed only at higher concentrations than those required for activation of DA_1 receptors and it is unlikely that α-adrenoreceptor antagonism contributes to the hemodynamic and renal effects of therapeutic doses of fenoldopam. Peripheral DA_1 receptors are located postsynaptically in the systemic and renal vasculature, and at various sites in the nephron and GI tract. These receptors mediate systemic, renal, and mesenteric vasodilation. Fenoldopam exerts its hypotensive effect by decreasing systemic vascular resistance. Unlike sodium nitroprusside, it also increases renal blood flow and causes a natriuresis and diuresis. It is six times as potent as dopamine in causing renal vasodilation. In patients with severe hypertension, intravenous infusion of fenoldopam significantly increases renal blood flow, and decreases renal vascular resistance with a significant increase in creatinine clearance, urine flow rate, and sodium excretion.[244] Because of its selective receptor binding characteristics, fenoldopam exhibits minimal adrenergic effects. Although DA_1 receptors are present in the

CNS, fenoldopam does not have any direct CNS effect because it does not cross the blood–brain barrier. Fenoldopam is metabolized in the liver to a variety of nontoxic methyl, sulfate, and glucuronide metabolites. There are two principal inactive metabolites, 7- and 8-methoxy-fenoldopam, that are eliminated by the kidney (80%) and in the feces (20%). Less than 1% is excreted unchanged in the urine; therefore, dosage adjustment is not required in the setting of renal insufficiency. Moreover, pharmacokinetic parameters do not appear to be significantly altered in the setting of hepatic insufficiency.[244] Fenoldopam is not metabolized by the cytochrome P 450 system and has no major drug–drug interactions although concomitant acetaminophen administration may increase fenoldopam levels by 30% to 70%. Following intravenous administration, the onset of action is within 10 minutes, and the half-life is 9.8 minutes. There is no evidence of rebound hypertension after stopping the infusion. The volume of distribution is 0.6 L per kg.

Dosage and Administration

Fenoldopam is available in 5-mL ampules at a concentration of 10 mg per mL. Following dilution, the solution, which is light stable, can be used for up to 24 hours. For the treatment of severe hypertension or hypertensive crises, fenoldopam is administered by continuous infusion with an initial dose of 0.1 μg/kg/minute. The infusion may be increased in increments of 0.1 μg/kg/minute every 20 minutes until the target blood pressure is achieved. The maximum recommended dosage is 1.7 μg/kg/minute. The average infusion rate required is 0.25 to 0.5 μg/kg/minute. Mean plasma fenoldopam levels after a 2-hour infusion at 0.5 μg/kg/minute is between 13 to 50 ng per mL. When the desired response has been achieved, fenoldopam infusion may be discontinued gradually or abruptly, as rebound elevation of blood pressure has not been observed. Oral antihypertensive medications may be started as the fenoldopam infusion is weaned.

Adverse Effects

Adverse events attributed to fenoldopam in the treatment of hypertensive emergencies and urgencies were generally mild, occurred within the first 24 hours, and were related to the vasodilatory action of the drug.[244] Headache was reported in 11% to 36% of patients, flushing in 7% to 11%, nausea in 20%, and dizziness in 10%. Asymptomatic ST-segment abnormalities occurred in 6% to 33% of patients. The etiology of these nonspecific ST- and T-wave abnormalities, which are similar to those seen with the use of other vasodilators, is unknown. They appear to be a benign phenomenon related to blood pressure lowering with alterations in myocardial repolarization rather than an indication of subclinical myocardial ischemia.[244] Less frequently reported adverse events included palpitations, transient hypotension, asthenia, and sinus bradycardia. Fenoldopam, unlike sodium nitroprusside, produced a reversible, dose-related increase in intraocular pressure and should be used with caution in patients with glaucoma. In comparative trials, the adverse event profiles of fenoldopam and sodium nitroprusside were generally similar, although fenoldopam may be associated with a lower incidence of transient hypotension than sodium nitroprusside.

Use for Treatment of Hypertensive Crises

Fenoldopam has been compared mostly with sodium nitroprusside in patients with acute severe hypertension (either severe uncomplicated hypertension or true hypertensive crises).[243,245,246] Treatment with fenoldopam or sodium nitroprusside reduced mean diastolic blood pressure to a similar extent and to goal levels in most patients.[244] The time to achievement of goal blood pressure was similar to that with sodium nitroprusside. There was no evidence of rebound hypertension following cessation of either drug. There was no evidence of tolerance to the antihypertensive effect of either drug during maintenance infusion. In patients with hypertension following noncardiac surgery or coronary artery bypass grafting, fenoldopam and sodium nitroprusside were equally efficacious in lowering blood pressure.[247,248]

The efficacy, safety, and cost of sodium nitroprusside versus fenoldopam has been compared in a retrospective analysis of consecutive patients with hypertensive crises admitted to a level 1 trauma center and treated with nitroprusside (n = 21) or fenoldopam (n = 22).[246] Neither the mean pretreatment mean arterial pressure (nitroprusside 168 ± 19; fenoldopam 163 ± 9; P = .45), time to reach MAP goal (3.6, range 0.4 to 30 hours vs. 4.0, range 1 to 22 hours; P = .5), nor the duration of infusion (18, range 0.7 to 113 hours vs. 18, range 3 to 74 hours; P = .45) differed between the treatment groups. Time to imitation of oral antihypertensive therapy was similar between nitroprusside (4.5, range 0.5 to 22 hours) and fenoldopam (6.5, range 1 to 100 hours) treated patients (P = .45). Change in creatinine clearance and the incidence of tachycardia did not differ between the two groups. No symptoms of cyanide toxicity were detected in nitroprusside-treated patients. Cost of therapy was less with nitroprusside ($2.66, range $1.68 to $3.48) than with fenoldopam ($567, range $199 to $6,675 dollars). Thus, treatment of hypertensive crises with fenoldopam appears to result in patient outcomes equal to those with nitroprusside but at substantially higher cost.

Additional studies are needed to compare fenoldopam and sodium nitroprusside in the treatment of true hypertensive crises. Because fenoldopam preferentially dilates the renal vasculature, it has theoretical advantages in the treatment of patients with severe hypertension associated with renal impairment. Moreover, fenoldopam is not associated with the risk of toxicity from thiocyanate accumulation or cyanide. It is possible that it may also offer advantages in patients in whom cross clamping of the aorta above the level of the renal arteries is required.

Esmolol Hydrochloride

Pharmacology and Pharmacokinetics

Esmolol hydrochloride is a $beta_1$-selective (cardioselective) adrenergic receptor blocking agent with rapid onset of action and a very short duration of action. At therapeutic doses it has no significant intrinsic sympathomimetic activity. Elimination half-life after intravenous infusion is approximately 9 minutes. Esmolol inhibits $beta_1$ receptors located chiefly in cardiac muscle, but this preferential effect is not absolute and at higher doses it begins to inhibit $beta_2$ receptors located chiefly in the bronchial and vascular smooth muscle. Esmolol hydrochloride is rapidly metabolized by hydrolysis of the ester linkage by the esterases in the cytosol of red blood cells. Total body clearance is about 20 L/kg/hour which is greater than cardiac output, thus metabolism is not limited by the rate of hepatic or renal blood flow. Esmolol at doses of 200 μg/kg/minute produces reductions in heart rate, systolic blood pressure, rate-pressure product, left and right ventricular ejection fraction, and cardiac index.

Dosage and Administration

Esmolol hydrochloride is supplied as ready-to-use 10-mL vials containing 100 mg per 10 mL (10 mg per mL) and 250-mL bags containing 2,500 mg per 250 mL (10 mg per mL) and double strength vials containing 100 mg per 5 mL (20 mg per mL) and double strength bags containing 2,000 mg per 100 mL (20 mg per mL). Esmolol hydrochloride is titrated based on ventricular rate response and blood pressure. An initial loading dose of 500 μg per kg is infused over 1 minute followed by a maintenance infusion of 50 μg/kg/minute for 4 minutes. If adequate therapeutic response is not observed within 5 minutes, repeat the same loading dose and follow with a maintenance infusion increased to 100 μg/kg/minute. Higher maintenance infusion rates (250–300 μg/kg/minute) may be required for adequate control of blood pressure in up to one third of patients with postoperative hypertensive crises. In the absence of loading doses, constant infusion of any given maintenance dose reaches pharmacokinetic and pharmacodynamics steady-state in about 30 minutes. Maintenance infusions (with or without loading doses) may be continued for as long as 24 hours.

Adverse Effects

Adverse reaction rates are based on use of esmolol hydrochloride in clinical trials involving nearly 1000 patients with supraventricular tachycardia or intraoperative and postoperative hypertension enrolled in clinical trials. Symptomatic hypotension with diaphoresis and dizziness was the most common side effect occurring in 12% of patients. Asymptomatic hypotension occurred in about 25% of patients. Pallor, flushing, bradycardia with heart rate less than 50 beats per minute, chest pain, syncope, pulmonary edema, and heart block have each been reported in less than 1% of treated patients. Bronchospasm, wheezing, and dyspnea were reported in less than 1% of patients.

Use for Treatment of Hypertensive Crises

Esmolol hydrochloride is indicated for treatment of tachycardia and hypertension that occur during induction of anesthesia and tracheal intubation, during surgery, on emergence from anesthesia, and in the postoperative period. Esmolol hydrochloride may also be useful in conjunction with sodium nitroprusside to achieve blood pressure reduction and reduction in heart rate and dV/dP in patients with dissecting aortic aneurysm.

Intravenous Nitroglycerin

Intravenous nitroglycerin is particularly useful for the management of hypertension complicating acute myocardial infarction and hypertension occurring after coronary artery bypass. Nitroglycerin causes relaxation of vascular smooth muscle. The predominant effect at lower doses is venodilation. At higher doses, both venous and arterial dilation occur in a dose-dependent fashion.[249] As with nitroprusside, the effects of intravenous nitroglycerin on stroke volume and cardiac output vary, depending on the presence or absence of left ventricular dysfunction. In patients without heart failure, the reduction in preload usually predominates and stroke volume falls. In contrast, in patients with left ventricular systolic dysfunction, the decrease in afterload results in a decrease in the impedance to left ventricular ejection such that stroke volume is maintained despite a reduction in preload.

For the treatment of hypertension complicating acute myocardial infarction or postcardiac bypass hypertension, nitroglycerin may have an advantage over sodium nitroprusside.[250] Nitroglycerin and nitroprusside have different effects on regional myocardial blood flow.[251–253] Although both drugs dilate coronary vessels, nitroglycerin has a predominant effect on large coronary conductance arteries, including intercoronary collaterals, and relatively little effect on small resistance arterioles. This phenomenon is explained by the fact that coronary resistance vessels less than 100 microns in diameter cannot convert nitrates to nitric oxide such that there is preferential dilation of the larger epicardial collateral vessels.[254] In contrast, sodium nitroprusside predominantly dilates the resistance vessels and has less effect on intercoronary collaterals. In the setting of regional myocardial ischemia, resistance vessels in the ischemic region are already maximally dilated. Thus, sodium nitroprusside may dilate resistance vessels in nonischemic areas and shunt blood away from ischemic areas (coronary steal). Nitroglycerin, by predominantly dilating conductance vessels, improves blood flow to the ischemic region. Given the potentially deleterious effect of nitroprusside on regional myocardial blood flow, it has been recommended that intravenous nitroglycerin be used in preference to nitroprusside for the treatment of hypertension with left ventricular dysfunction in association with acute myocardial infarction.[250]

Nitrates produce vasodilation through the formation of nitric oxide (endothelium-derived relaxing factor), which

activates guanylate cyclase.[250] There appears to be tight coupling between the cyclic guanosine monophosphate (cGMP) production and smooth muscle relaxation. A cGMP-dependent protein kinase is stimulated, resulting in alterations in the phosphorylation of various proteins in smooth muscle. Dephosphorylation of the light chain of myosin leads to smooth muscle relaxation.[256]

Intravenous nitroglycerin has a rapid onset and brief duration of action with a half-life of 1 to 4 minutes. It is metabolized in the liver by a glutathione-dependent organic nitrate reductase. Intravenous nitroglycerin is supplied in 10-mL bottles containing 50 mg, which should be diluted in 5% dextrose in water or 0.9% sodium chloride. Usually one bottle is diluted in a 250 mL volume to yield a final concentration of 200 μg per mL. Nitroglycerin interacts with many types of plastic. Thus, the drug should be diluted only in glass parenteral solution bottles. Special infusion sets that have been developed absorb fewer nitroglycerins than standard polyvinyl chloride tubing. The initial infusion rate should not exceed 5 μg per minute. The dose is titrated in 5 μg per minute increments every 3 to 5 minutes until the desired hypotensive response is achieved. There is no standard optimal dose of nitroglycerin. There tends to be great variability in response from patient to patient. Blood pressure should be monitored every 30 seconds during the titration phase and every 15 minutes thereafter. As with nitroprusside, close monitoring in an intensive care unit setting is required. In the setting of acute myocardial infarction, monitoring of cardiac output and left ventricular filling pressure is essential.

Intravenous nitroglycerin has also been recommended for the management of the potentially dangerous posttreatment hypertensive response that inevitably follows electroconvulsive therapy.[257]

Labetalol

Intravenous labetalol may be of value in a variety of hypertensive crises including malignant hypertension, hypertensive encephalopathy,[258] aortic dissection,[259] and hypertensive crises during pregnancy.[259]

Labetalol has selective α_1- and nonselective beta-blocking properties.[260,261] The ratio of beta- to α-blocking potency is 7:1 for intravenous labetalol. The acute antihypertensive effect after intravenous administration appears to be caused by a decrease in systemic vascular resistance without an appreciable change in cardiac output.[261] However, when used in the treatment of hypertension following open heart surgery, labetalol causes a significant reduction in cardiac output.[262] The beta-blocking effect offsets the baroreceptor-mediated sympathetic response to hypotension. Thus, heart rate remains unchanged or decreases slightly.

After intravenous bolus injection, the full antihypertensive effect occurs within 5 to 10 minutes, and the blood pressure gradually rises to pretreatment levels over 16 to 18 hours. The duration of action, defined as the time from the last injection until the diastolic blood pressure rises 10 mm Hg above the nadir pressure, ranges from 2.0 to 6.5 hours. The major route of elimination is via glycuronide conjugation in the liver. Thus, the labetalol dose must be decreased in patients with liver dysfunction but need not be modified in patients with renal failure.

Labetalol is supplied in 20-mL ampules containing 100 mg of drug. It is usually administered by repeated mini-bolus injections through an intravenous line. The initial dose is 20 mg (4 mL) injected slowly over a 2-minute period. The maximum hypotensive response usually occurs within 5 minutes of the injection. If the desired hypotensive response is not obtained after 10 minutes, a 40-mg bolus is administered over 2 minutes. Additional injections of 40 to 80 mg can be given at 10-minute intervals until the desired hypotensive response is obtained or the maximum total dose of 300 mg has been given.

Labetalol can also be given by continuous infusion. The contents of two ampules (200 mg, 40 mL) are added to 160 mL of diluent to yield a volume of 200 mL with a final concentration of 1 mg per mL. The infusion is begun at 2 mg per minute. The infusion is continued until the desired response is obtained and then discontinued. Again, the maximum total dose of 300 mg should not be exceeded. Continuous infusion may be preferable to bolus therapy in patients at risk for ischemic complications due to overshoot hypotension.

After the blood pressure is controlled with either the mini-bolus or the continuous infusion technique, oral therapy can be initiated with labetalol as soon as the supine diastolic pressure increases by 10 mm Hg above the minimum obtained with parenteral therapy. The initial oral dose is 200 mg. Thereafter the oral dose is titrated beginning at 200 mg twice daily and increased to 600 mg twice daily as required. The addition of a diuretic often enhances the long-term blood pressure response.

As with other parenteral antihypertensive agents, intravenous labetalol can cause precipitous hypotension, which can result in cerebral ischemia. Exaggerated hypotensive responses are usually reported when the initial injection is large (1.5 to 2.0 mg per kg); however, overshoot hypotension can also develop with either the mini-bolus or the continuous infusion technique. Chronically hypertensive patients sometimes develop paradoxical hypertension in response to volume depletion.[263] In this setting treatment with labetalol leads to reduction in systemic vascular resistance leading to sustained overshoot hypotension. Thus, before labetalol is used to treat a patient with postoperative hypertension and tachycardia, the possibility of physiologic tachycardia due to volume depletion should be considered.

Other side effects of labetalol are related to its nonselective beta-blocking properties. It should be avoided in patients with severe sinus bradycardia, heart block greater than first degree, bronchial asthma, or congestive heart failure.

Oral labetalol has been used safely for treatment of hypertensive crises of pregnancy.[264] However, intravenous labetalol should be used with caution because it has been associated with evidence of neonatal β-adrenergic blockade such as hypoglycemia, bradycardia, and hypotension.[265]

Labetalol can cause a significant reduction in cardiac index when used in the setting of hypertension after open heart surgery.[262] The hypotensive action of the drug in this setting appears to result from a decrease in cardiac output rather than from a decrease in systemic vascular resistance. Thus, labetalol should be avoided after open heart surgery, a setting in which nitroglycerin or sodium nitroprusside is preferred for management of hypertension.

Although there are reports of preoperative management of pheochromocytoma with labetalol,[266] beta-blockade can result in exacerbation of hypertension if α-blockade is incomplete. In this regard, there have been reports of paradoxical hypertension when labetalol was used to treat pheochromocytoma.[267] Therefore, routine use of labetalol for the preoperative management of pheochromocytoma is not recommended.

Several deaths have been reported to the FDA with use of parenteral labetalol injection to treat intraoperative hypertension (including cases where it is used to control intraoperative bleeding).

Although intravenous labetalol has been recommended as an effective agent for the treatment of severe acute hypertension in patients with chronic renal failure,[268] life-threatening hyperkalemia has been reported in patients with renal failure that received intravenous labetalol for the treatment of hypertensive crises.[269,270] Beta-adrenergic stimulation is known to shift potassium into cells and beta-agonists have been proposed as acute therapy for hyperkalemia in dialysis patients. Conversely, hyperkalemia may be caused by nonselective beta-blockers through inhibition of Na-K-ATPase with decreased cellular uptake of potassium, independent of effects on insulin or aldosterone.[271] Thus, labetalol and other nonselective beta-blockers should probably be avoided for the acute management of postoperative hypertension and other hypertensive crises in patients with renal failure.

In summary, although intravenous labetalol has been used to treat a variety of hypertensive crises, its long duration of action and beta-blocking properties are potential disadvantages. Slow continuous infusion may be preferred over intravenous bolus therapy to minimize the risk of sustained overshoot hypotension. For this reason, sodium nitroprusside or nicardipine usually represents more logical choices for the acute management of patients with hypertensive crises requiring parenteral therapy.

Phentolamine

Phentolamine is useful in the management of catecholamine-related hypertensive crises including pheochromocytoma, monoamine oxidase (MAO) inhibitor–tyramine interactions, and clonidine, methyldopa, or guanabenz withdrawal reactions. It is not consistently effective in other hypertensive crises. In fact, phentolamine has largely been replaced by sodium nitroprusside in the management of catecholamine-related hypertensive crises.

Phentolamine is a nonselective α-adrenergic blocking agent that competitively inhibits the effect of norepinephrine on vascular smooth muscle α_1-receptors. It does not have beta-blocking activity and therefore does not block the cardiac effects associated with β_1-receptor activation by catecholamines. Phentolamine produces dilation of both arteriolar resistance vessels and venous capacitance vessels.[273]

The intravenous injection of 1 to 5 mg produces a hypotensive effect within 2 to 3 minutes; however, the duration of action may be only 15 to 30 minutes, so that frequent dosing is required to control blood pressure. Phentolamine is supplied in ampules containing 5 mg. The initial dose should be 1 mg. Subsequent boluses of 1 to 5 mg are administered up to a total dose of 20 to 30 mg or until the blood pressure is controlled. After the desired blood pressure is achieved, intermittent injections are given as necessary to maintain the response.

Side effects due to phentolamine are common. Tachycardia and arrhythmias can occur due to β-adrenergic cardiac stimuli that are not blocked by phentolamine. GI side effects include abdominal pain, nausea, vomiting, and diarrhea. Exacerbation of peptic ulcer disease can occur, so phentolamine should be used with caution in patients with a history of gastritis or peptic ulcer disease.

Hydralazine

In the past, parenteral hydralazine was often used for the treatment of hypertensive crises. Most obstetricians still consider hydralazine to be the drug of choice for the management of hypertensive crises during pregnancy.[273] However, aside from its use during pregnancy, hydralazine has largely been replaced by other agents in the treatment of hypertensive crises.

The hypotensive response to either intramuscular or intravenous hydralazine is unpredictable. The onset of action occurs 10 to 30 minutes after a parenteral dose. The duration of action is 3 to 9 hours. The dose and frequency of administration needed to control the blood pressure are highly variable.[274] Profound and sustained hypotension can occur with an intravenous dose as low as 10 mg. Hydralazine is a direct-acting arteriolar vasodilator. It causes reflex activation of the adrenergic nervous system.[274] Because venous capacitance vessels are not affected, venous return is maintained. In association with activation of the adrenergic system, there are increases in heart rate and stroke volume.[274] Hydralazine is contraindicated in the treatment of aortic dissection because the increase in myocardial contractility can result in propagation of the dissection. It is also contraindicated in patients with ischemic heart disease because the increased myocardial oxygen demand can precipitate angina or myocardial infarction.

Parenteral hydralazine is still used in acute hypertensive crises of pregnancy. In the majority of patients, hydralazine reduces the blood pressure to acceptable levels and is well tolerated by both mother and fetus, despite reflex activation of the adrenergic system.[273] Dosing guidelines for the use of parenteral hydralazine during pregnancy are well established.[273] Because maternal hypertension helps to maintain placental perfusion, there is concern that aggressive treatment aimed at normalization of blood pressure might

further compromise placental perfusion to the detriment of the fetus. Therefore, hydralazine treatment is usually instituted only if the diastolic blood pressure is more than 110 mm Hg and the goal of therapy is a diastolic pressure in the 90 to 100 mm Hg range. After an initial intravenous dose of 5 mg, additional 5- to 10-mg doses are administered every 15 to 20 minutes until the desired response is obtained. Because preeclampsia is associated with intravascular volume depletion, it is important to initiate therapy with a low dose to avoid overshoot hypotension. Intramuscular injection of hydralazine is unsatisfactory because the onset of action and magnitude of response are unpredictable.

Calcium Channel Blockers

Intravenous nicardipine has been reported to be effective in the acute treatment of severe hypertension in both adults and children.[275–277] It may be useful in the management of postoperative hypertension in both cardiac and noncardiac patients.[277] Intravenous nicardipine is also effective in preventing circulatory responses to laryngoscopy and tracheal intubation in hypertensive patients.[278] Safe use of nicardipine in preeclamptic patients has also been reported.[279]

Nicardipine is a dihydropyridine calcium channel blocker that inhibits the transmembrane influx of calcium into vascular smooth muscle, resulting in vasodilation with a decrease in systemic vascular resistance. The effect on heart rate is dependent on the intrinsic state of the myocardium. In patients with intact systolic function, reflex increases in heart rate may occur in response to blood pressure reduction. In patients with impaired left ventricular function, cardiac output may increase in response to afterload reduction.

Compared to other parenteral medications available for the treatment of hypertensive crises, the pharmacokinetic properties of nicardipine (as well as other calcium channel blockers) are unfavorable. The currently available dihydropyridine calcium channel blockers have very long half-lives. The beta half-life of nicardipine is 40 minutes, whereas its gamma half-life is approximately 13 hours. Because about 14% of the drug is eliminated during the gamma phase, the hypotensive effect is prolonged. Discontinuation of the infusion is followed by a 50% reduction in the hypotensive action within 30 minutes but a gradually decreasing antihypertensive effect may last for about 50 hours. Thus, nicardipine may not be the best choice for true hypertensive crises in which moment-to-moment titration of the blood pressure is the desired therapeutic goal.

In the past, nimodipine had been recommended for the treatment of patients undergoing cardiac valve replacement to decrease the incidence of postoperative neurologic sequelae by increasing cerebral blood flow and protecting against anoxic brain cell damage. However, a recent placebo controlled trial of oral nimodipine following cardiac valve replacement was terminated prematurely because of a lack of evidence of benefit of nimodipine and an unexpected increase in the death rate of patients treated with nimodipine compared to placebo.[280] The higher mortality rate was attributed to an increased risk of major bleeding in patients treated with nimodipine. Excess bleeding in patients treated with calcium channel blockers may be explained by the combination of vasodilation and the antiplatelet action of calcium antagonists.

The clinical use of nifedipine for severe uncomplicated hypertension and hypertensive crises has been reviewed.[281,282] Nifedipine produces a prompt fall in systemic arterial pressure after a single oral dose. The antihypertensive effect results from arteriolar vasodilation with a decrease in systemic vascular resistance. Nifedipine produces a prompt reduction in systolic, diastolic, and mean arterial pressures of about 25% below the baseline value in most patients.[282]

The major acute side effects of nifedipine include a burning sensation in the face and legs, facial flushing, headache, and palpitations. Overshoot hypotension has been observed, especially in hypovolemic patients or patients pretreated with diuretics.[283,284] Exaggerated hypotension can cause myocardial ischemia in patients with underlying coronary atherosclerosis.[285]

Oral nifedipine may be useful in the management of patients with malignant hypertension who do not have an absolute indication for parenteral antihypertensive therapy. However, in patients with hypertensive crises requiring careful titration of the hypotensive response, the prolonged duration of action and the potential risk of overshoot hypotension with nifedipine are major disadvantages. Sodium nitroprusside is clearly preferable for the management of true hypertensive crises. The role of nifedipine in the acute treatment of severe uncomplicated hypertension in the emergency room setting prior to discharge is discussed in the section entitled Severe Uncomplicated Hypertension.

Minoxidil

Minoxidil is a potent antihypertensive agent that is available only for oral use. In combination with a potent diuretic and a beta-blocker, it is very useful in the control of hypertension refractory to conventional antihypertensive regimens. The efficacy of a triple drug regimen with minoxidil in the management of the patient with malignant hypertension and azotemia has already been discussed. Minoxidil is often employed for the long-term control of blood pressure in patients with malignant hypertension after initial control of the blood pressure with parenteral medications. Furthermore, in patients with malignant hypertension not requiring immediate blood pressure reduction, an oral triple drug regimen consisting of minoxidil, a beta-blocker, and a loop diuretic can effectively control the blood pressure over a period of hours to days and thereby eliminate the need for parenteral antihypertensive therapy (see Treatment subsection under Malignant Hypertension earlier in this chapter).

Minoxidil is a direct-acting arteriolar vasodilator. Its antihypertensive effect results from a decrease in systemic vascular resistance.[286] It has no effect on venous capacitance vessels. The hypotensive response to minoxidil is accompanied by a baroreceptor-mediated reflex increase in

sympathetic tone, which results in an increase in heart rate, contractility, and cardiac output. Unopposed, the cardiac output may increase threefold to fourfold and attenuate the fall in blood pressure.[286,287] The resulting increase in myocardial oxygen demand may precipitate ischemia in patients with limited coronary reserve. For this reason minoxidil is usually given concomitantly with a β-adrenergic blocking drug.

As with other peripheral vasodilators, minoxidil induces profound renal salt and water retention.[286] This fluid retention is probably related to the hypotensive effect of the drug. A similar antinatriuresis occurs with both hydralazine and diazoxide. Minoxidil causes more fluid retention because it is a more potent arteriolar vasodilator. Several factors enhance renal salt and water retention.[287] Decreased peritubular capillary pressure is a potent stimulus for salt and water reabsorption in the proximal tubule. Increased adrenergic tone also enhances proximal tubular salt and water reabsorption. Like other vasodilators, minoxidil increases renin release, which leads to increased aldosterone production and enhanced distal tubular sodium reabsorption.[287] Pseudotachyphylaxis to the original hypotensive effect of minoxidil can occur if either beta-blockade or diuretic therapy is inadequate.

The serum half-life of minoxidil is 4.5 hours; however, the duration of action is longer than the half-life would predict.[287] After oral administration, the antihypertensive effect begins within 30 to 60 minutes, reaches a maximum in 2 to 4 hours, and slowly abates over the next 12 to 18 hours. The prolonged hypotensive effect is probably due to persistent binding of minoxidil at the site of action in vascular smooth muscle. About 15% of the parent compound is excreted in the urine, whereas the remainder is metabolized in the liver by glucuronide conjugation.[287]

Although the serum half-life is 4 hours, the persistent hypotensive effect allows for a twice daily dosing schedule. Prior to the initiation of minoxidil, all other antihypertensives except diuretics and beta-blockers should be discontinued. Minoxidil is started at a dose of 2.5 mg twice daily and increased in 5-mg per day increments every 2 to 3 days until the desired response is obtained. The usual effective dose is 10 to 40 mg per day. The doses of loop diuretic and beta-blocker are titrated to maintain dry weight and prevent tachycardia, respectively.

When more rapid control of arterial pressure is required, incremental changes in minoxidil dosage can be made every 6 hours. The initial 2.5-mg dose is doubled every 6 hours up to a maximum dose of 20 mg, or until the desired response is obtained. The effective dose should then be administered every 12 hours and the dose of diuretic and beta-blocker titrated as necessary.[287]

The dose of beta-blocker required to prevent reflex tachycardia in patients treated with minoxidil is often in excess of the usual beta-blocking dose. This is because the sympathetic nervous system is activated by minoxidil and beta-blockers compete with catecholamines for receptor binding.[287] The dose of beta-blocker should be titrated to maintain resting heart rate at 70 to 80 beats per minute.

In general, thiazide diuretics are not potent enough to counteract minoxidil-induced antinatriuresis, especially if renal insufficiency is present. The starting dose of furosemide is 40 mg twice daily. However, a dose of 300 to 400 mg per day may be required to prevent fluid retention and maintain dry weight.

The most common side effects of minoxidil are related to its pharmacologic properties. Fluid retention can lead to weight gain, edema, anasarca, congestive heart failure, and pericardial effusion. With inadequate beta-blockade, reflex sympathetic stimulation can lead to angina or myocardial infarction in patients with underlying coronary disease. Electrocardiographic changes following the initiation of minoxidil have been reported. In more than 90% of patients flattening or inversion of T waves develops.[287] Although often marked, these changes do not necessarily indicate myocardial ischemia, and they usually resolve with continued therapy.[286,287]

Pericardial effusion has been reported with minoxidil treatment; however, progression to cardiac tamponade is rare. The cause of the effusion is unknown, but it occurs most commonly in patients with renal failure, collagen vascular diseases, or inadequate diuretic therapy. A hemodynamically insignificant effusion is not necessarily a reason to discontinue minoxidil, but the patient should be treated aggressively with diuretics and followed closely for signs of tamponade.[286,287] Patients on dialysis should have a trial of intensive daily dialysis to achieve and maintain dry weight.

Reversible hypertrichosis of the face, back, and arms occurs in almost all patients taking minoxidil and is the most frequent reason for discontinuation of the drug, especially among female patients. Calcium thioglycolate depilatory agents and shaving are used to control this cosmetic side effect.

Triple therapy with minoxidil, a beta-blocker, and a loop diuretic is often dramatically effective in the long-term management of malignant hypertension, even when conventional antihypertensive regimens are unsuccessful or produce intolerable side effects.[288]

SEVERE UNCOMPLICATED HYPERTENSION

The benefits of acute reduction of blood pressure in the setting of true hypertensive crises are obvious. Fortunately, hypertensive crises are relatively rare events that never affect the vast majority of hypertensive patients. Another type of presentation that is more common than true hypertensive crisis is the patient who presents with severe hypertension (diastolic blood pressure greater than 115 mm Hg) in the absence of the hypertensive neuroretinopathy or other acute end-organ damage that would signify a true crisis. This entity, which is known as severe uncomplicated hypertension, is very common in the emergency department setting. In a recent study of severe uncomplicated hypertension treated in an emergency room, 60% of the patients were

entirely asymptomatic and had presented for prescription refills or routine blood pressure checks, or were found to have elevated blood pressure during routine examinations. The other 40% presented with nonspecific symptoms such as headache, dizziness, and weakness in the absence of evidence of acute end-organ dysfunction.[289]

In the past, this entity has been referred to as urgent hypertension, reflecting the widely accepted notion that acute reduction of blood pressure, over a few hours prior to discharge from the emergency room, was essential to minimize the risk of short-term complications from the severe hypertension.[290] Commonly used treatment regimens include oral clonidine loading, or sublingual nifedipine given to acutely reduce the blood pressure prior to initiation of a maintenance antihypertensive regimen.[289,290]

In recent years, however, the urgency of treatment in patients with severe uncomplicated hypertension has been questioned.[3,5,291] Although it is clear that in comparison to patients with mild or moderate hypertension, patients with severe uncomplicated hypertension are at increased long-term risk of cardiovascular complications,[292] they are generally not in any immediate danger of an untoward event. The argument supporting the acute reduction of blood pressure is based on the following assumptions: (1) it is important to reduce blood pressure immediately to avoid complications; (2) oral antihypertensive loading prior to initiation of maintenance therapy produces improved immediate and long-term blood pressure control; and (3) there are no adverse consequences of this form of treatment.[3] Two classical studies provided some useful information regarding the need to reduce blood pressure immediately with the aim of preventing hypertensive complications. In the Veterans Administration Cooperative Study of patients with severe hypertension,[293] there were 70 untreated patients who had no evidence of malignant hypertension or significant end-organ dysfunction despite the presence of diastolic blood pressures averaging 121 mm Hg. Among these patients, 27 experienced morbid events at an average of 11 ± 8 months into follow-up. The earliest morbid event occurred after 2 months. Likewise, a similar study in Baltimore showed that among 42 untreated patients with severe but uncomplicated hypertension, 19 patients experienced morbid events (congestive heart failure, onset of malignant hypertension, cerebrovascular accident, or evidence of declining renal function) at a mean of 12 ± 7 months into follow-up. The earliest morbid event occurred at 2 months.[293] These data suggest that patients who have severe but uncomplicated hypertension need not be exposed to the risk of "urgent" blood pressure reduction in the emergency room setting because hypertensive complications tend to occur over a matter of months to years rather than hours to days.

Another study addressed the question of whether antihypertensive loading prior to the initiation of maintenance therapy improves or hastens blood pressure control.[5] Sixty-four asymptomatic patients with severe hypertension were randomized to treatment with hourly doses of clonidine followed by clonidine and thiazide diuretic maintenance therapy, or an initial dose of clonidine followed by hourly placebo and then subsequent maintenance therapy, or initiation of maintenance therapy without prior antihypertensive loading. There was no difference between the first two groups with regard to the time required to achieve acceptable blood pressure control during loading therapy. Furthermore, there were no differences between the three groups with regard to adequacy of blood pressure control at 24 hours or 1 week. The authors conclude that sustained blood pressure control resulted solely from maintenance therapy and that the time to adequate control and eventual level of blood pressure were independent of the administration of an initial loading dose. They suggest that the common practice of acute oral antihypertensive loading to treat severe, asymptomatic hypertension should be reconsidered.[5] In this regard, a study of 32 patients with severe uncomplicated hypertension found that a significant decrease in blood pressure frequently occurred in the emergency department even before pharmacologic intervention was initiated. The mean arterial pressure decreased by 6% without treatment within 1 hour after the initial blood pressure reading.[294] The authors suggest that given a short period of observation, many patients with severe uncomplicated hypertension will experience a decrease in blood pressure to mildly or moderately hypertensive levels, which would clearly make acute blood pressure reduction with an antihypertensive loading regimen unnecessary.

Although generally safe, the oral antihypertensive loading regimens occasionally cause significant adverse effects. Sublingual nifedipine can produce severe headache and profound overshoot hypotension.[284] The marked blood pressure reduction can exacerbate underlying ischemic heart disease, resulting in angina or myocardial infarction. It has even been suggested that a moratorium be placed on the use of sublingual nifedipine for the treatment of severe uncomplicated hypertension.[295] Loading doses of clonidine cause sedation in 60% of patients and some of these patients are difficult to awaken and require assistance in returning home.[289] Furthermore, the recommended conversion from the oral loading dose to a twice-daily dose of clonidine may represent special problems in the treatment of patients with severe uncomplicated hypertension. Clonidine produces a number of common side effects including dry mouth, drowsiness, and constipation, which may interfere with long-term compliance with medical therapy. The risk of hypertensive rebound on abrupt discontinuation of clonidine[290] should also be considered since many patients with this form of hypertension are noncompliant with medical therapy.[5]

Although the acute reduction of blood pressure in patients with severe uncomplicated hypertension with sublingual nifedipine or oral clonidine loading regimens has become the de facto standard of care in the acute care setting, this practice is often an emotional response on the part of the treating physician to the dramatic elevation of blood pressure. This aggressive approach may also be motivated

by fear of medicolegal repercussions in the unlikely event that an untoward hypertensive complication occurs shortly after the emergency room visit. Although observing and documenting the dramatic fall in blood pressure prior to discharge is a satisfying therapeutic maneuver, there is no scientific basis for this approach and it is unclear if even the small but definite risks of acute blood pressure reduction are justified. There is, at present, no literature to support the notion of an absolute level of blood pressure above which the acute reduction of blood pressure is mandatory before the patient can be discharged from the acute care setting. For asymptomatic patients with severe uncomplicated hypertension, acute reduction of blood pressure in the emergency room is often counterproductive because it can produce untoward symptoms that render the patient less likely to comply with long-term drug therapy. Because the available data suggest that the risks to the patient are not immediate, therapeutic intervention should focus on tailoring an effective, well-tolerated maintenance antihypertensive regimen with emphasis on patient education to enhance long-term compliance.[5] Therefore, oral antihypertensive loading in this setting is of little value. If the patient has simply run out of medications, reinstitution of the previous regimen should suffice. If the patient is thought to be compliant with an existing drug regimen, a sensible change in therapy such as an increase in a suboptimal dosage of an existing drug or the addition of a drug of another class is appropriate. Addition of a low dose of a thiazide diuretic as a second-step agent to existing monotherapy with converting enzyme inhibitor, calcium channel blocker, beta-blocker, or central α_2-agonist is often efficacious. Another essential goal of the intervention should be to arrange for suitable outpatient follow-up within a few days. Gradual reduction of blood pressure to normotensive levels over the next few days to a week should be accomplished in conjunction with frequent outpatient follow-up visits to modify drug regimens and reinforce the importance of lifelong compliance with therapy. Although less dramatic than acute reduction of blood pressure in the emergency room, this type of approach to the treatment of this chronic disease is more likely to prevent long-term hypertensive complications as well as recurrent episodes of severe uncomplicated hypertension.

A recent multicenter study evaluated the level of adherence to current guidelines that recommend that patients in the emergency department with severely elevated blood pressure be evaluated for target organ damage (to exclude hypertensive crisis requiring immediate blood pressure reduction), have their outpatient medical regimen adjusted, and be instructed to follow-up promptly for reassessment.[296] This observational study was conducted during 1 week at four urban academic emergency departments. Severely elevated blood pressure was defined as systolic blood pressure greater than or equal to 180 mm Hg or diastolic blood pressure greater than or equal to 110 mm Hg on at least one measurement. Among 423 patients with severely elevated blood pressure, serum chemistry was obtained in 73%, ECG in 53%, chest radiograph in 46%, urinalysis in 43%, and funduscopic examination to exclude malignant hypertension in 36%. All recommended studies were performed in only 6% of patients. Acute reduction of blood pressure with oral medications was undertaken in 36% of patients and intravenous antihypertensive agents were given to 4% of patients. Modification of the outpatient antihypertensive regimen was documented in only 19% of discharged patients. The authors conclude that the majority of emergency department patients with severely elevated blood pressure do not receive the evaluation, medical regimen modification, and discharge instructions advised by current guidelines.

Finally, an important entity that can masquerade as severe uncomplicated hypertension deserves special mention. Pseudohypertension is a condition in which indirect measurement of arterial pressure using a cuff sphygmomanometer is artificially high in comparison to direct intra-arterial pressure measurements.[297] Failure to recognize pseudohypertension can result in unwarranted and sometimes frankly dangerous treatment. Pseudohypertension can result from Mönckeberg's medial calcification, advanced atherosclerosis with widespread calcification of intimal plaques, or azotemic arteriopathy (metastatic vascular calcification in patients with ESRD).[297] In these entities, stiffening of the arterial wall may prevent its collapse by externally applied pressure, resulting in artificially high indirect blood pressure readings affecting both systolic and diastolic measurements. Pseudohypertension should be suspected in the patient with severe hypertension in the absence of significant target-organ damage. The presence of a positive Osler's maneuver, in which the radial or brachial artery remains clearly palpable despite being made pulseless by proximal inflation of a cuff above systolic blood pressure, is an important physical examination finding that should suggest the diagnosis.[298] Roentgenograms of the extremities will often reveal calcified vessels.[297] However, the diagnosis can only be made definitively by direct measurement of intra-arterial pressure. If unrecognized, pseudohypertension may result in unwarranted treatment. Patients with pseudohypertension are often older adults and therefore may have critical limitation of blood flow to the brain or heart such that inappropriate blood pressure reduction may precipitate life-threatening ischemic events.[297]

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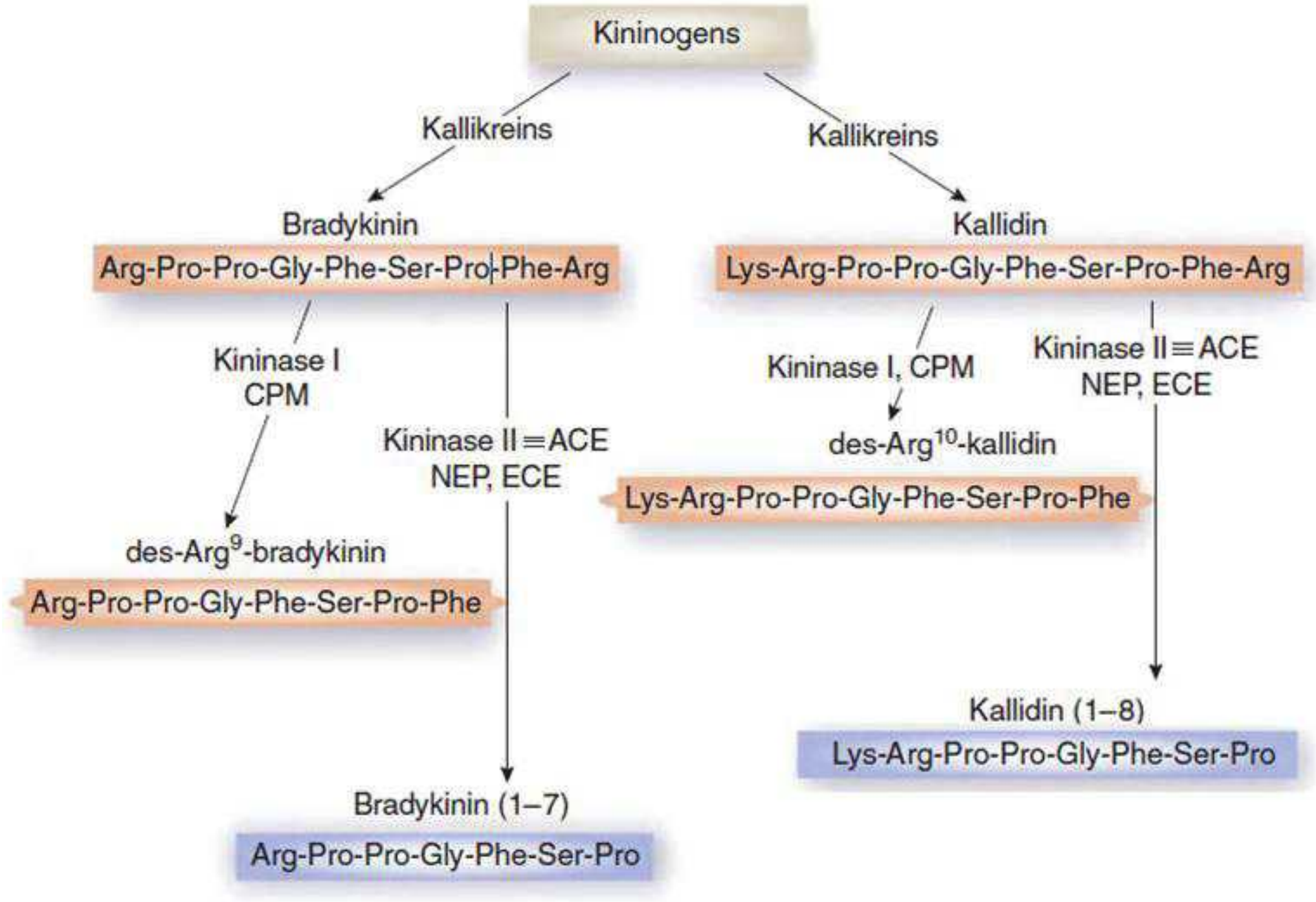


FIGURE 8.5

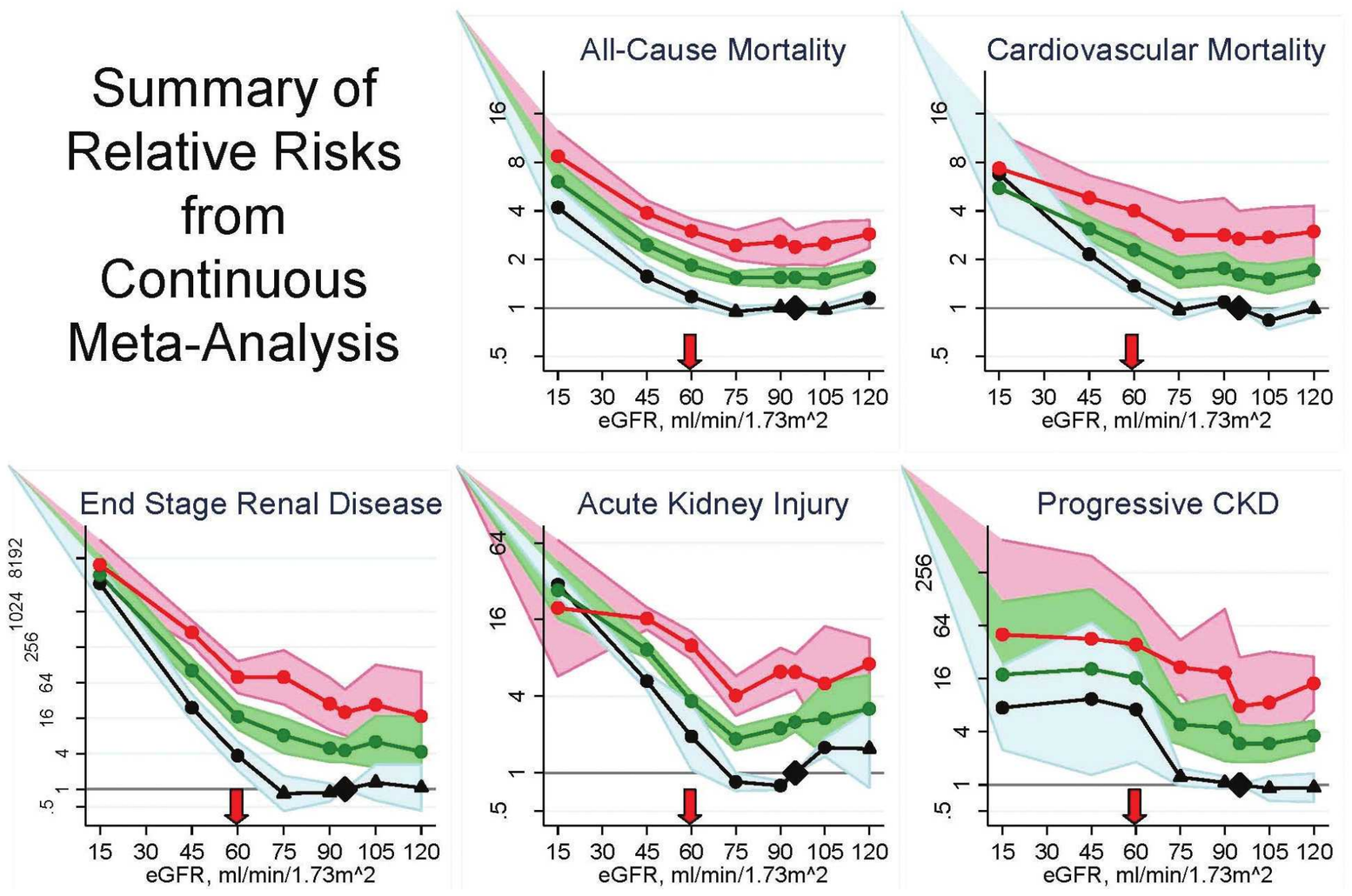


FIGURE 9.2

Summary of Relative Risks from Categorical Meta-Analysis

(dipstick included [-, ±, +, ≥++])

All-Cause Mortality

	ACR <10	ACR 10-29	ACR 30-299	ACR ≥300
eGFR > 105	1.1	1.5	2.2	5.0
eGFR 90-105	Ref	1.4	1.5	3.1
eGFR 75-90	1.0	1.3	1.7	2.3
eGFR 60-75	1.0	1.4	1.8	2.7
eGFR 45-60	1.3	1.7	2.2	3.6
eGFR 30-45	1.9	2.3	3.3	4.9
eGFR 15-30	5.3	3.6	4.7	6.6

Cardiovascular Mortality

	ACR <10	ACR 10-29	ACR 30-299	ACR ≥300
eGFR > 105	0.9	1.3	2.3	2.1
eGFR 90-105	Ref	1.5	1.7	3.7
eGFR 75-90	1.0	1.3	1.6	3.7
eGFR 60-75	1.1	1.4	2.0	4.1
eGFR 45-60	1.5	2.2	2.8	4.3
eGFR 30-45	2.2	2.7	3.4	5.2
eGFR 15-30	14	7.9	4.8	8.1

Kidney Failure (ESRD)

	ACR <10	ACR 10-29	ACR 30-299	ACR ≥300
eGFR > 105	Ref	Ref	7.8	18
eGFR 90-105	Ref	Ref	11	20
eGFR 75-90	Ref	Ref	3.8	48
eGFR 60-75	Ref	Ref	7.4	67
eGFR 45-60	5.2	22	40	147
eGFR 30-45	56	74	294	763
eGFR 15-30	433	1044	1056	2286

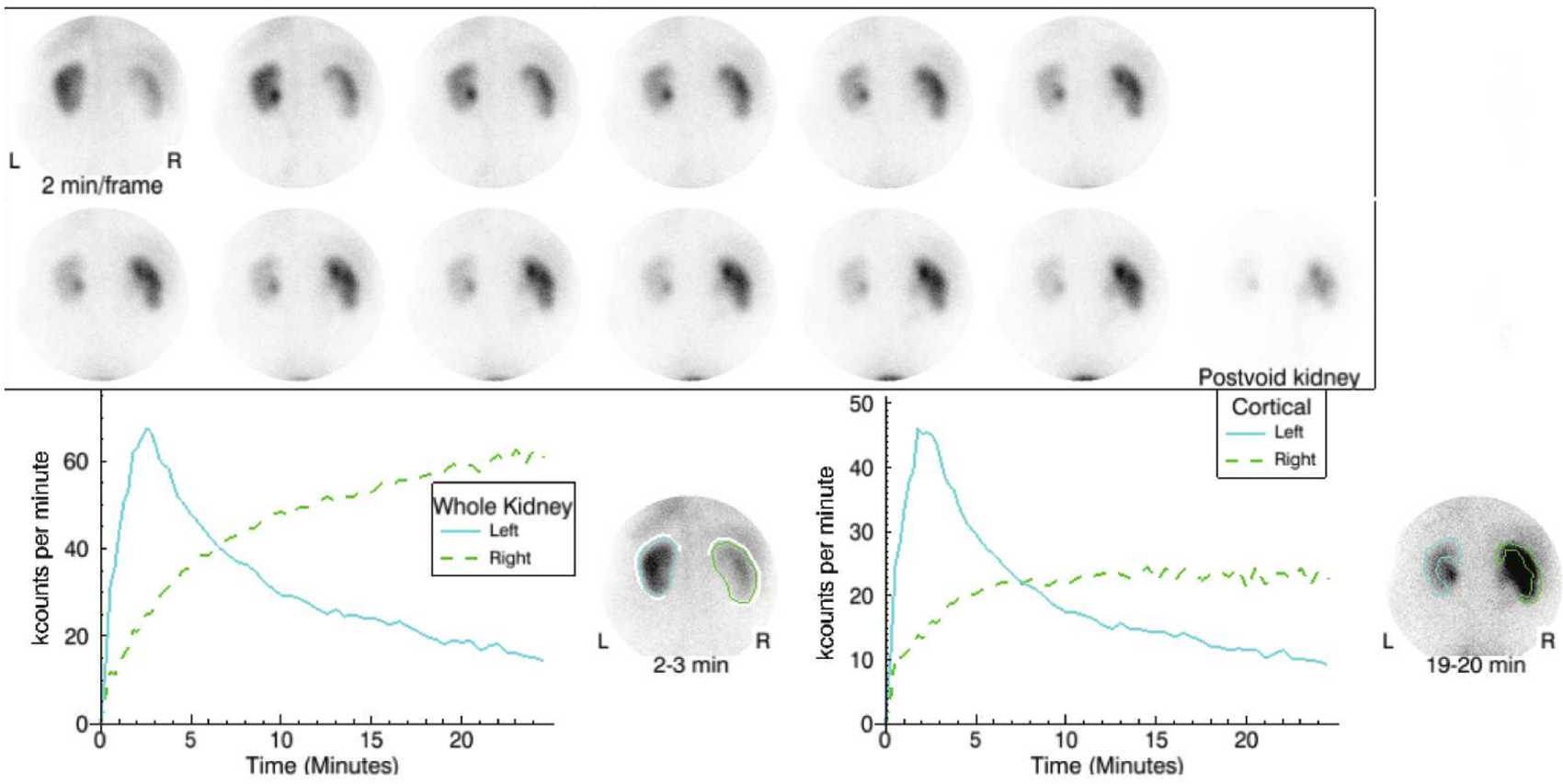
Acute Kidney Injury (AKI)

	ACR <10	ACR 10-29	ACR 30-299	ACR ≥300
eGFR > 105	Ref	Ref	2.7	8.4
eGFR 90-105	Ref	Ref	2.4	5.8
eGFR 75-90	Ref	Ref	2.5	4.1
eGFR 60-75	Ref	Ref	3.3	6.4
eGFR 45-60	2.2	4.9	6.4	5.9
eGFR 30-45	7.3	10	12	20
eGFR 15-30	17	17	21	29

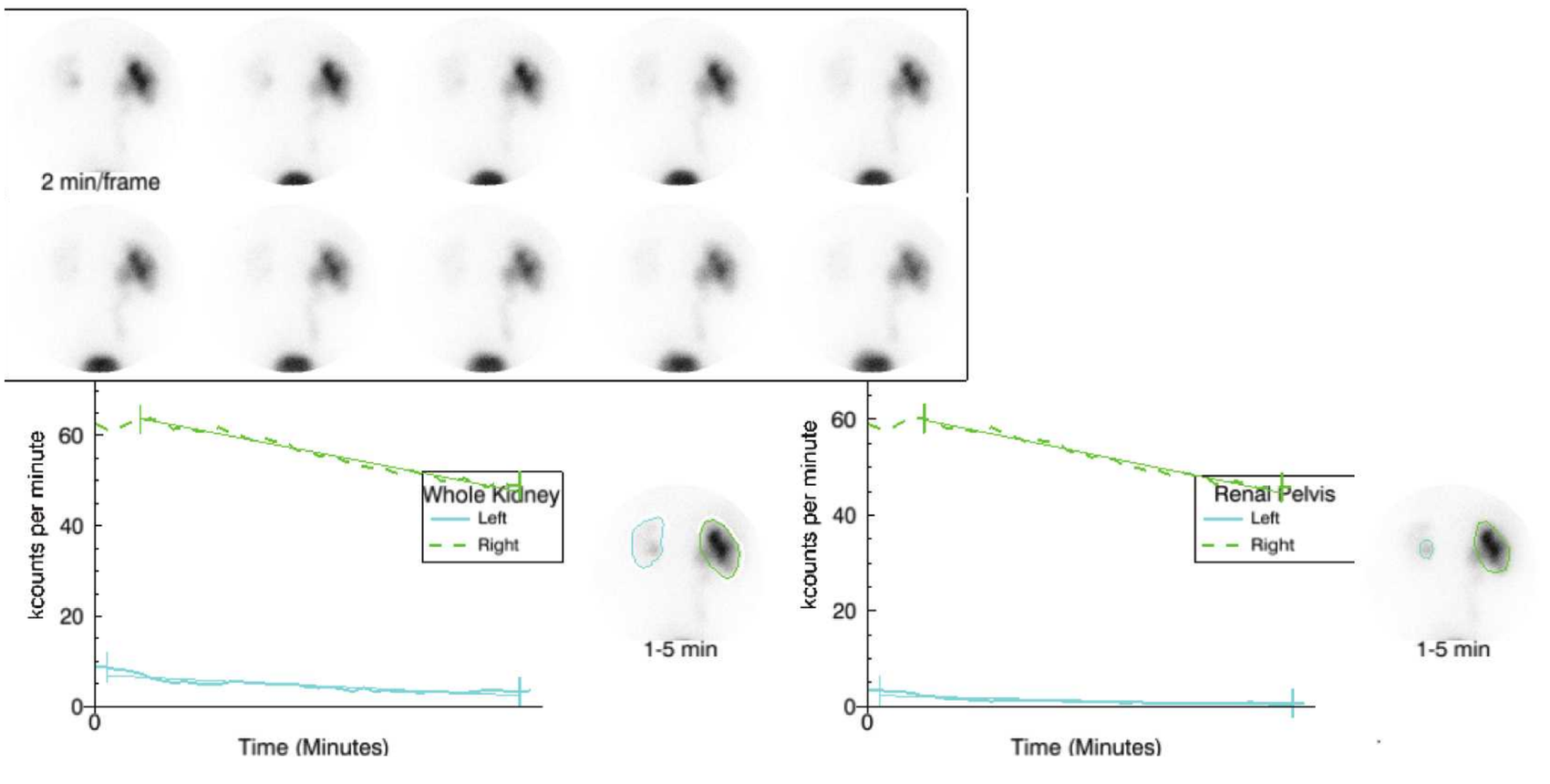
Progressive CKD

	ACR <10	ACR 10-29	ACR 30-299	ACR ≥300
eGFR > 105	Ref	Ref	0.4	3.0
eGFR 90-105	Ref	Ref	0.9	3.3
eGFR 75-90	Ref	Ref	1.9	5.0
eGFR 60-75	Ref	Ref	3.2	8.1
eGFR 45-60	3.1	4.0	9.4	57
eGFR 30-45	3.0	19	15	22
eGFR 15-30	4.0	12	21	7.7

FIGURE 9.3

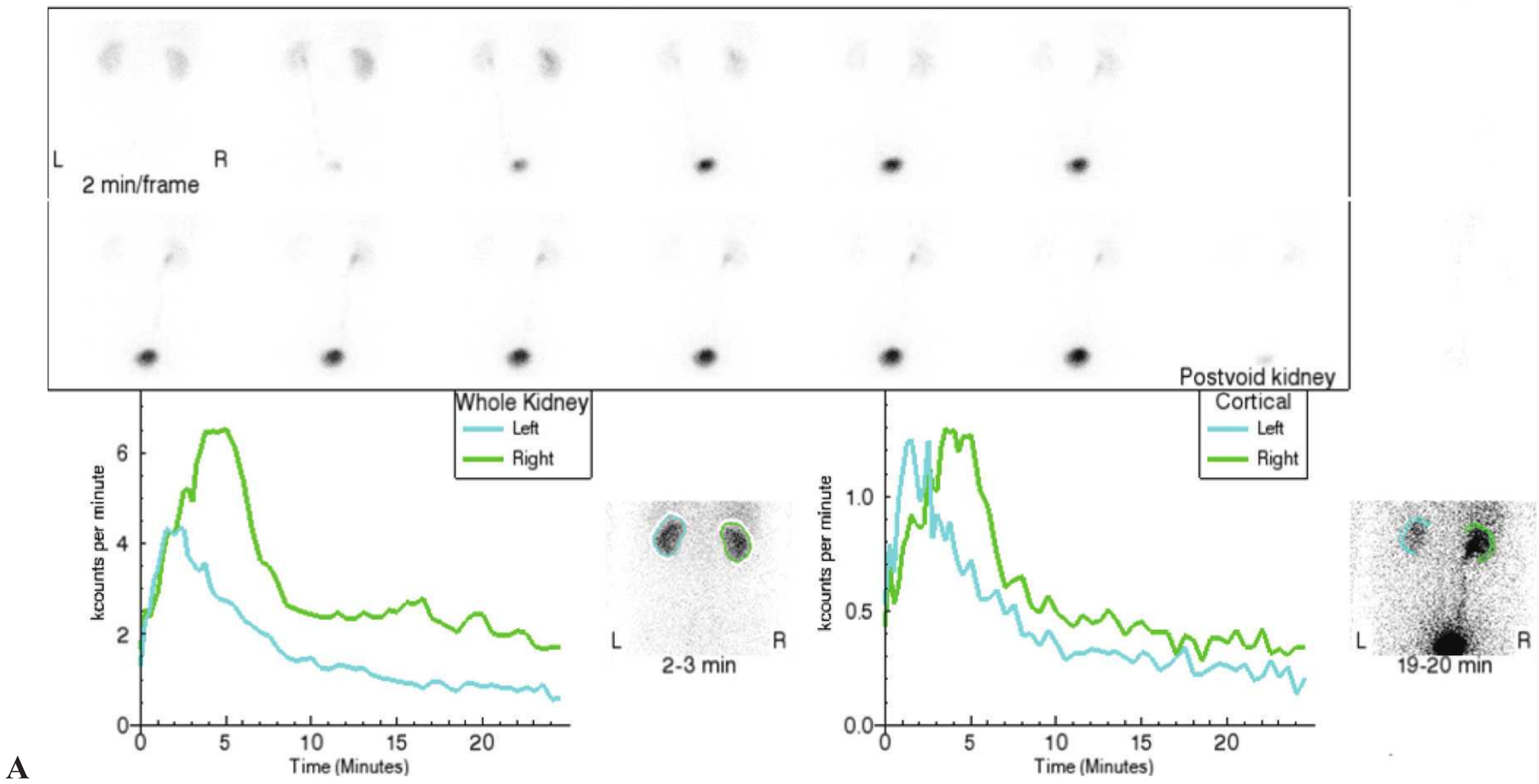


A

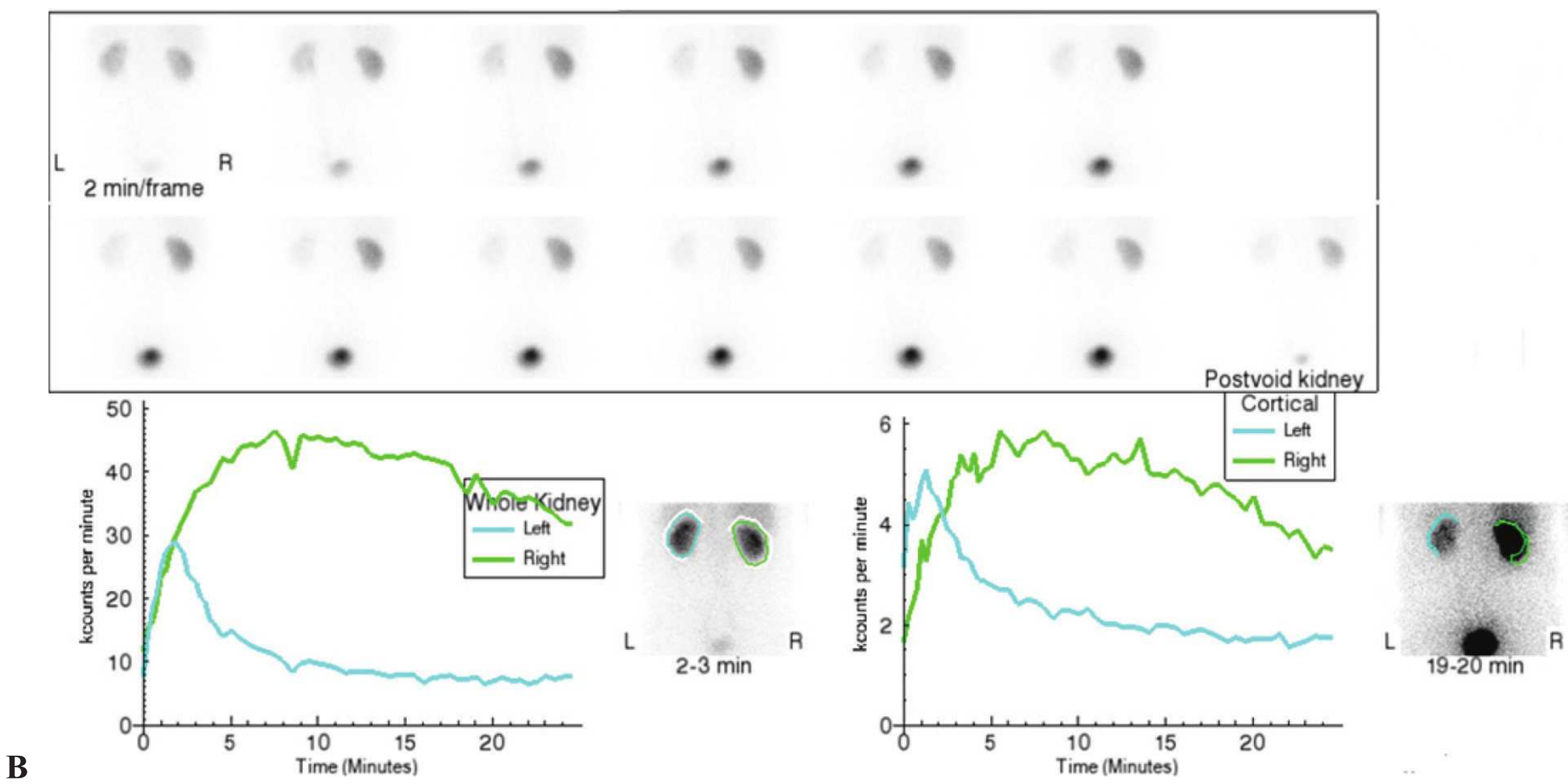


B

FIGURE 10.37A,B



A



B

FIGURE 10.38A,B

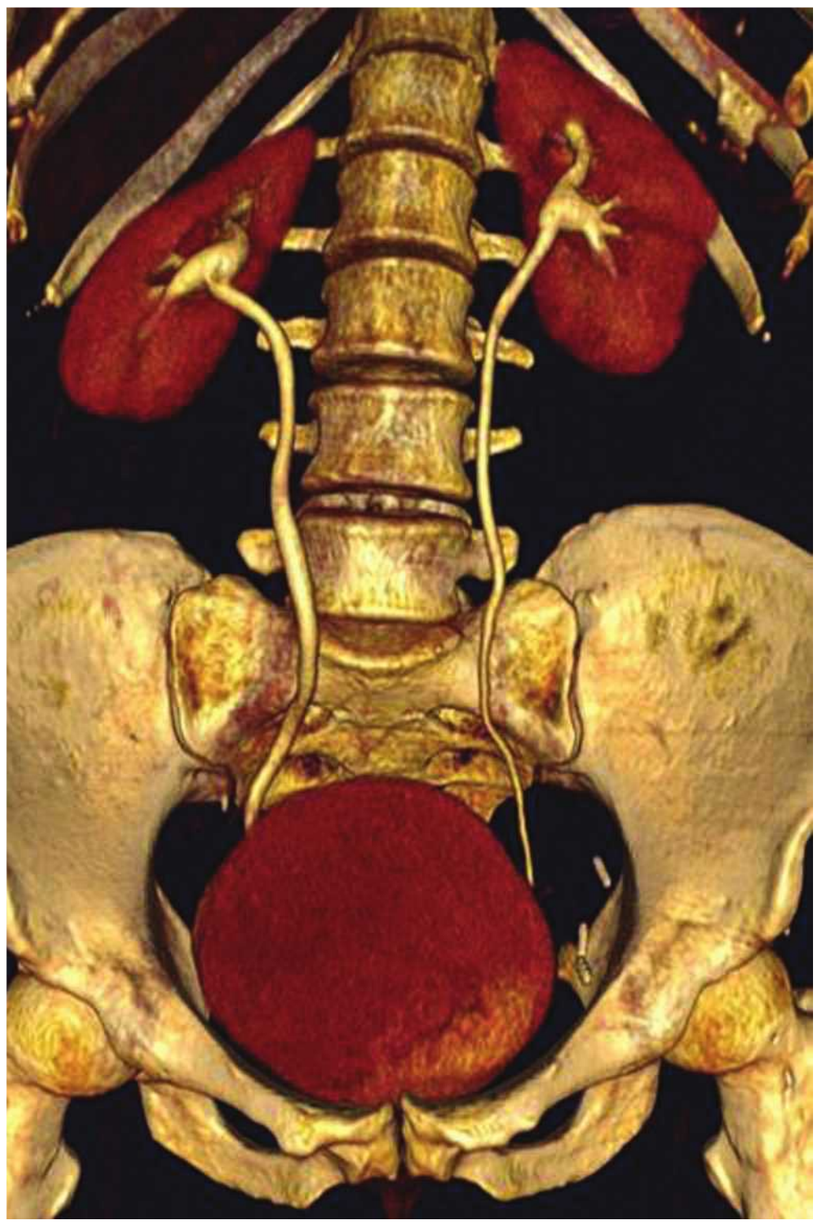
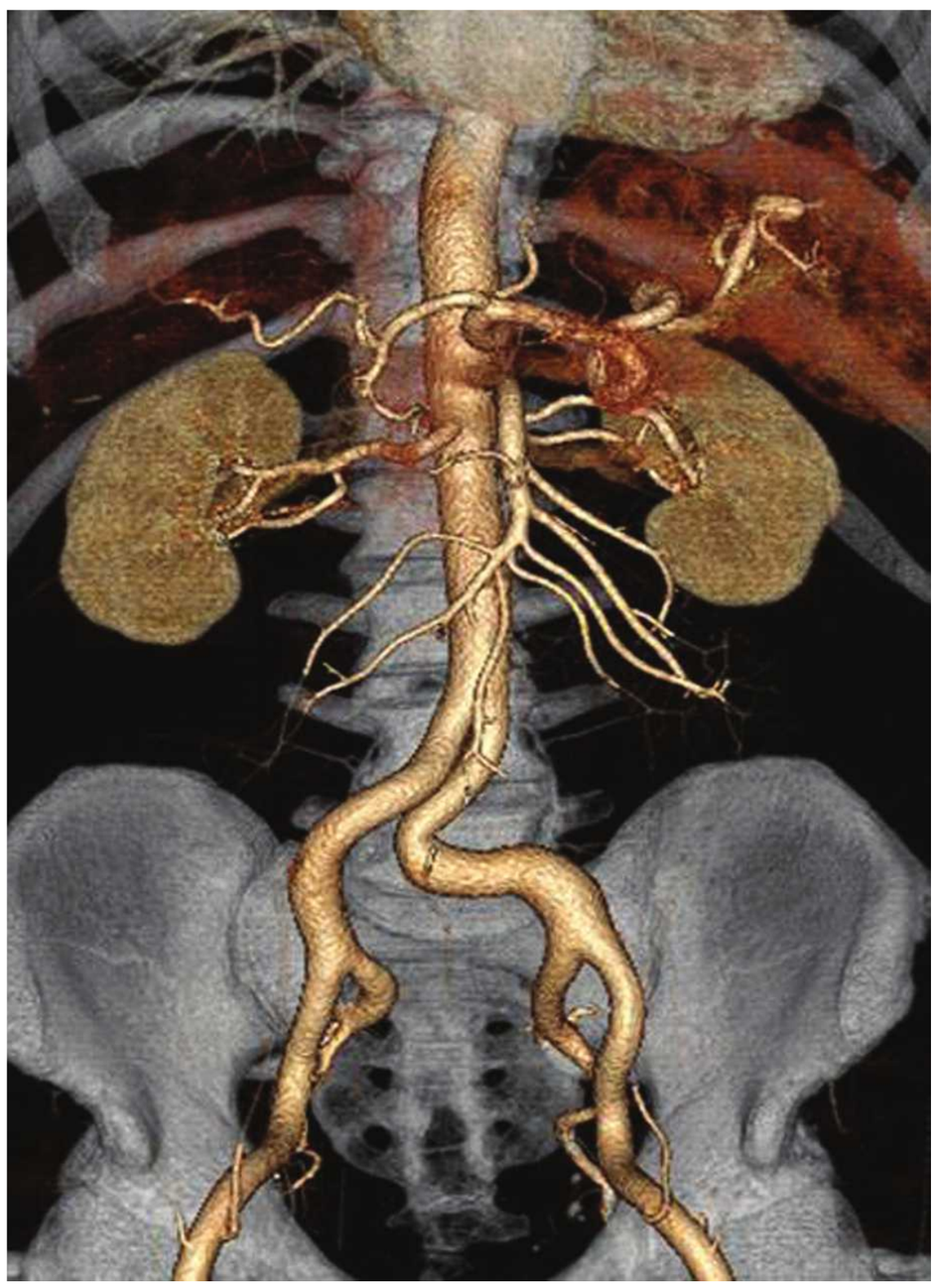


FIGURE 11.1B



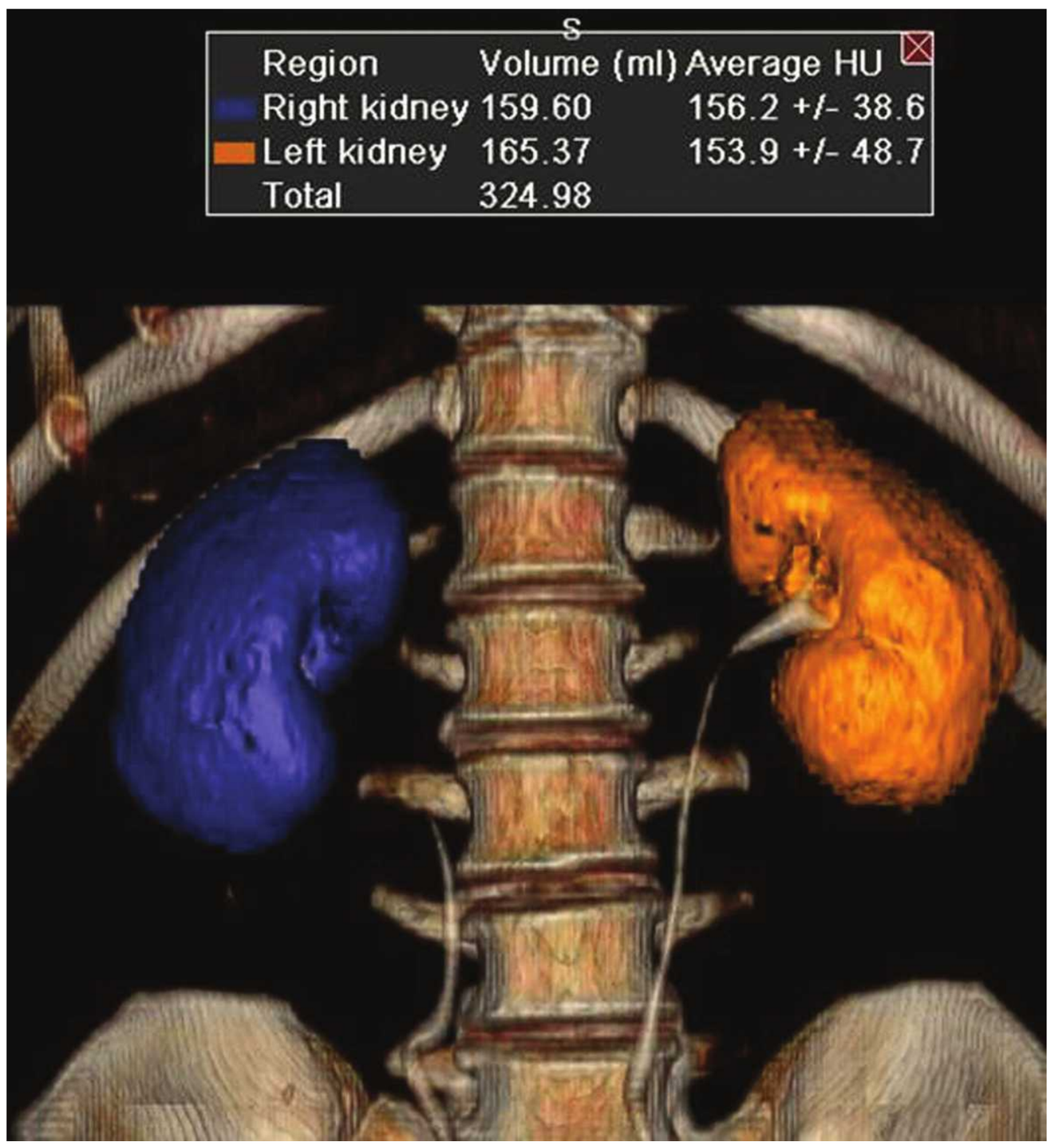
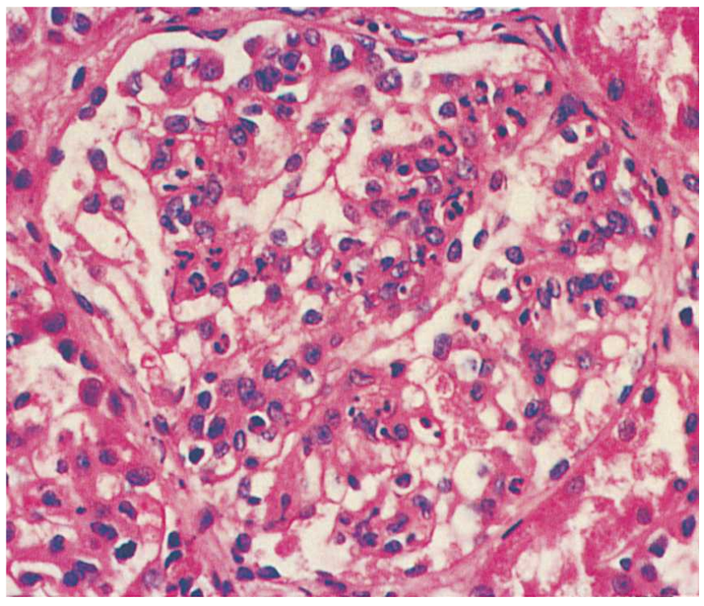
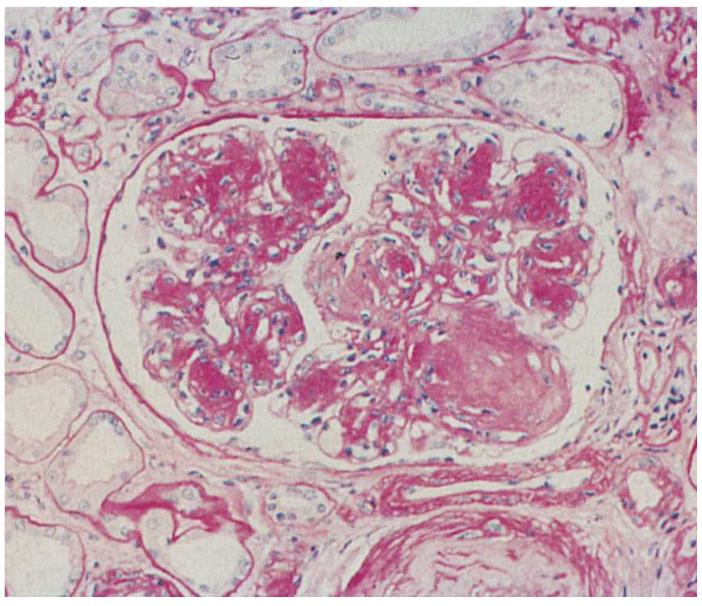
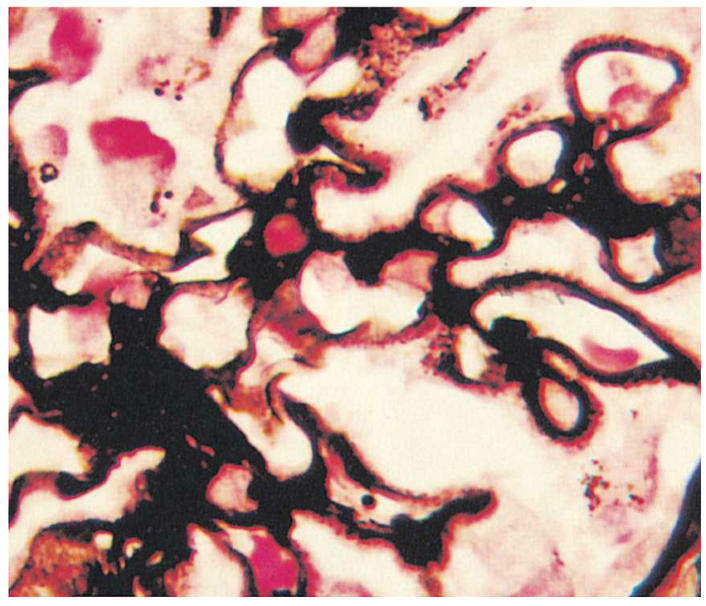


FIGURE 11.2A,C



A,B





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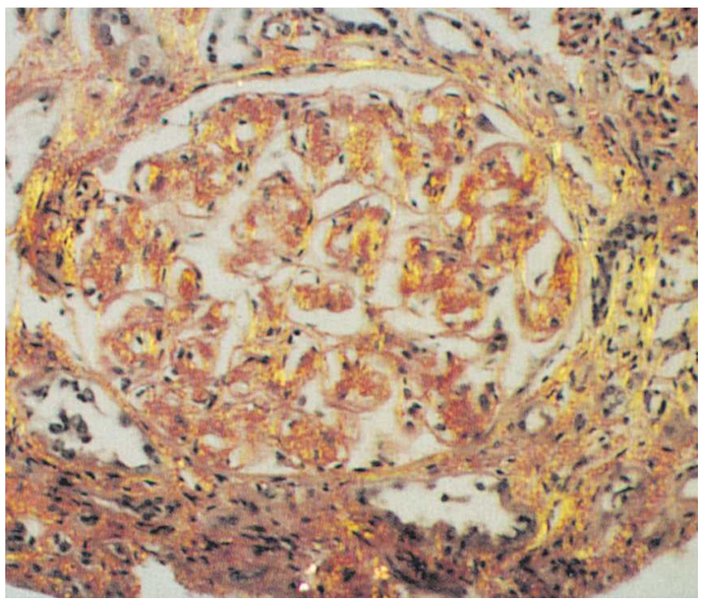
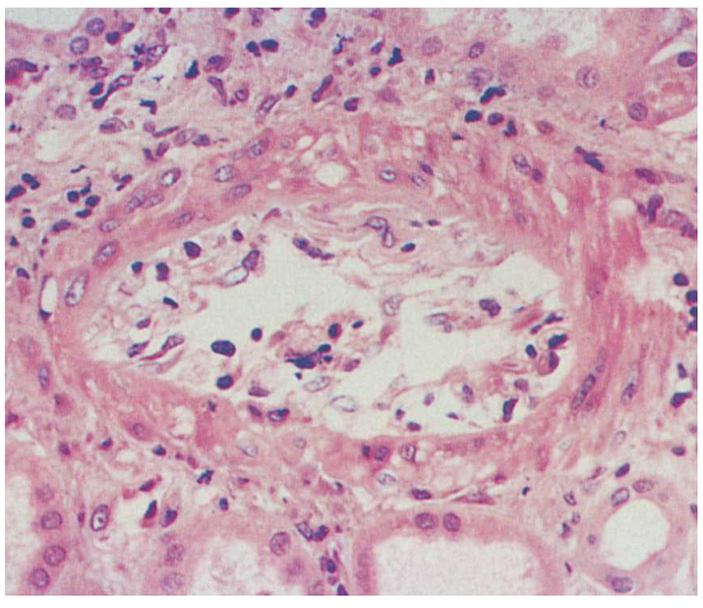
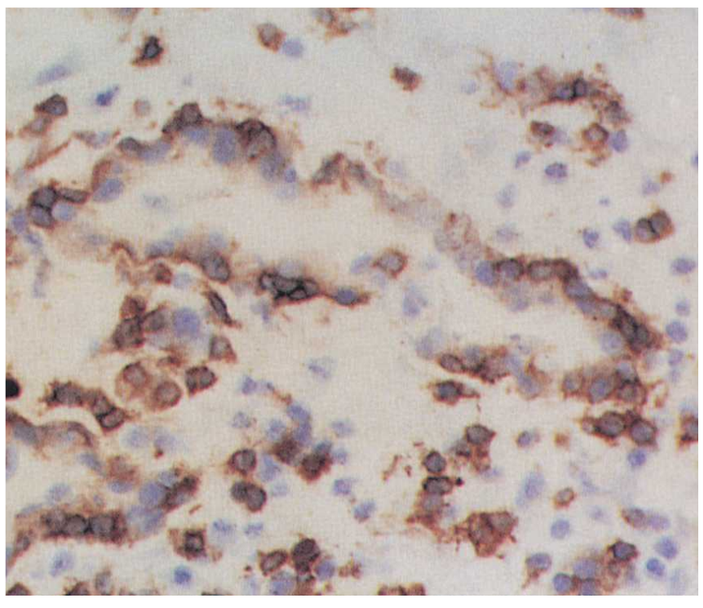


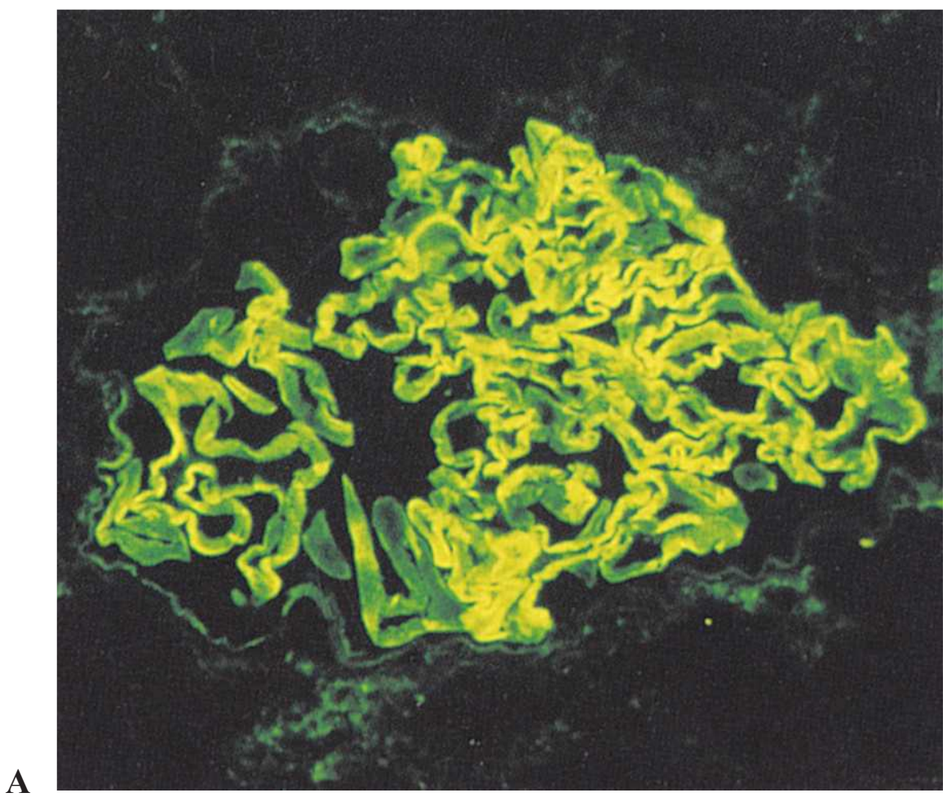
D,E





F

FIGURE 13.4



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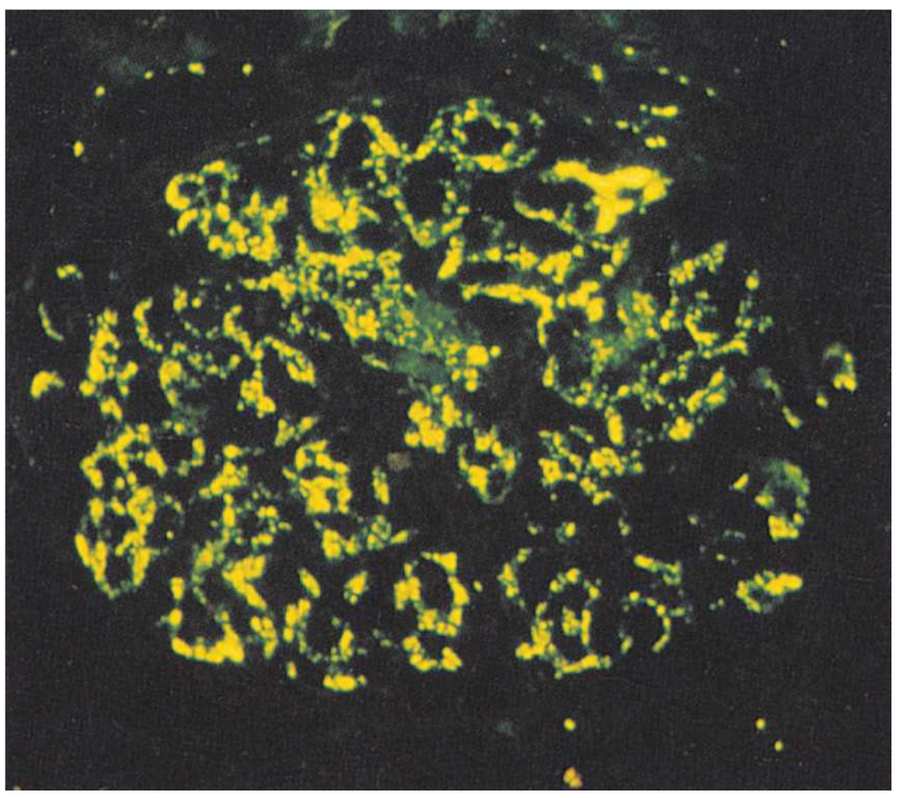

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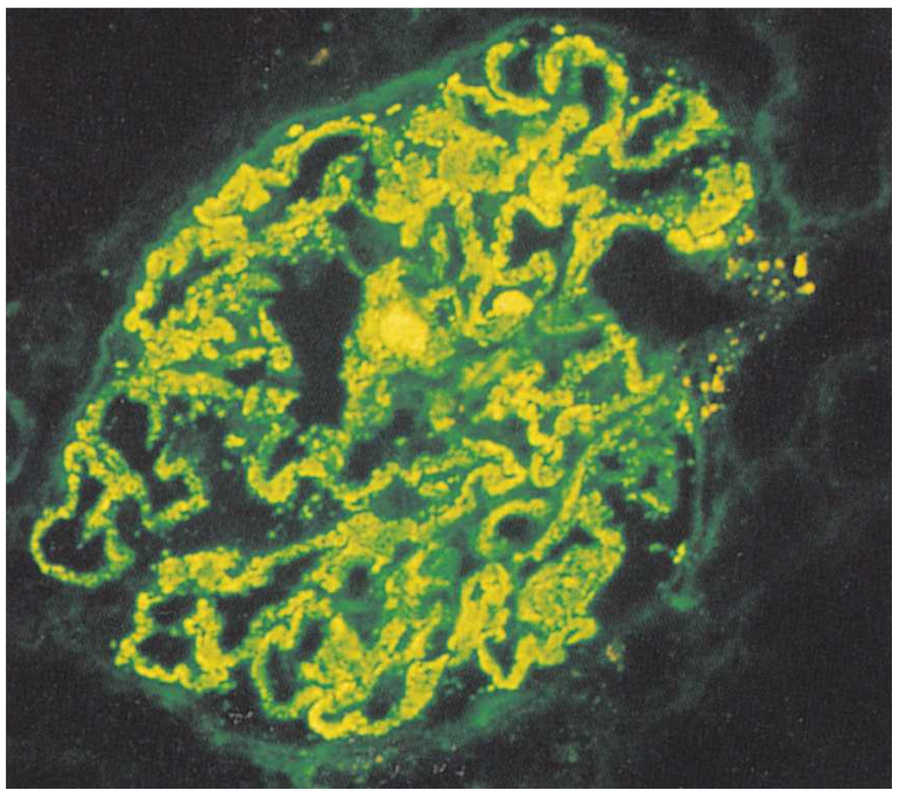

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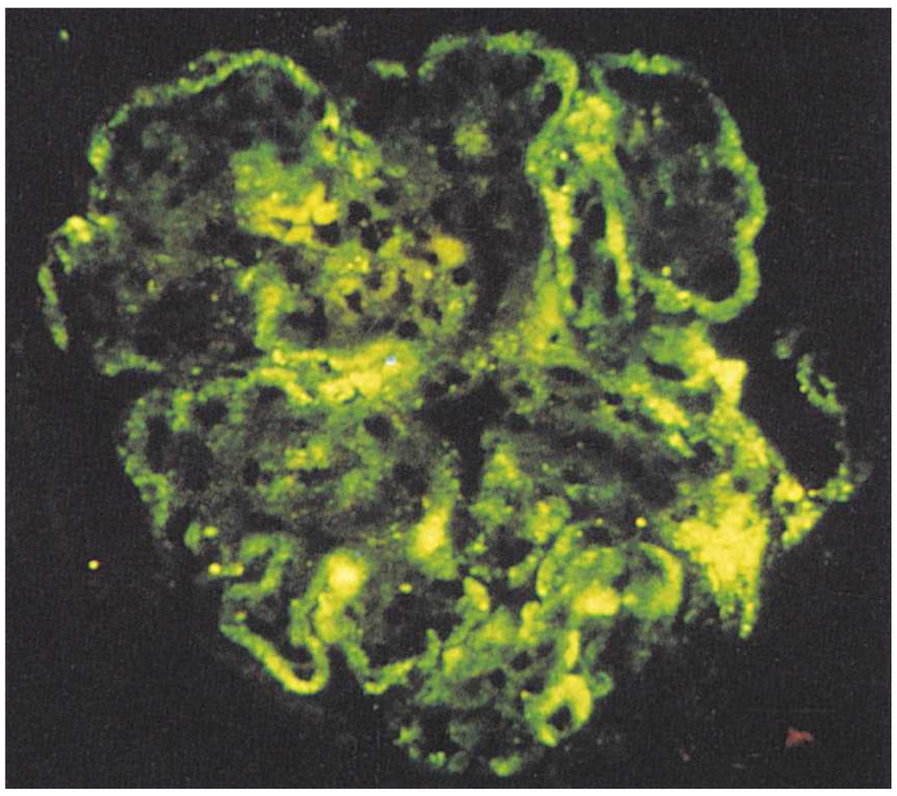
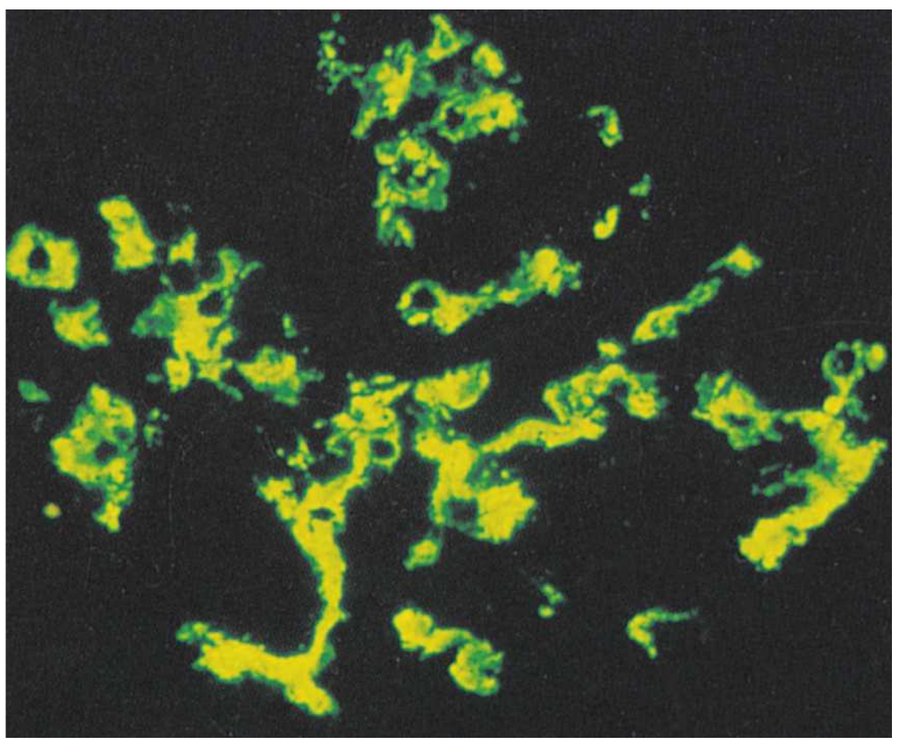


D



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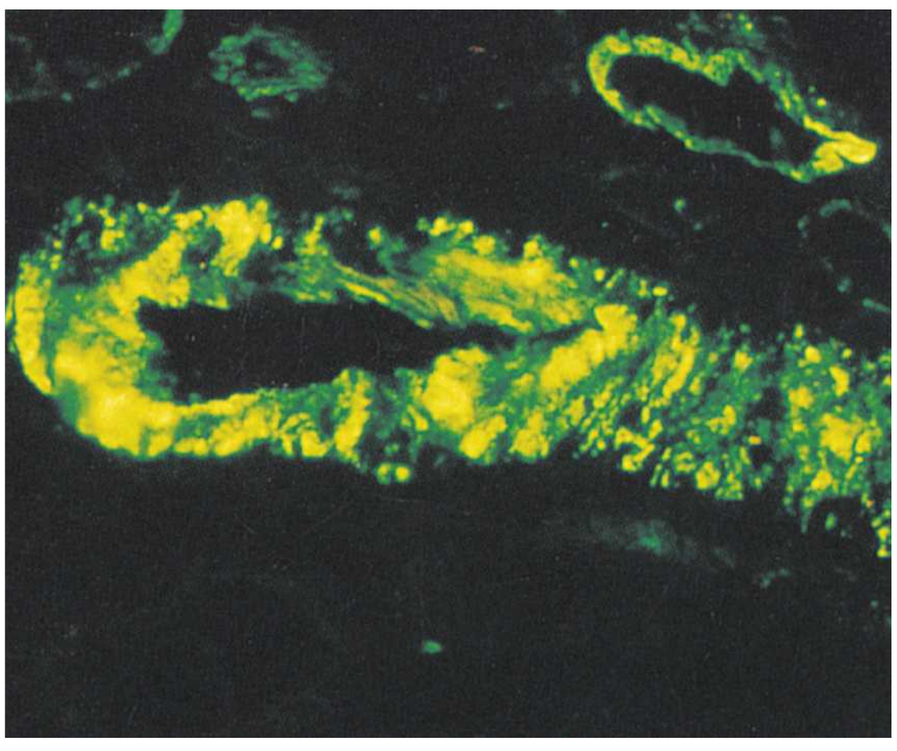
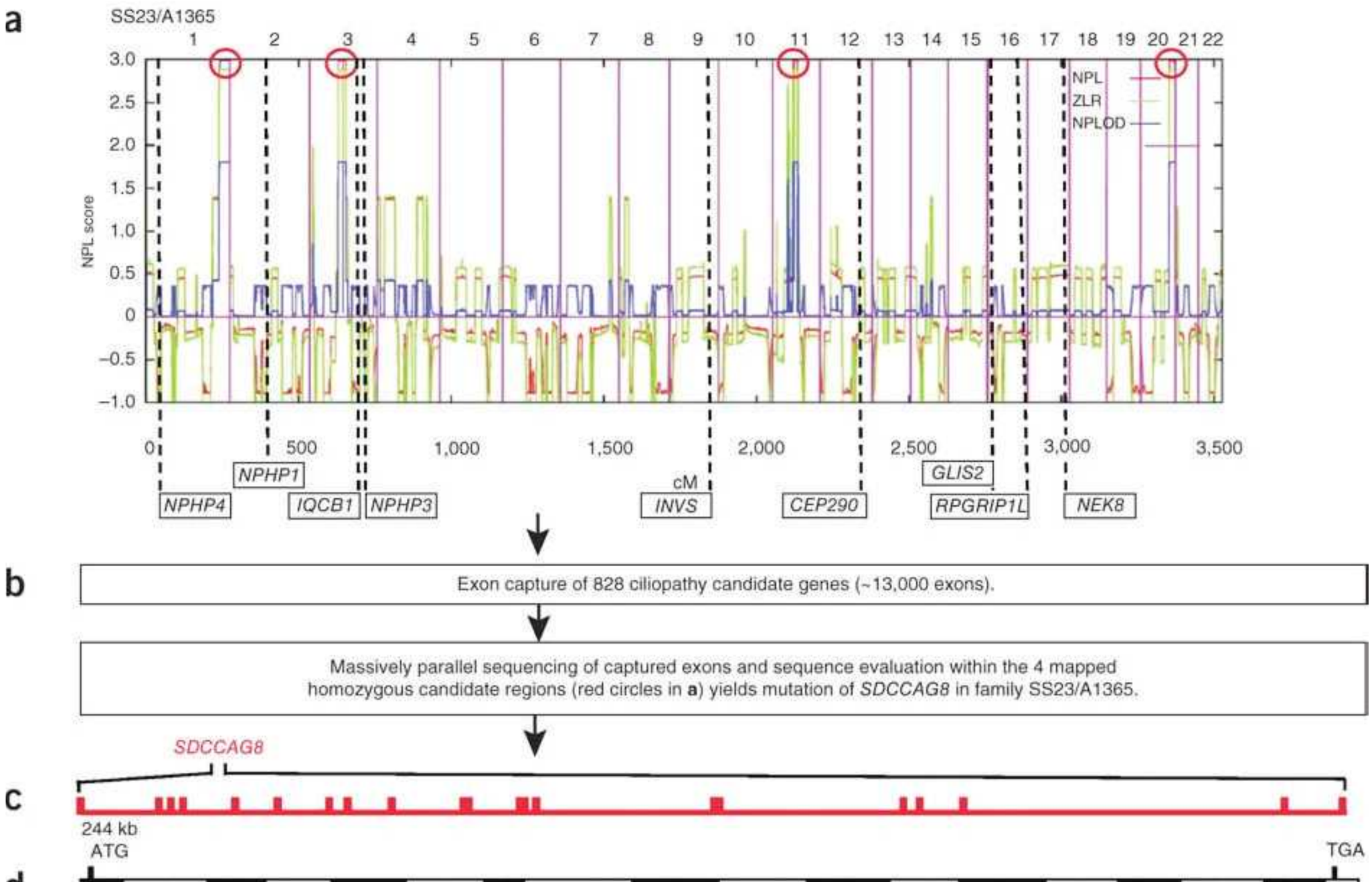


F

FIGURE 13.22 A,F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SS23/A1365
NPL score
NPL
ZLR
NPLOD
cM
NPHP4
NPHP1
IQCB1
NPHP3
INVS
CEP290
GLIS2
RPGRIP1L
NEK8
b
Exon capture of 828 ciliopathy candidate genes (~13,000 exons).
Massively parallel sequencing of captured exons and sequence evaluation within the 4 mapped homozygous candidate regions (red circles in a) yields mutation of SDCCAG8 in family SS23/A1365.
c
SDCCAG8
244 kb
ATG
TGA
d
2,142 bp
< Deletion >
e
NGD
NLS
CC1
CC2
CC3
CC4
CC5
CC6
CC7
CC8
713 aa
PN
PC
Gln_rich (PS50322)
f
F159
Δ exon 5–7
FII.22
679A>T
(K227X)
FI.2
740+356C>T
(ESE loss)
A2290
1068+1G>A
(splice)
SS-F336
1339-40insG
(447fsX463)
SS23/A1365
1420delG
(E474fsX493)
F1054
1796T>G
(L599X)
F195
1946-1949
delGTGT
(C649fsX658)
Control
F159-21
F159-22
Exon 8
Exon 5
Exon 6
Exon 8
Exon 8
Exon 7

FIGURE 14.1

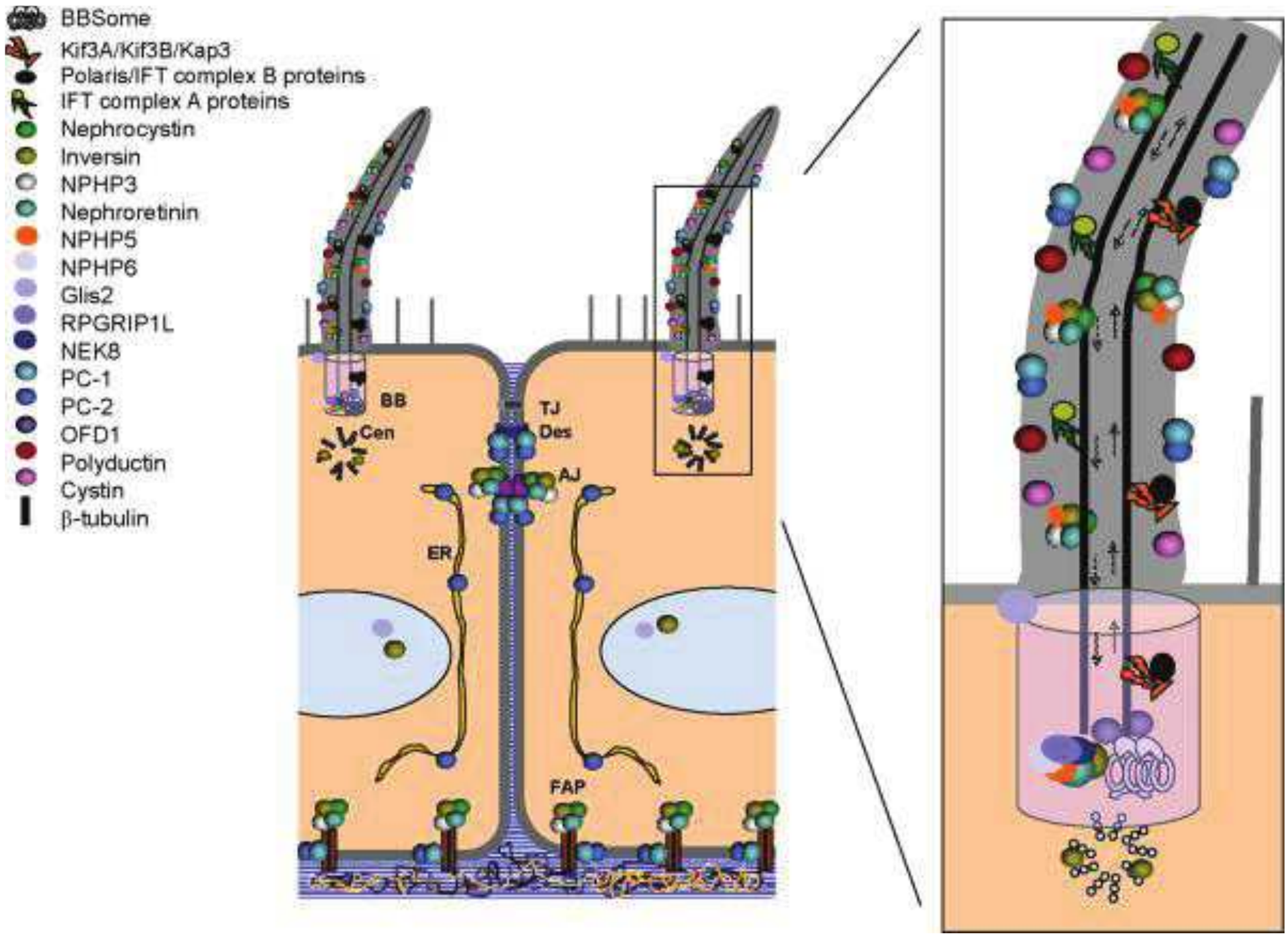


FIGURE 14.7

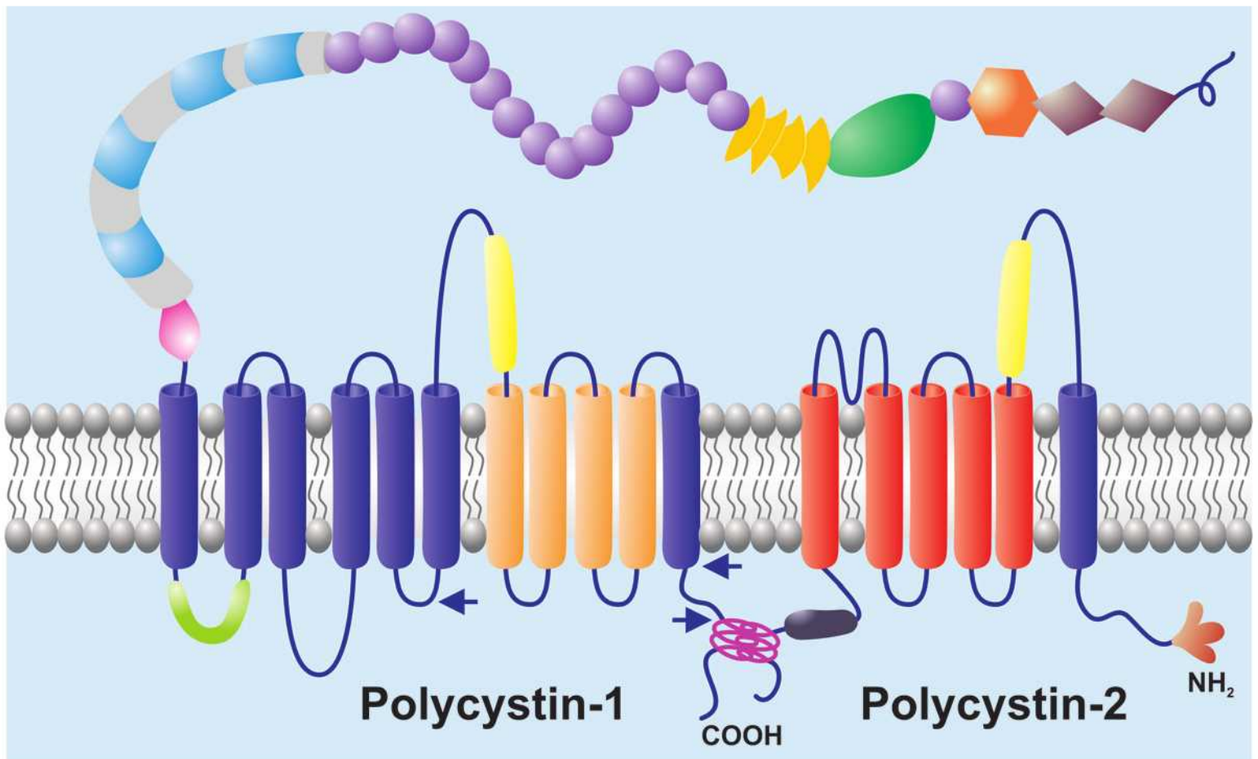


FIGURE 16.2

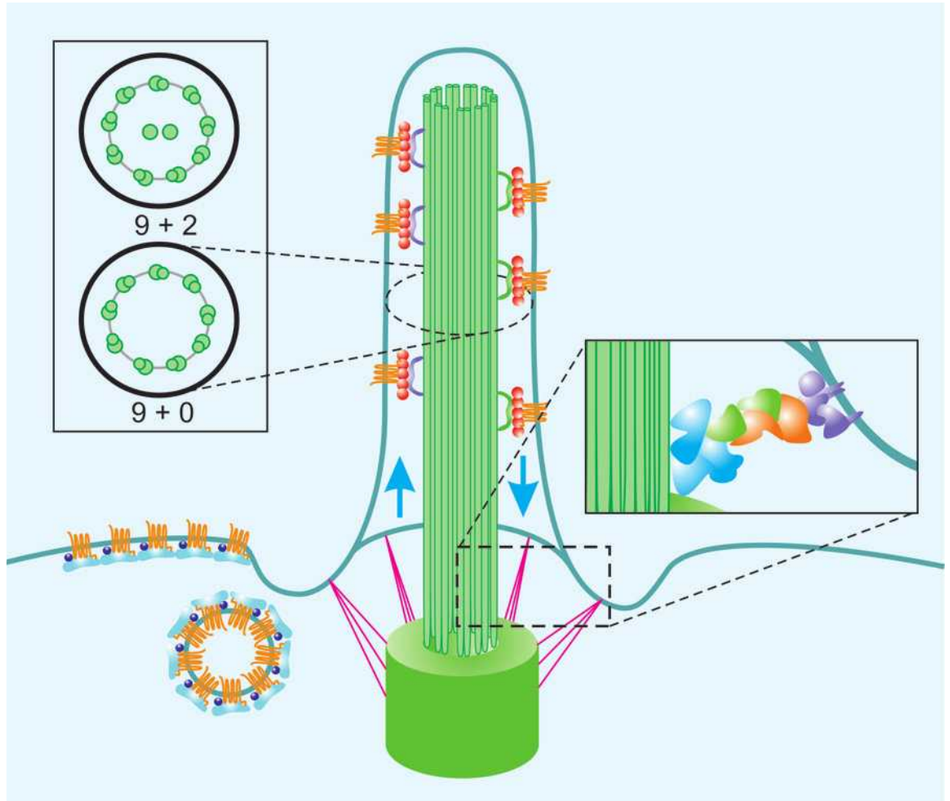


FIGURE 16.5

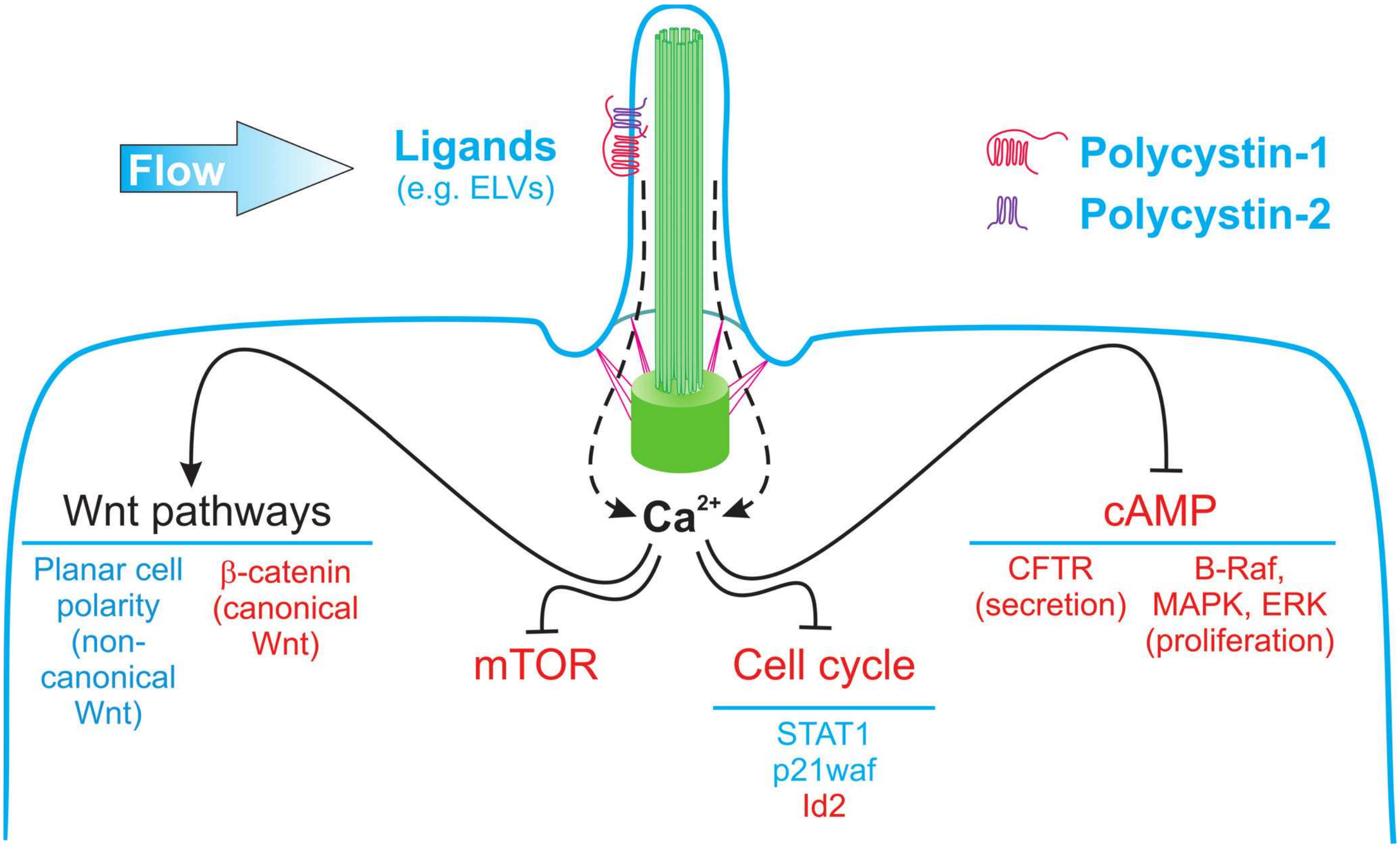


FIGURE 16.6

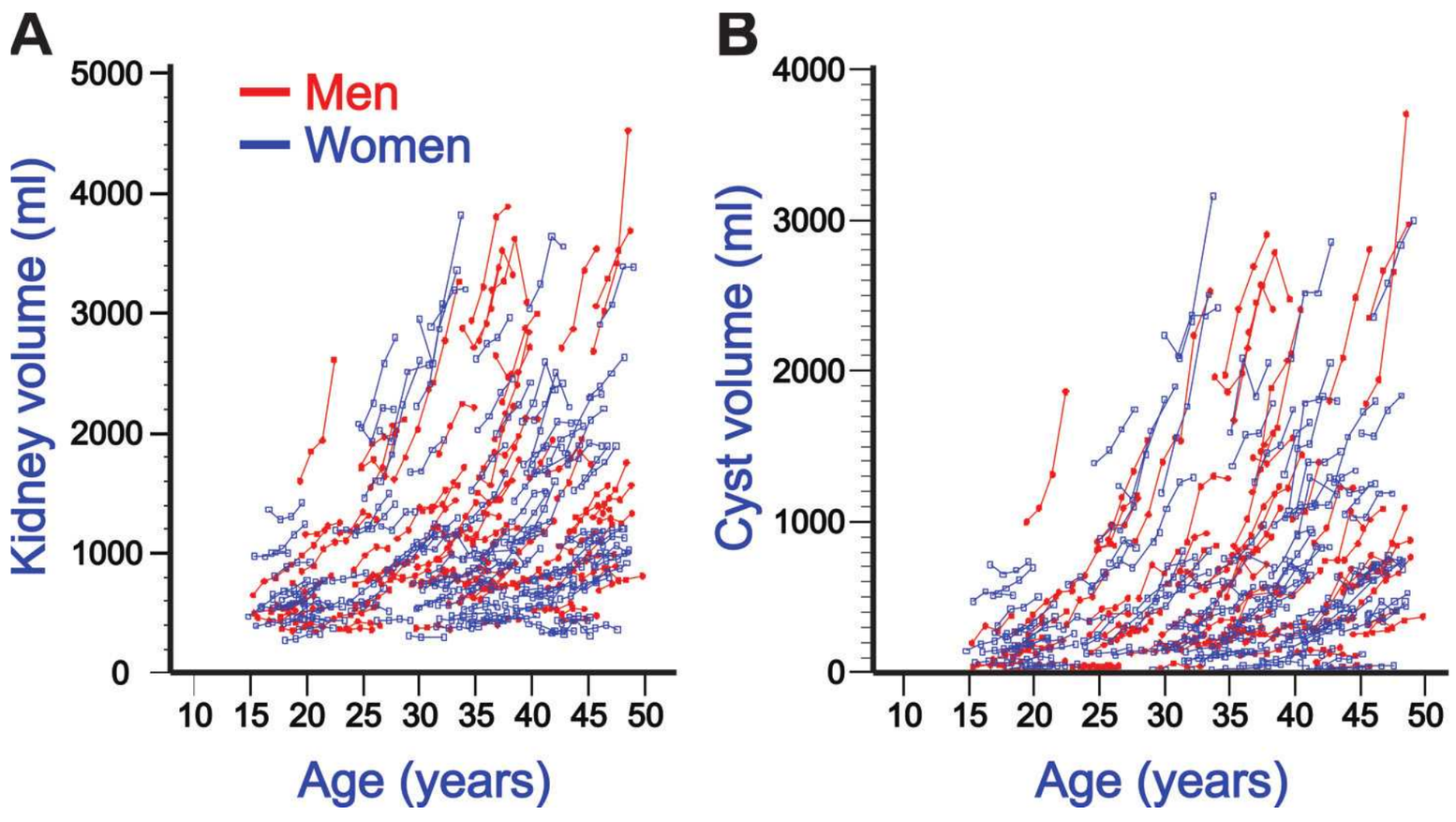


FIGURE 16.9

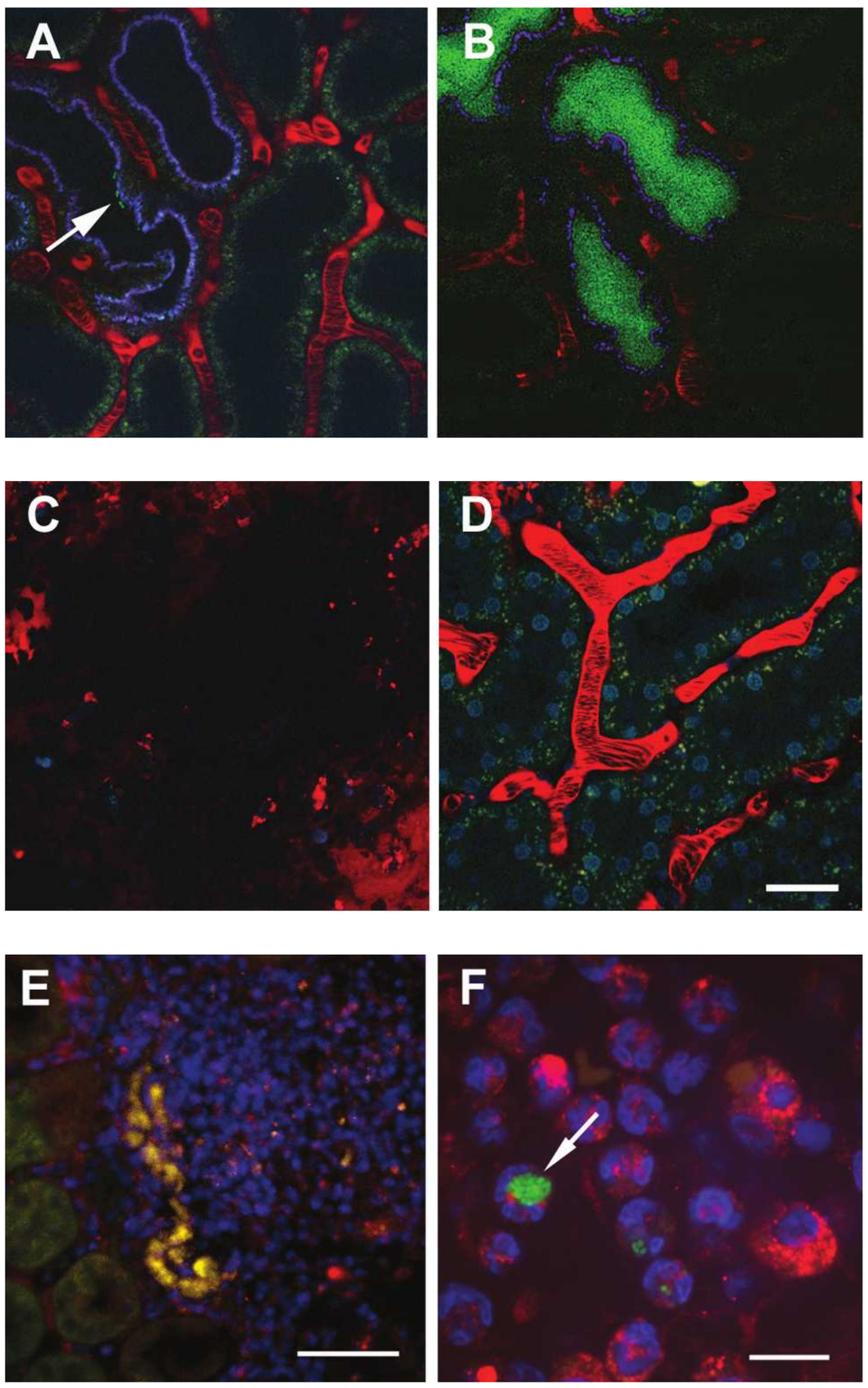


FIGURE 21.1

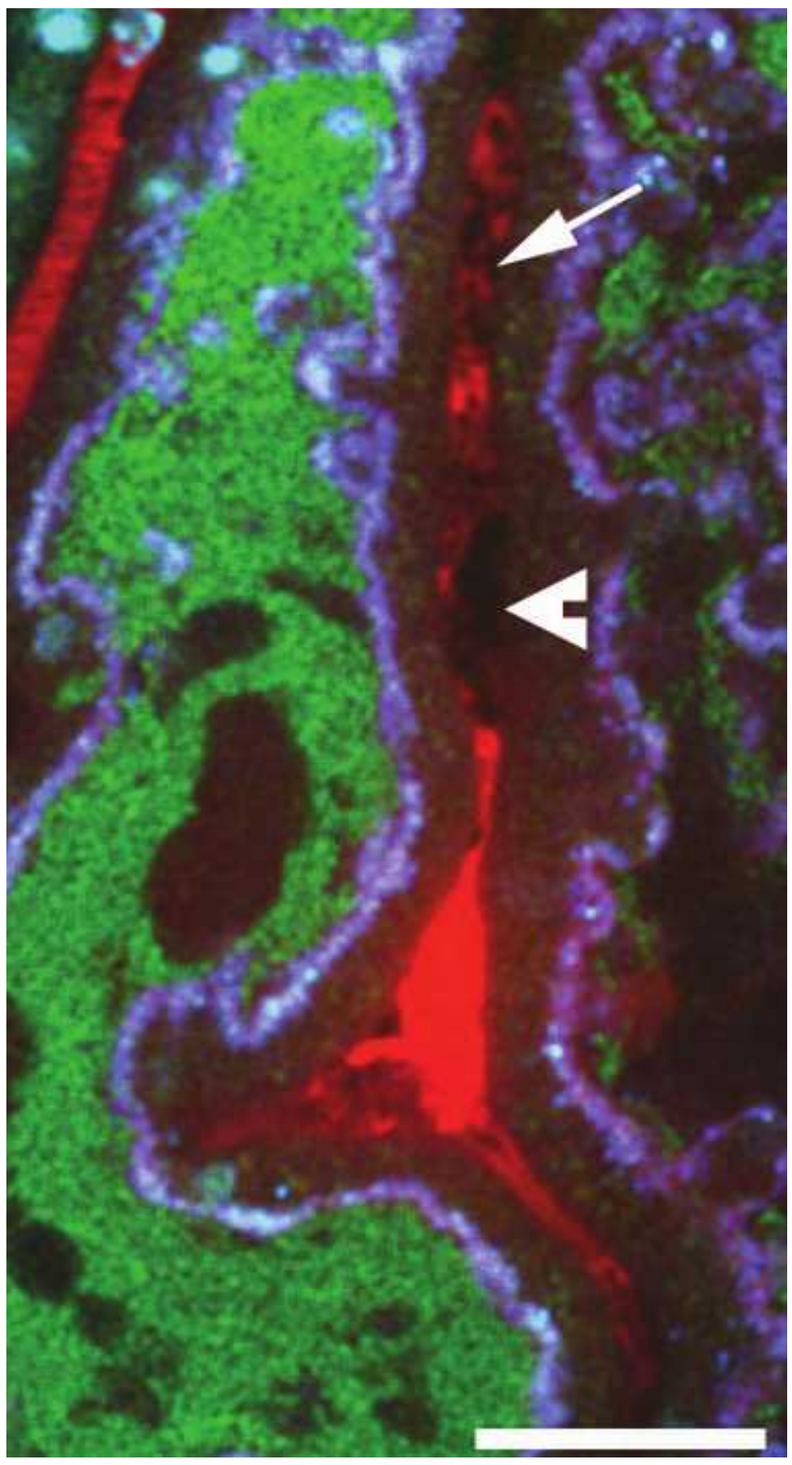


FIGURE 21.2

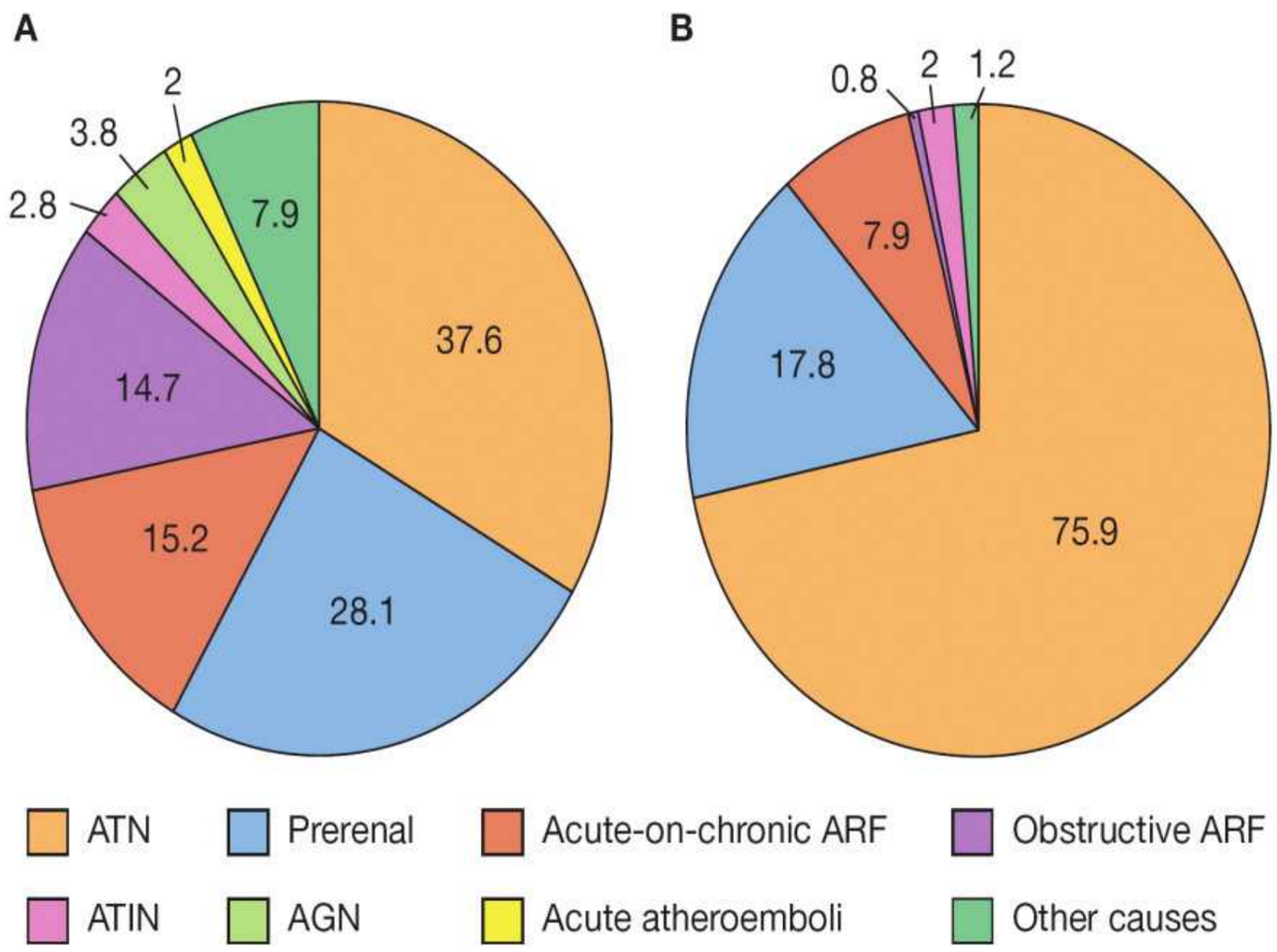


FIGURE 28.7

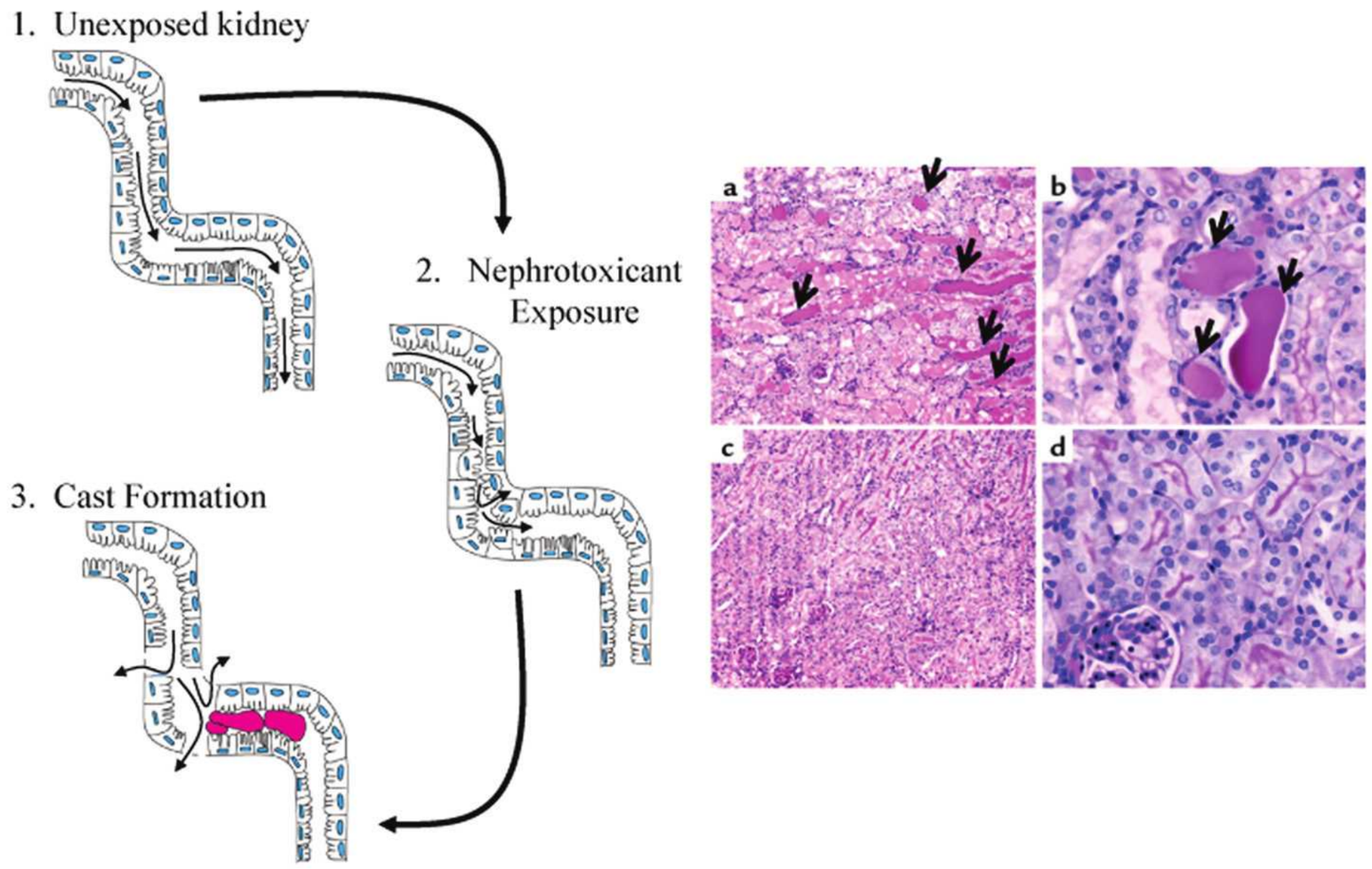


FIGURE 30.1

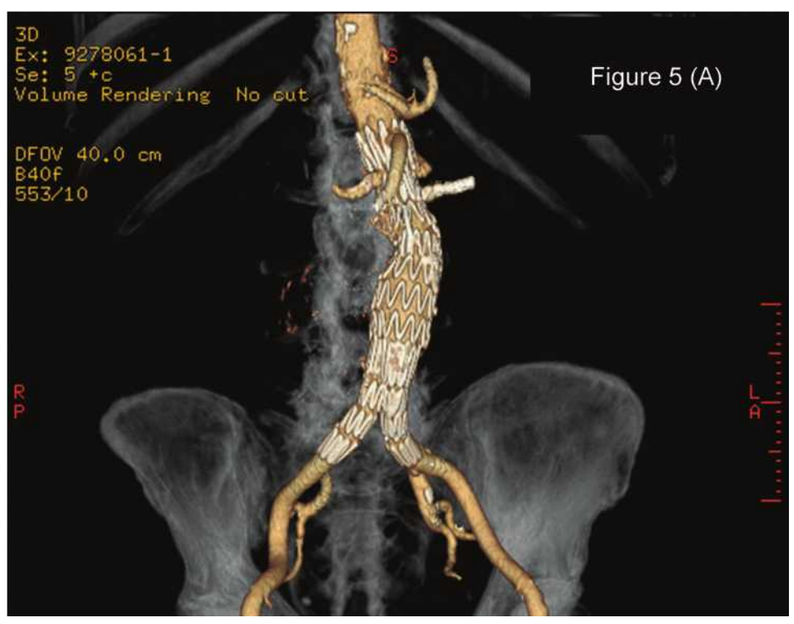
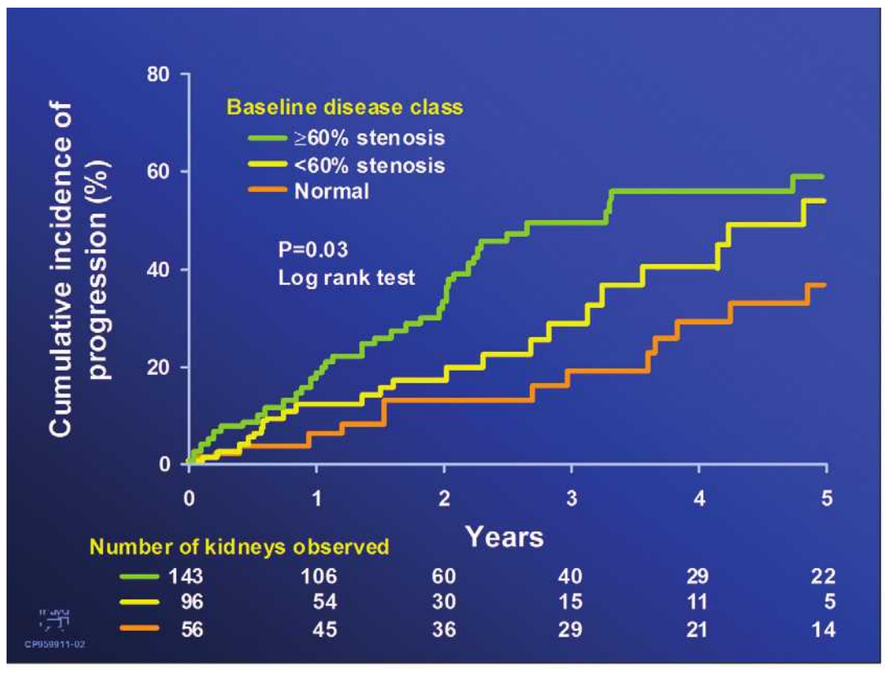


FIGURE 42.5A

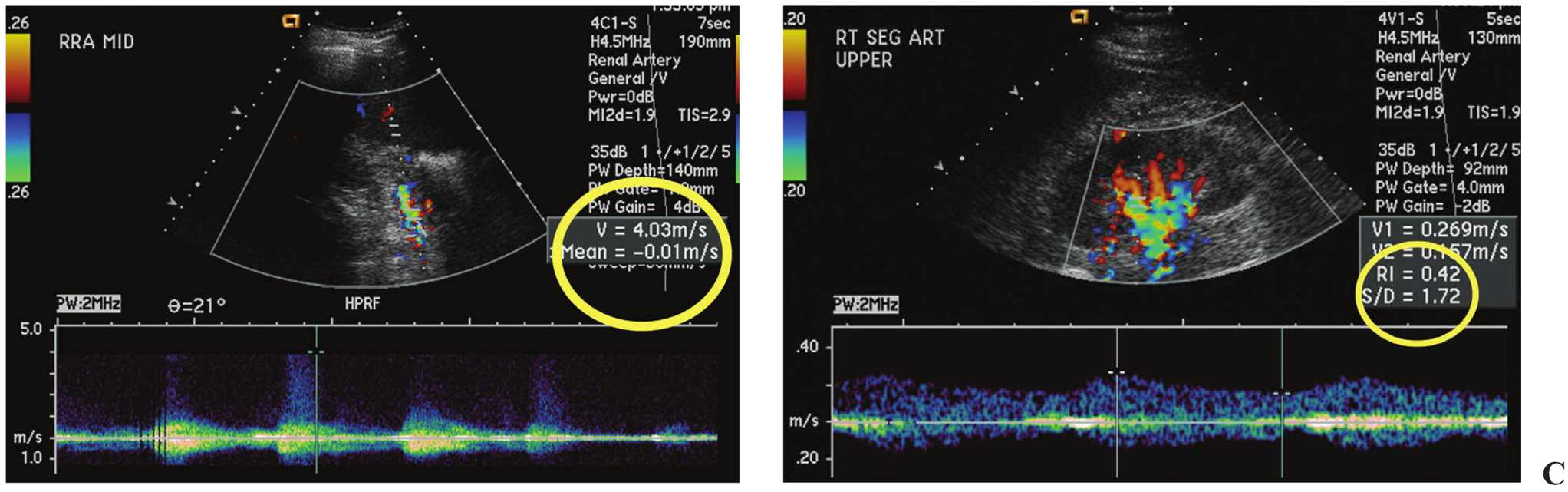


N=170 patients with study of 295 renal arteries by serial duplex scans between 1990 and 1997.

Total Occlusion: 9/295 arteries (3%)

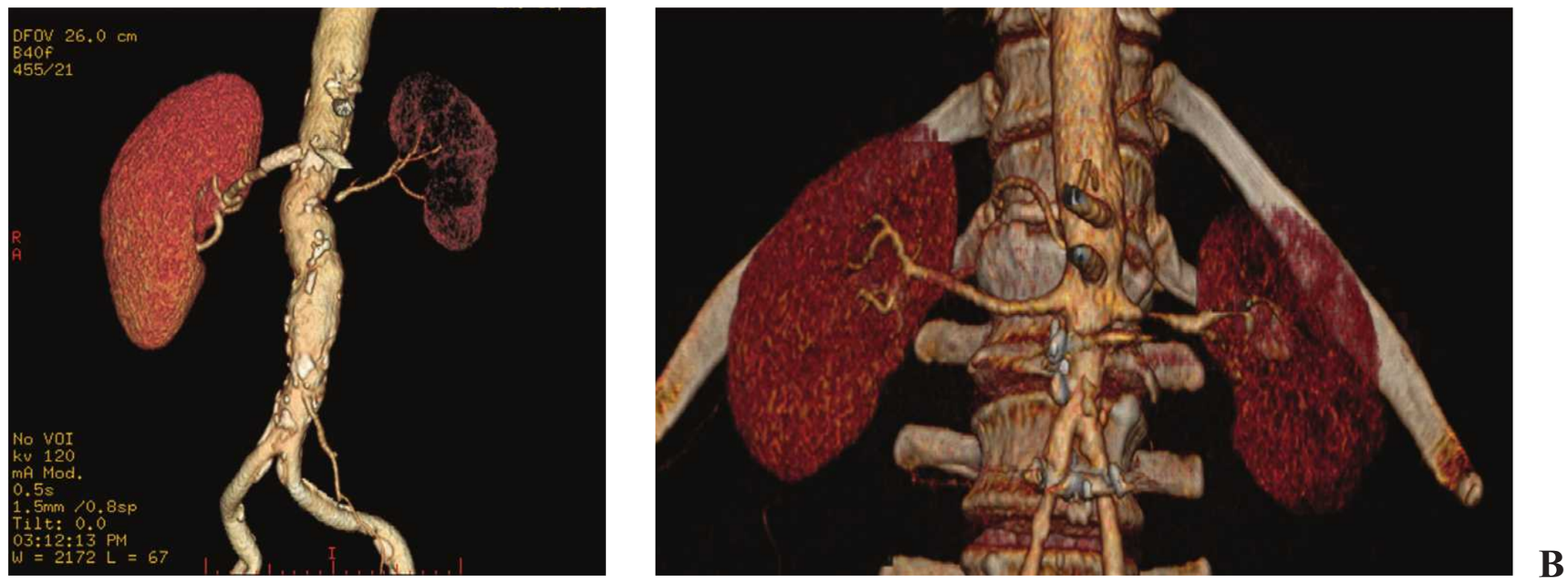
Caps, et. al. : Circulation 98: 2866-2872, 1998

FIGURE 42.6



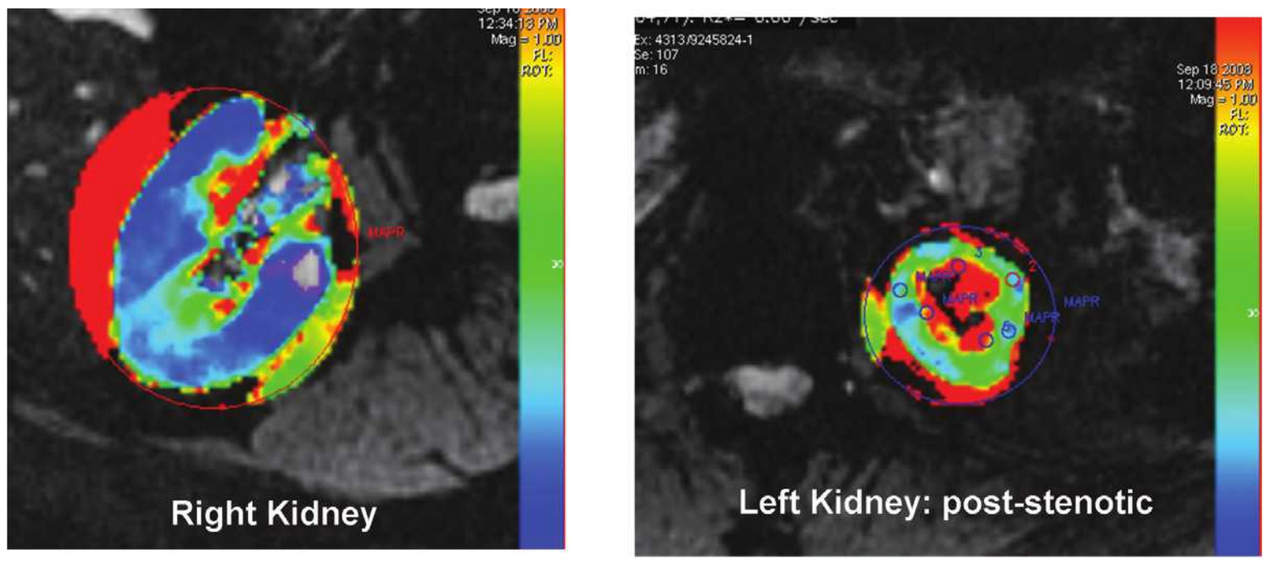
B C

FIGURE 42.13B,C



A B

FIGURE 42.16A,B



B C

FIGURE 42.18B,C