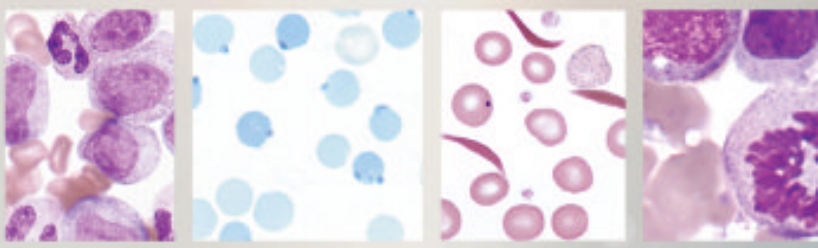
Fourth Edition

Clinical Hematology Atlas



Bernadette F. Rodak
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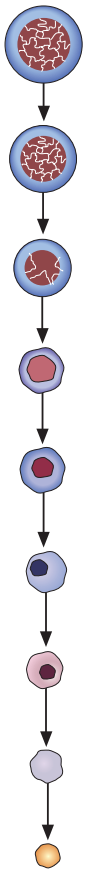

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To our husbands,
Robert Hartman
and
Charles Carr,
daughters,
Kimberly Carr Mayrose
and
Alexis Carr,
and all of the students
who have taught us hematology

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PREFACE

Because the emphasis of an atlas is morphology, the *Clinical Hematology Atlas* is intended to be used with a textbook, such as Rodak BF, Fritsma GA, Keohane EM: *Hematology: Clinical Principles and Applications*, fourth edition, that addresses physiology and diagnosis along with morphology.

This atlas is designed for a diverse audience that includes clinical laboratory science students, medical students, residents, and practitioners. It is also a valuable resource for clinical laboratory practitioners who are being retrained or cross-trained in hematology. It is not intended to be a detailed comprehensive manual for diagnosis.

ORGANIZATION

As is frequently expounded, morphology on a peripheral blood film is only as good as the quality of the smear and the stain. Chapter 1 reviews smear preparation, staining, and the appropriate area in which to evaluate cell distribution and morphology. A table that summarizes the morphology of leukocytes found in a normal differential, along with multiple examples of each cell type, facilitates early instruction in blood smear review.

Chapter 2 schematically presents hematopoietic features of cell maturation. General cell maturation, along with an electron micrograph with labeled organelles, will help readers correlate the substructures with the appearance of cells under light microscopy. Visualizing normal cellular maturation is essential to the understanding of disease processes. This correlation of schematic, electron micrograph, and Wright-stained morphology is carried throughout the maturation chapters. Figure 2-1 has been reformatted to reflect recent hematopoietic theory. In addition, the chart aids readers in recognizing the anatomical sites at which each stage of maturation normally occurs.

Chapters 3 to 9 present the maturation of each cell line individually, repeating the respective segment of the overall hematopoietic scheme from Chapter 2, to assist the student in seeing the relationship of each cell line to the whole. In these chapters, each maturation stage is presented as a color print, a schematic, and an electron micrograph. A description of each cell, including overall size, nuclear-to-cytoplasmic ratio, morphologic features, and reference ranges in peripheral blood and bone marrow, serves as a convenient summary. The final figure in each of these chapters summarizes lineage maturation by repeating the hematopoietic segment with the corresponding photomicrographs. Multiple nomenclatures for erythrocyte maturation are used to accommodate use in multiple settings and demographic groups.

Chapters 10 to 12 present discrete cellular abnormalities of erythrocytes, that is, variations in size, color, shape, and distribution, as well as inclusions found in erythrocytes. Each variation is presented along with a description of the abnormality, or composition of the inclusion, and associated disorders.

Because diseases are often combinations of the cellular alterations, Chapter 13 integrates morphologic findings into the diagnostic features of disorders primarily affecting erythrocytes.

In Chapter 14, nuclear and cytoplasmic changes in leukocytes are displayed and correlated with non-malignant leukocyte disorders.

Diseases of excessive or altered production of cells may be caused by maturation arrest, asynchronous development, or proliferation of one cell line,

as presented in Chapters 15 to 19. Cytochemical stains are presented with disorders in which they are useful.

The therapeutic use of myeloid growth factors causes morphologic changes that mimic severe infections or malignancies. New to this edition is Chapter 20 in which examples of peripheral blood morphology following G-CSF or GM-CSF are presented.

It is the authors' design that the cellular defects in leukocyte disorders be visually compared with the process of normal hematopoiesis for a more thorough comprehension of normal and altered development. Readers are encouraged to refer to the normal hematopoiesis illustration, Figure 2-1, for comparison of normal and abnormal cells and the progression of diseases.

Microorganisms, including parasites, may be seen on peripheral blood smears. A brief photographic overview is given in Chapter 21. Readers are encouraged to consult a microbiology reference, such as Mahon CM, Lehman DC, Manuselis G: *Textbook of Diagnostic Microbiology*, fourth edition, for a more detailed presentation.

Chapter 22 includes photomicrographs that are not categorized into any one particular area, such as fat cells, mitotic figures, metastatic tumor cells, and artifacts.

Chapter 23 describes findings expected in the peripheral blood of neonates, including anticipated variations in morphology and cellular distribution. New to this edition is comparison of the hematogone, normal for newborns, with the blast cell of acute leukemia.

Chapter 24 is intended to be an overview of the most frequent microscopic findings in body fluids. It is not proposed as a comprehensive review of the cytology of human body fluids, but rather a quick reference for the beginning microscopist as well as the seasoned professional.

The majority of the images for the fourth edition were taken using digital photography. As with the third edition, the fourth edition also features spiral binding, making the atlas more convenient when used at the microscope bench.

All of these chapters combine into what we believe is a comprehensive and valuable resource for any clinical laboratory. The quality of the schematic illustrations, electron micrographs, and color photographs stand for themselves. We hope that this atlas will enrich the learning process for the student and serve as an important reference tool for the practitioner.

EVOLVE

The Evolve website provides free materials for both students and instructors. Instructors have access to an electronic image collection that features all of the images from the atlas. Students and instructors have access to summary tables and chapter quizzes.

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A special thank you goes to the professionals at Elsevier who navigated us through the production of this atlas. **Ellen Wurm-Cutter**, Content Manager, who has the patience of a saint and persevered with us, even when the going got rough; **Jennifer Byington Geistler**, Production Editor (of Graphic World); and **Catherine Jackson**, Publishing Services Manager.

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CONTENTS

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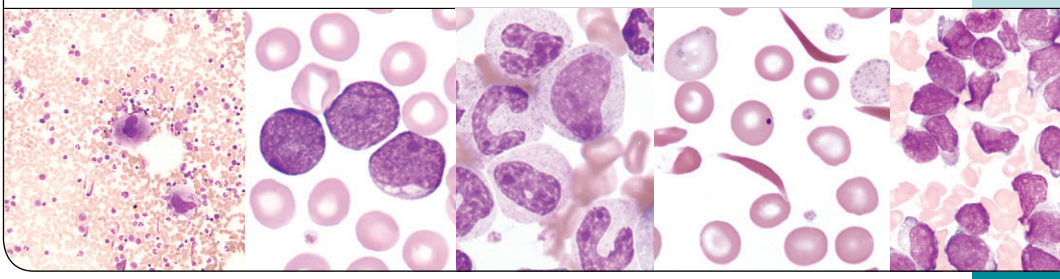
COMMONLY USED ABBREVIATIONS IN HEMATOLOGY

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
Baso	Basophil
BM	Bone marrow
CD	Cluster of differentiation
CLL	Chronic lymphocytic leukemia
CML	Chronic myelogenous leukemia
CNS	Central nervous system
CSF	Cerebrospinal fluid
EDTA	Ethylenediaminetetraacetic acid
Eos	Eosinophil
ET	Essential thrombocythemia
FAB	French-American-British (classification of tumors of the hematopoietic and lymphoid systems)
G-CSF	Granulocyte colony-stimulating factor
LAP	Leukocyte alkaline phosphatase
LE	Lupus erythematosus
Lymph	Lymphocyte
M:E ratio	Myeloid: erythroid ratio
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDS	Myelodysplastic syndrome
MK	Megakaryocyte
Mono	Monocyte
MPN	Myeloproliferative neoplasms
N/C ratio (or N:C ratio)	Nucleus/cytoplasm ratio
NRBC	Nucleated red blood cells
PB	Peripheral blood
PMF	Primary myelofibrosis
Poly, PMN	Polymorphonuclear neutrophil
PV	Polycythemia vera
RBC	Red blood cell
RDW	Red blood cell distribution width
Seg	Segmented neutrophil
WBC	White blood cell
WHO	World Health Organization

1

INTRODUCTION TO PERIPHERAL BLOOD SMEAR EXAMINATION



A properly prepared blood smear is essential to accurate assessment of cellular morphology. A variety of methods are available for preparing and staining blood smears, the most common of which are discussed in this atlas. It is beyond the scope of this atlas to discuss other methodologies; however, detailed descriptions of these procedures can be found in textbooks on hematology, such as Rodak, Fritsma, and Keohane's *Hematology: Clinical Principles and Applications.*

WEDGE SMEAR PREPARATION

MAKING THE PERIPHERAL BLOOD SMEAR Although some automated analyzers prepare and stain blood smears according to established criteria, manual blood smear preparation is still used in many places. The wedge smear is a convenient and commonly used technique for making peripheral blood smears. This technique requires at least two 3 × 1-inch (75 × 25-mm) clean glass slides. High-quality, beveled-edge microscope slides are recommended. One slide serves as the blood smear slide and the other as the spreader slide. These can then be reversed to prepare a second smear. A drop of ethylenediaminetetraacetic acid (EDTA) anticoagulated blood about 3 mm in diameter is placed at one end of the slide. Alternatively, a similar size drop of blood directly from a finger or heel puncture is acceptable. The size of the drop of blood is important. Too large a drop creates a long or thick smear, and too small a drop often makes a short or thin smear. In preparing the smear, the technician holds the pusher slide securely in front of the drop of blood at a 30- to 45-degree angle to the smear slide (Figure 1-1, *A*). The pusher slide is pulled back into the drop of blood and held in that position until the blood spreads across the width of the slide (Figure 1-1, *B*). It is then quickly and smoothly pushed forward to the end of the smear slide, creating a wedge smear (Figure 1-1, *C*). It is important that the whole drop of blood is picked up and spread. Moving the pusher slide forward too slowly accentuates poor leukocyte distribution by pushing larger cells, such as monocytes and granulocytes, to the very end and sides of the smear. Maintaining a consistent angle between the slides and an even, gentle pressure is essential. It is frequently necessary to adjust the angle between the slides to produce a satisfactory smear. For higher than normal hematocrit, the angle between the slides must be lowered so that the smear is not too short and thick. For extremely low hematocrit, the angle must be raised. A well-made peripheral blood smear (Figure 1-2) has the following characteristics:

1. About two-thirds to three-fourths of the length of the slide is covered by the smear.
2. It is slightly rounded at feather edge (thin portion), not bullet shaped.
3. Lateral edges of the smear should be visible. The use of slides with chamfered (beveled) corners may facilitate this appearance.
4. It is smooth without irregularities, holes, or streaks.
5. When the slide is held up to light, the feather edge of the smear should have a "rainbow" appearance.
6. The whole drop is picked up and spread.

Figure 1-3 shows examples of unacceptable smears.

STAINING OF PERIPHERAL BLOOD SMEARS The purpose of staining blood smears is to identify cells and recognize morphology easily through the microscope. Wright or Wright-Giemsa stain is the most commonly used stain for peripheral blood and bone marrow smears. These stains contain both eosin and methylene blue, and are therefore termed *polychrome*

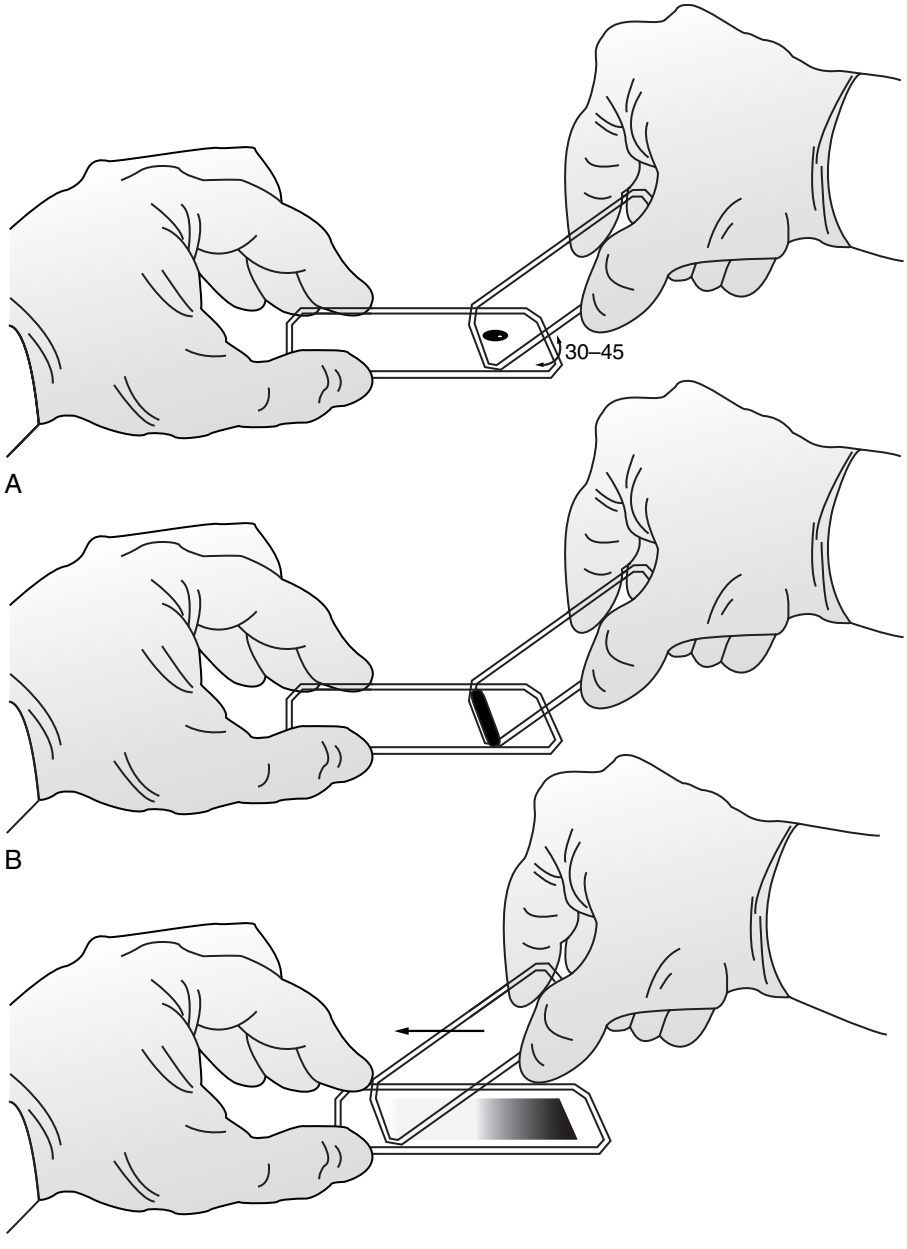


FIGURE 1–1 Wedge technique of making a peripheral blood smear. **A,** Correct angle to hold spreader slide. **B,** Blood spread across width of slide. **C,** Completed wedge smear. (From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.)

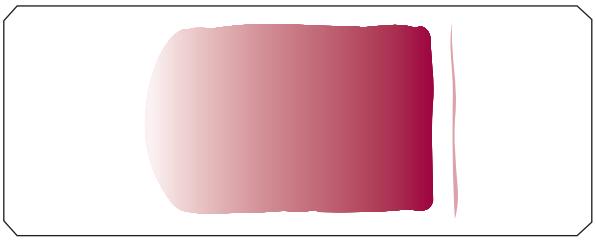


FIGURE 1–2 Well-made peripheral blood smear. (From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.)

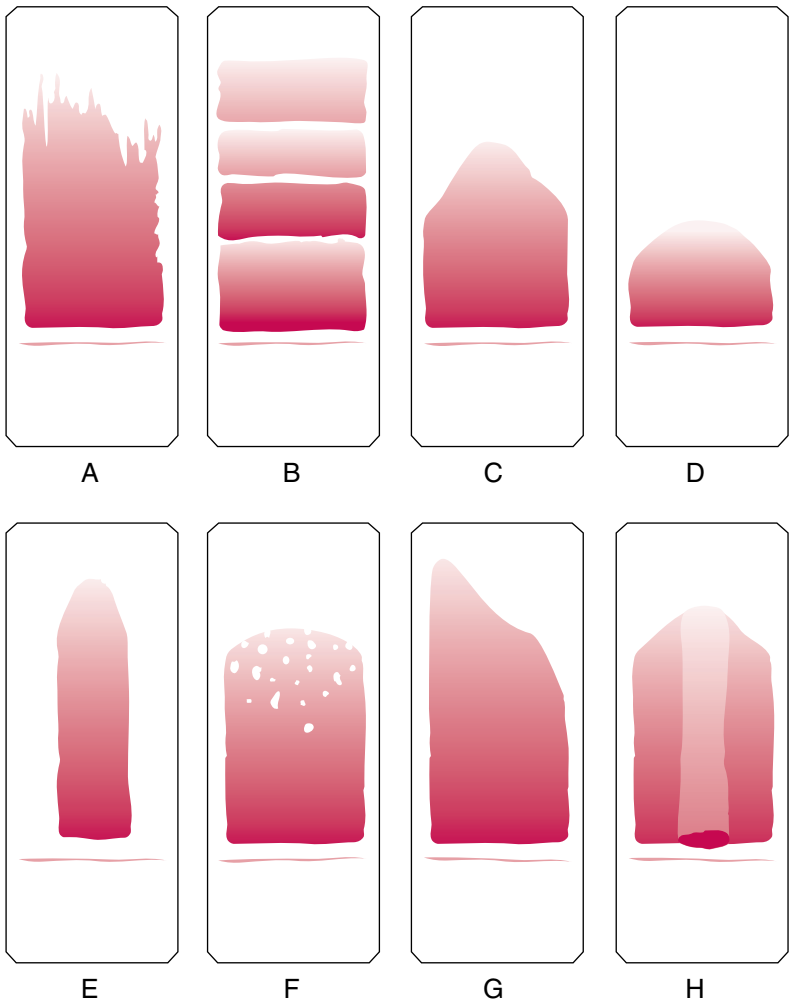


FIGURE 1–3 Unacceptable peripheral blood films. Slide appearances associated with the most common errors are shown, but note that a combination of causes may be responsible for unacceptable films. **A,** Chipped or rough edge on spreader slide. **B,** Hesitation in forward motion of spreader slide. **C,** Spreader slide pushed too quickly. **D,** Drop of blood too small. **E,** Drop of blood not allowed to spread across the width of the slide. **F,** Dirt or grease on the slide; may also be caused by elevated lipids in the blood specimen. **G,** Uneven pressure on the spreader slide. **H,** Time delay; drop of blood began to dry. (From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.)

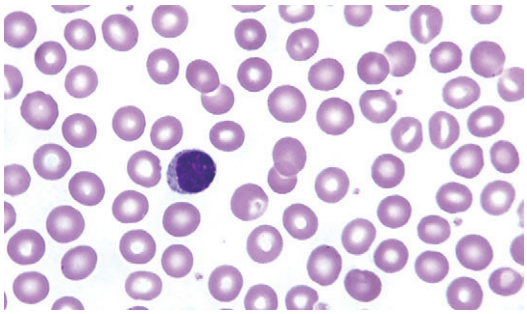


FIGURE 1–4 Optimally stained peripheral blood smear demonstrating the appropriate area in which to perform the white blood cell differential and morphology assessment and the platelet estimate. Only the center of the field is shown; an entire field would contain 200 to 250 red blood cells (×1000).

stains. The colors vary slightly from laboratory to laboratory, depending on the method of staining.

The cells are fixed to the glass slide by the methanol in the stain. Staining reactions are pH dependent, and the actual staining of the cellular components occurs when a buffer (pH 6.4) is added to the stain. Free methylene blue is basic and stains acidic cellular components, such as RNA, blue. Free eosin is acidic and stains basic components, such as hemoglobin or eosinophilic granules, red. Neutrophils have cytoplasmic granules that have a neutral pH and accept some characteristics from both stains. Details for specific methods of staining peripheral blood and bone marrow smears, including automated methods, may be found in a standard textbook of hematology.

An optimally stained smear (Figure 1-4) has the following characteristics:

1. The red blood cells (RBCs) should be pink to salmon.
2. Nuclei are dark blue to purple.
3. Cytoplasmic granules of neutrophils are lavender to lilac.
4. Cytoplasmic granules of basophils are dark blue to black.
5. Cytoplasmic granules of eosinophils are red to orange.
6. The area between the cells should be colorless, clean, and free of precipitated stain.

A well-stained slide is necessary for accurate interpretation of cellular morphology. The best staining results are obtained from freshly made slides that have been prepared within 2 to 3 hours of blood collection. Slides must be allowed to dry thoroughly before staining. Box 1-1 lists common reasons for poorly stained slides and may be used as a guide when troubleshooting.

PERIPHERAL SMEAR EXAMINATION

10× EXAMINATION Examination of the blood smear is a multistep process. Begin the smear examination with a scan of the slide using the 10× or low-power objective (total magnification = 100×). This step is necessary to assess the overall quality of the smear, including abnormal distribution of RBCs, suggesting the presence of rouleaux or autoagglutination and/or the presence of a disproportionate number of large nucleated cells, such as monocytes or neutrophils, at the edges of the smear. If the latter exists, another smear should be prepared. In addition, the 10× smear examination allows for the rapid detection of large abnormal cells such as blasts, reactive lymphocytes, and parasites.

BOX 1-1 Troubleshooting Poorly Stained Blood Smears

First Scenario

Problems

- Red blood cells appear gray
- White blood cells are too dark
- Eosinophil granules are gray, not orange

Causes

- Stain or buffer too alkaline (most common)
- Inadequate rinsing
- Prolonged staining
- Heparinized blood sample

Second Scenario

Problems

- Red blood cells too pale or are red color
- White blood cells barely visible

Causes

- Stain or buffer too acidic (most common)
- Underbuffering (too short)
- Over-rinsing

From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.

40× or 50× EXAMINATION Using the 40× (high dry) objective or the 50× oil objective (400× and 500× total magnification, respectively), find an area of the smear in which the RBCs are evenly distributed and barely touching one another (two or three cells may overlap; Figure 1-5). Scan 8 to 10 fields in this area of the smear and determine the average number of white blood cells (WBCs) per field. Although an exact factor varies with the make and model of microscope, in general, an approximate WBC count per cubic millimeter can be determined by multiplying the average number of WBCs per high-power

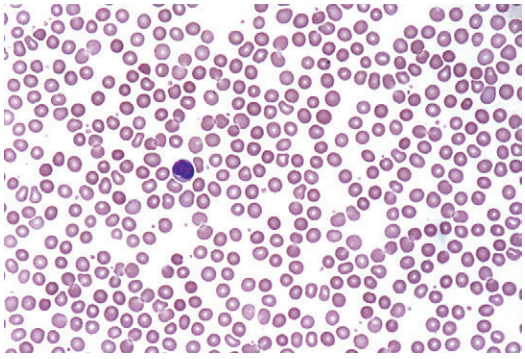


FIGURE 1–5 Correct area of blood smear in which to evaluate cellular distribution and perform white blood cell estimate (×400).

field by 2000 (if 40× is used) or 2500 (if 50× is used). This estimate is a useful quality-control tool for validating WBC counts from hematology analyzers. Any discrepancy between the instrument WBC count and the slide estimate must be resolved. Some reasons for discrepancy include the presence of WBC or platelet clumps, fibrin strands, severe RBC agglutination, cryoprecipitate, giant platelets, as well as a mislabeled smear, a smear made from the wrong patient's sample, and an instrument malfunction.

100× EXAMINATION The next step in smear evaluation is to perform the WBC differential. This is done in the same area of the smear as the WBC estimate but using the 100× oil immersion objective (1000× total magnification). When the correct area of the smear from a patient with a normal RBC count is viewed, about 200 to 250 RBCs per oil immersion field are seen (see Figure 1-4). Characteristically, the differential count includes counting and classifying 100 consecutive WBCs and reporting these classes as percentages. The differential count is performed in a systematic manner using the "battlement" track (Figure 1-6), which minimizes WBC distribution errors. The results are reported as percentages of each type of WBC seen during the count. An example of a WBC differential count is 3% bands, 55% segmented neutrophils, 30% lymphocytes, 6% monocytes, 4% eosinophils, and 2% basophils (Table 1-1). Any WBC abnormalities, such as toxic changes, Döhle bodies, reactive lymphocytes, and Aüer rods, are also reported. When present, nucleated red blood cells (NRBCs) are counted and reported as number of NRBCs per 100 WBCs. The RBC, WBC, platelet morphology evaluation, and platelet estimates are also performed under the 100× oil immersion objective. RBC inclusions, such as Howell-Jolly bodies, and WBC inclusions, such as Döhle bodies, can be seen at this magnification. Each laboratory should have established protocols for standardized reporting of abnormalities.

Evaluation of the RBC morphology is an important aspect of the smear evaluation and is used in conjunction with the RBC indices to describe cells as normal or abnormal in size, shape, and color. Each laboratory should establish a standard reporting protocol. Most laboratories use concise statements describing overall RBC morphology that is consistent with the RBC indices. The microscopic evaluation of RBC morphology must be congruent with the information given by the automated hematology analyzer. If not, discrepancies must be resolved before reporting patient results.

The final step in the performance of the differential count is the estimation of the platelet number. This is done under the 100× oil immersion objective. In an area of the smear where RBCs barely touch, the number of platelets in 5 to 10 oil immersion fields is counted. The average number of platelets is multiplied by 20,000 to provide an estimate of the total number of platelets per cubic millimeter. This estimate is reported as adequate if

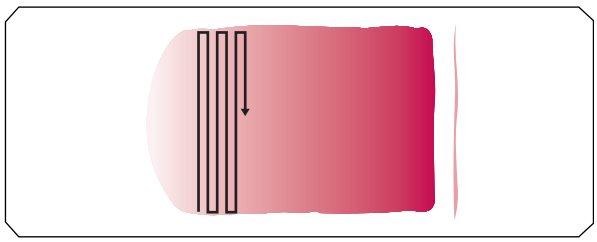


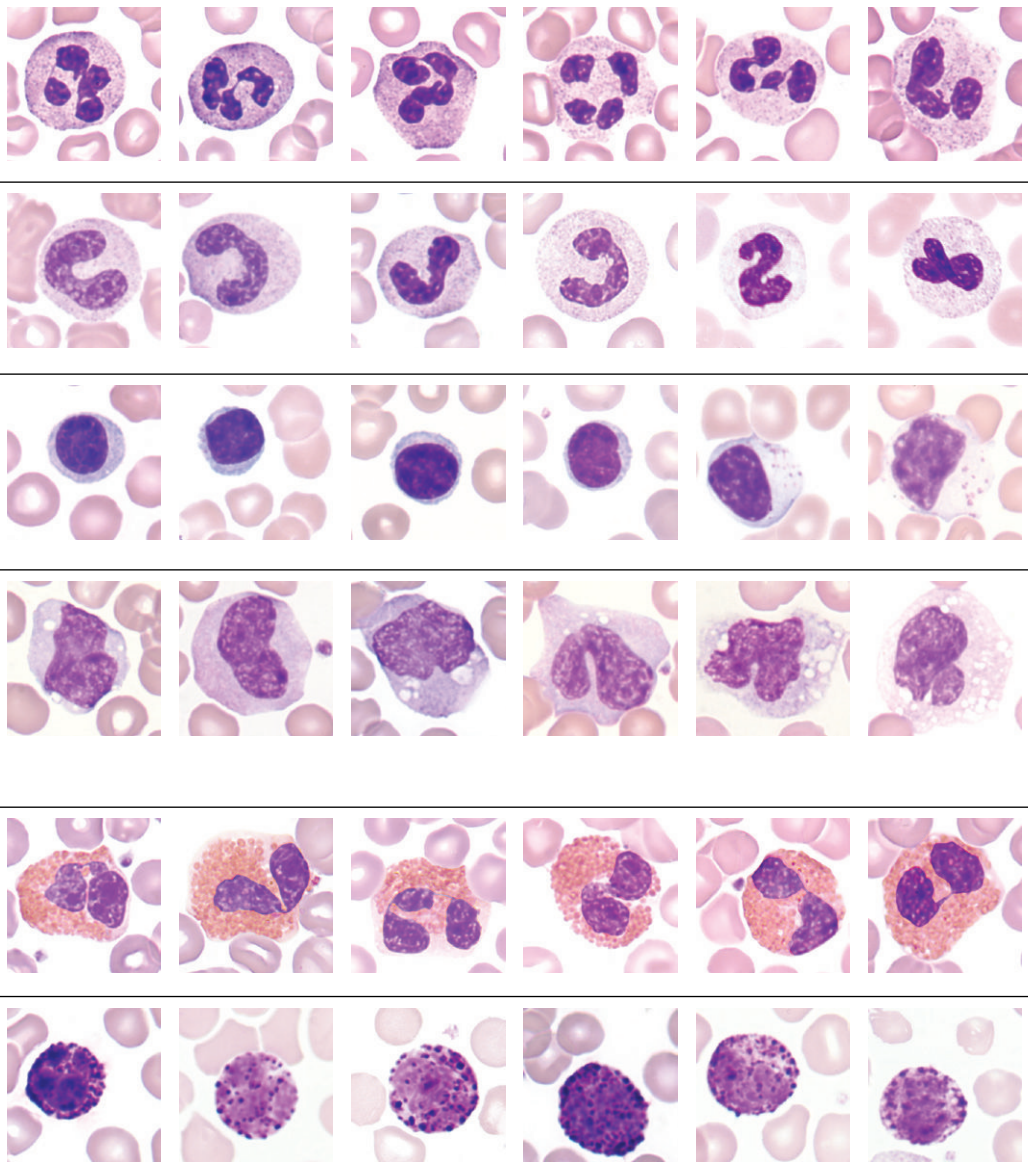
FIGURE 1–6 "Battlement" pattern for performing a white blood cell differential. (From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.)

TABLE 1-1 Cells Found in a Normal White Blood Cell Differential

Cell Type	Cell Size (μm)	Nucleus	Chromatin	Cytoplasm	Granules	*Adult Reference Range*	
						Peripheral Blood (%)	Cells × 10^9/L
Segmented neutrophil (Seg), polymorphonuclear neutrophil (Poly, PMN)	10-15	2-5 lobes connected by thin filaments without visible chromatin	Coarsely clumped	Pale pink, cream colored, or colorless	1°: Rare 2°: Abundant	50-70	2.3-8.1
Band neutrophil (Band)	10-15	Constricted, but chromatin must be visible within the thinnest part	Coarsely clumped	Pale blue to pink	1°: Few 2°: Abundant	0-5	0.0-0.6
Lymphocyte (Lymph)	7-18*	Round to oval; may be slightly indented; occasional nucleoli	Condensed to deeply condensed	Scant to moderate; sky blue	± Few azurophilic	20-40	0.8-4.8
Monocyte (Mono)	12-20	Variable; may be round, horseshoe, or kidney shaped; often has folds producing "brainlike" convolutions	Moderately clumped; lacy	Blue-gray; may have pseudopods; vacuoles may be absent or numerous	Many fine granules, frequently giving the appearance of ground glass	3-11	0.5 to 1.3
Eosinophil (Eos)	12-17	2-3 lobes connected by thin filaments without visible chromatin	Coarsely clumped	Cream to pink; may have irregular borders	1°: Rare 2°: Abundant red to orange, round	0-5	0.0-0.4
Basophil (Baso)	10-14	Usually two lobes connected by thin filaments without visible chromatin	Coarsely clumped	Lavender to colorless	1°: Rare 2°: Lavender to, dark purple; variable in number with uneven distribution; may obscure nucleus or wash out during staining, giving the appearance of empty areas in cytoplasm	0-1	0.0-0.1

*The difference in size from small to large lymphocyte is primarily due to a larger amount of cytoplasm. See Chapter 9 for more detailed information on lymphocyte size.

1^o = primary, 2^o = secondary.



the estimate is consistent with a normal platelet count, decreased if below the lower limit of normal for that laboratory, and increased if above the upper limit of normal. A general reference range is 150,000 to 450,000/mm^3 (150–450 $\times$ 10^9/L). When a patient is extremely anemic or has erythrocytosis, a more involved formula for platelet estimates may be used:

$$\frac{\text{Average number of platelets/field} \times \text{Total RBC count}}{\text{200 RBCs/field}}$$

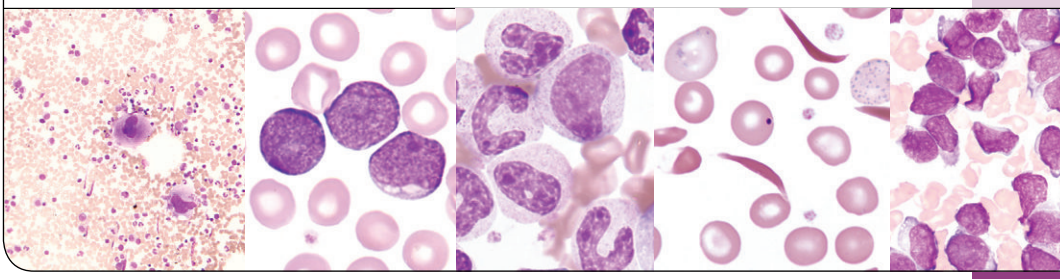
The estimate can be compared with an automated platelet count as an additional quality-control measure. If the estimate and the instrument platelet count do not agree, discrepancies must be resolved. Some causes for discrepancy include the presence of giant platelets, many schistocytes, and platelet satellitism. Notably, high-quality 40$\times$ or 50$\times$ oil immersion objectives can be used by the experienced technologist to perform the differential analysis of the blood smear. However, all abnormal findings must be verified under the 100$\times$ objective.

SUMMARY

A considerable amount of valuable information can be obtained from properly prepared, stained, and evaluated peripheral blood smears. Many laboratories use smears made by the wedge technique from EDTA anticoagulated blood and stained with Wright or Wright-Giemsa stain. The smears should be evaluated in a systematic manner using first the 10$\times$, then 40$\times$ high dry or 50$\times$ oil, and finally the 100$\times$ oil immersion objectives on the microscope. WBC differential and morphology and the RBC morphology and platelet estimate are included in the smear evaluation.

2

HEMATOPOIESIS



Hematopoiesis is a vigorous process of blood cell production and maturation that in the adult occurs primarily in the bone marrow. The process begins with the pluripotential hematopoietic stem cell (multipotent progenitor), which is capable of proliferation, replication, and differentiation. In response to cytokines (growth factors), the pluripotential stem cell will differentiate into a common myeloid or common lymphoid progenitor. Both the myeloid and lymphoid progenitors maintain their pluripotential capacity. The lymphoid progenitor proliferates and differentiates into T, B, and natural killer cells. The myeloid progenitor proliferates and differentiates into granulocyte, monocyte, erythrocyte, and megakaryocyte lineages. To this point in maturation, none of these stem cells can be morphologically identified, although it is postulated that they appear similar to a small resting lymphocyte. The blue shaded area in Figure 2-1 highlights the stem cell populations. Each lineage and maturation stage will be presented in detail in the following chapters.

Hematopoiesis is a dynamic continuum, that is, cells gradually mature from one stage to the next and may be between stages when viewed through the microscope. In general, the cell is then identified as the more mature stage. General morphological changes in blood cell maturation are demonstrated in Figure 2-2.

Figures 2-3 and 2-4 illustrate cell ultrastructure. A review of organelles will facilitate correlation of morphological maturation with cell function. This topic is explored in depth in hematology textbooks. Table 2-1 delineates the location, appearance, and function of individual organelles.

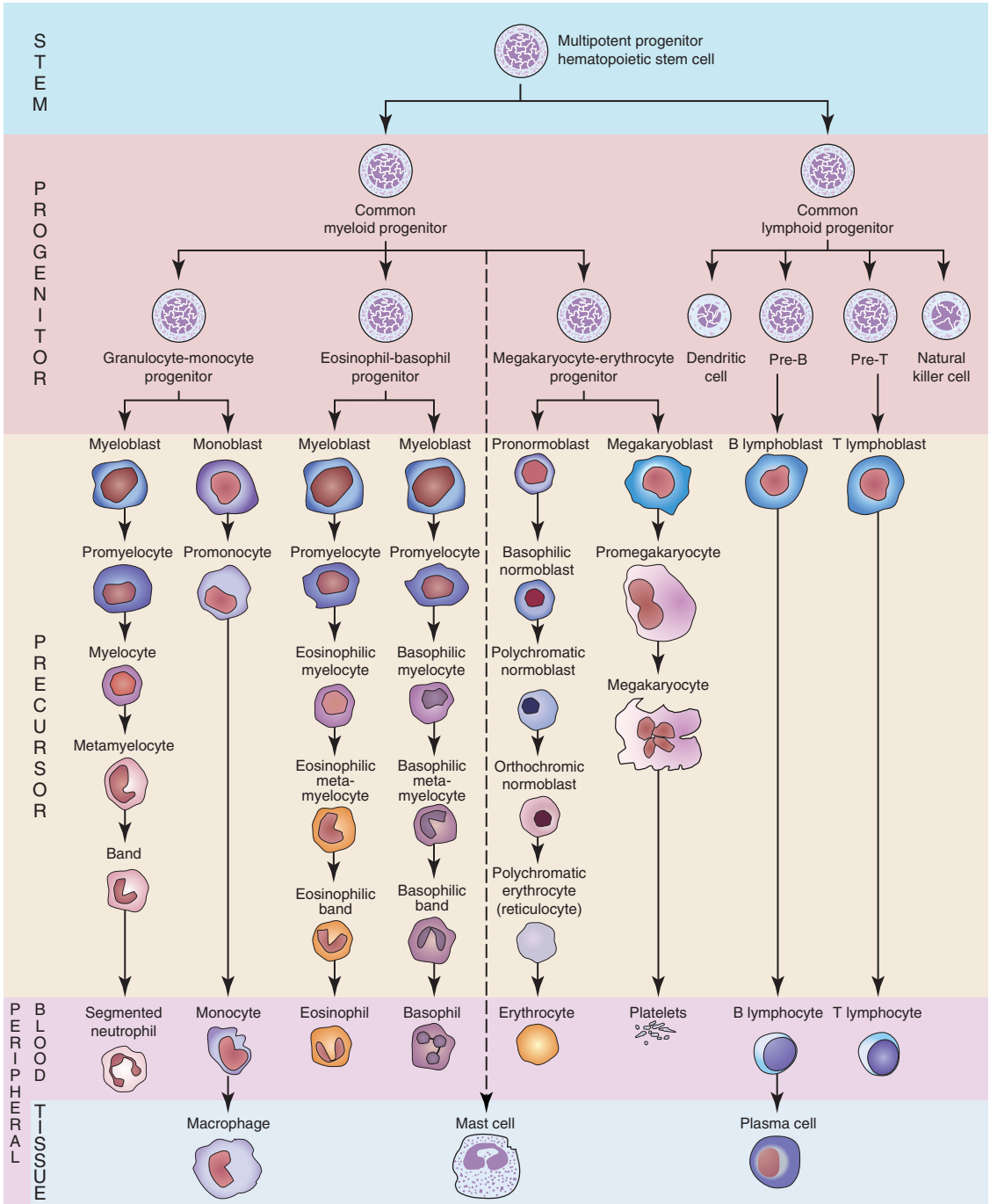


FIGURE 2–1 Chart of hematopoiesis.

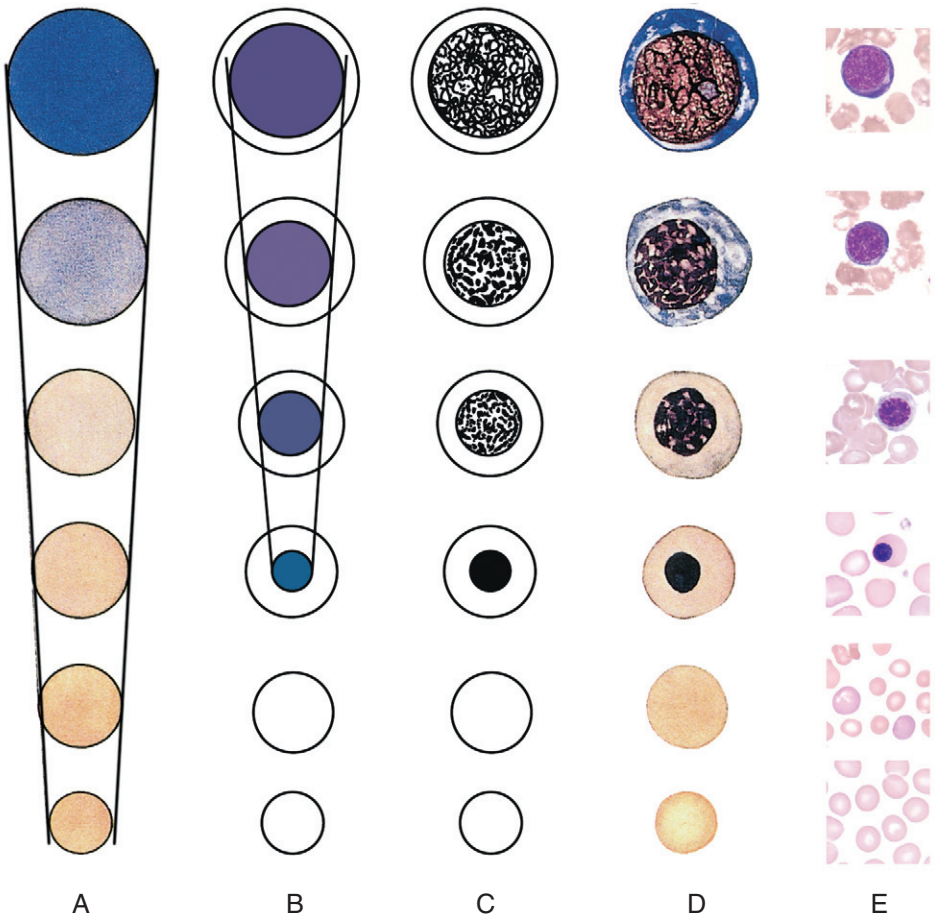


FIGURE 2–2 General trends that affect the morphology of blood cells during the developmental process.
A, Cell diameter decreases and cytoplasm becomes less basophilic.

- An exception to the diameter decreasing is that in the granulocytic series, the promyelocyte may be larger than its precursor, the myeloblast (see Chapter 5).
- In the erythroid series, hemoglobin development in the cytoplasm imparts a pink/salmon color.

B, Nuclear diameter decreases (N:C ratio decreases). Nuclear color changes from purplish red to dark blue.
C, Nuclear chromatin becomes coarser, clumped, and condensed.

- Nucleoli disappear.
- In the granulocytic series, the nuclear shapes changes and the nucleus becomes segmented. Granules appear in cytoplasm (see Chapter 5).
- In the erythroid series, the nucleus becomes fully condensed and is ejected.

D, Composite of changes during maturation process.
E, Representative cells from the erythroid series, demonstrating maturation changes.
(Modified from Diggs LW, Sturm D, Bell A: *The morphology of human blood cells*, ed 5, Abbott Park, Ill, 1985, Abbott Laboratories. Reproduction of *The Morphology of Human Blood Cells* has been granted with approval of Abbott Laboratories, all rights reserved by Abbott Laboratories.)

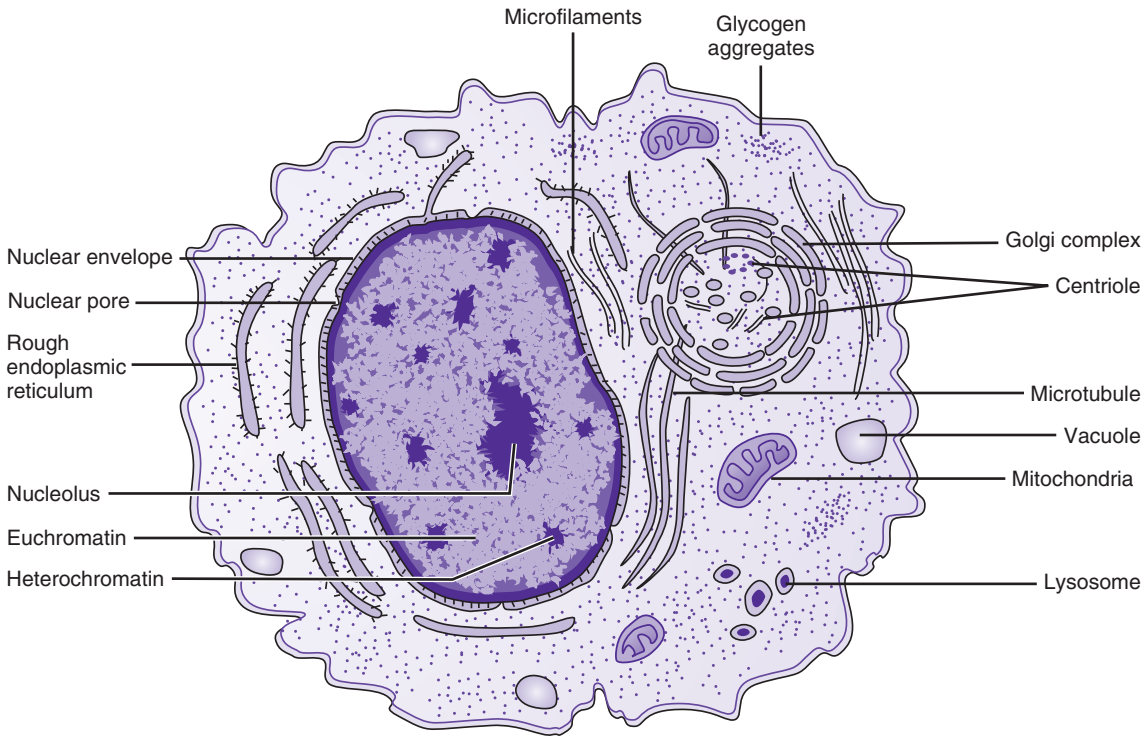


FIGURE 2–3 Schematic of electron micrograph. (From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.)

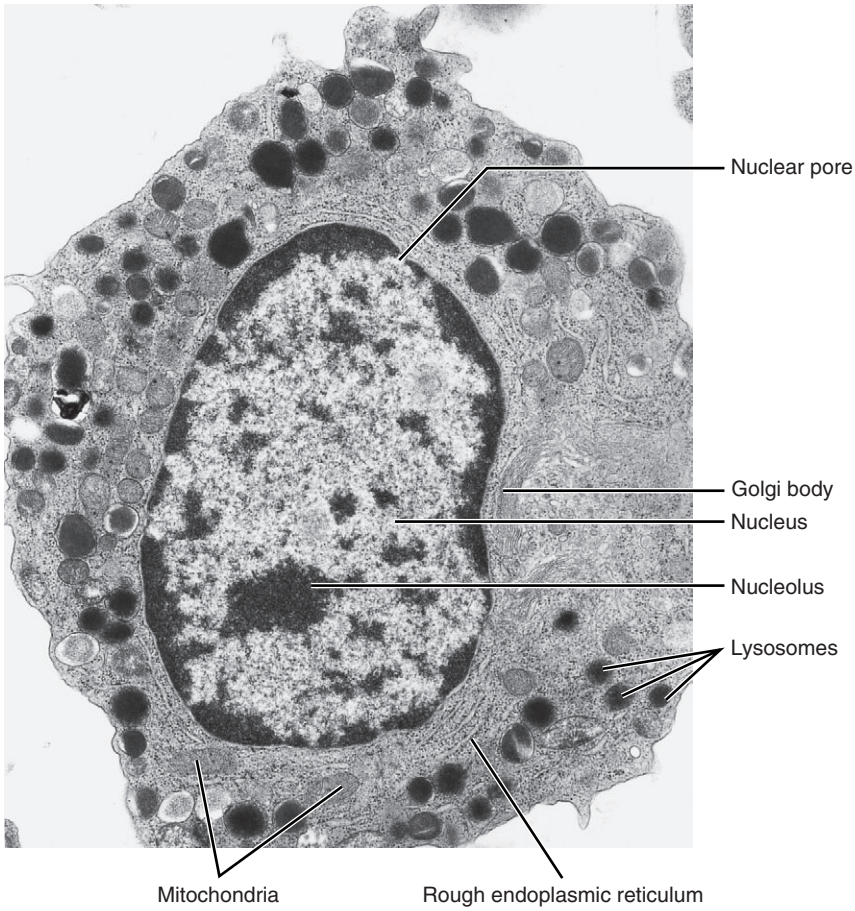


FIGURE 2–4 Electron micrograph with labeled organelles.

TABLE 2-1 Summary of Cellular Components and Function

Organelle	Location	Appearance and Size	Function	Comments
Membranes: plasma, nuclear, mitochondrial, endoplasmic reticulum	Outer boundary of cell, nucleus, endoplasmic reticulum, mitochondria, and other organelles	Usually a lipid bilayer consisting of proteins, cholesterol, phospholipids, and polysaccharides; membrane thickness varies with cell or organelle	Separates various cellular components; facilitates and restricts cellular exchange of substances	Membrane must be resilient and flexible
Nucleus	Within cell	Usually round or oval but varies depending on cell; varies in size; composed of DNA	Control center of cell containing the genetic blueprint	Governs cellular activity and transmits information for cellular control
Nucleolus	Within nucleus	Usually round or irregular in shape; 2-4 μm in size; composed of RNA; there may be 1-4 within nucleus	Site of synthesis and processing of ribosomal RNA	Appearance varies with activity of the cells; larger when cell is actively involved in protein synthesis
Golgi body	Next to nucleus	System of stacked, membrane-bound, flattened sacs; horseshoe shaped; varies in size	Involved in modifying and packaging macromolecules for secretion	Well developed in cells with large secretion responsibilities
Endoplasmic reticulum	Randomly distributed throughout cytoplasm	Membrane-lined tubules that branch and connect to nucleus and plasma membrane	Stores and transports fluids and chemicals	Two types: smooth with no ribosomes, rough with ribosomes on the surface
Ribosomes	Free in cytoplasm; outer surface of rough endoplasmic reticulum	Small granule, 100-300 Å; composed of protein and nucleic acid	Site of production of proteins, such as enzymes and blood proteins	Large proteins are synthesized from polyribosomes (chains of ribosomes)
Mitochondria	Randomly distributed in cytoplasm	Round or oval structures; 3-14 nm in length; 2-10 nm in width; membrane has two layers; inner layer has folds called *cristae*	Cell's "powerhouse"; make ATP, the energy source for the cell	Active cells have more present than do inactive ones

Continued

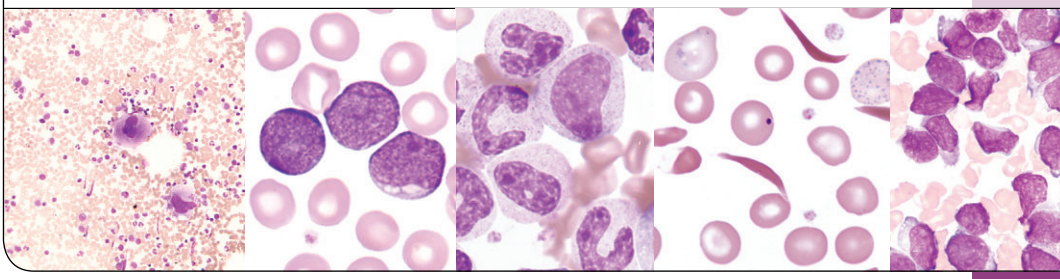
TABLE 2-1 Summary of Cellular Components and Function—cont'd

Organelle	Location	Appearance and Size	Function	Comments
Lysosomes	Randomly distributed in cytoplasm	Membrane-bound sacs; size varies	Contain hydrolytic enzymes for cellular digestive system	If the membrane breaks, hydrolytic enzymes can destroy the cell
Microfilaments	Near nuclear envelope and within proximity of mitotic process	Small, solid structure approximately 5 nm in diameter	Support cytoskeleton and motility	Consist of actin and myosin (contractile proteins)
Microtubules	Cytoskeleton, near nuclear envelope and component part of centriole near Golgi body	Hollow cylinder with protofilaments surrounding the outside tube; 20-25 nm in diameter, variable length	Maintains cell shape, motility, and mitotic process	Produced from tubulin polymerization; make up mitotic spindles and part of structure of centriole
Centriole	In centrosome near nucleus	Cylinders; 150 nm in diameter, 300-500 nm in length	Serves as insertion point for mitotic spindle fibers	Composed of nine sets of triplet microtubules

From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.
ATP, Adenosine triphosphate.

3

ERYTHROCYTE MATURATION



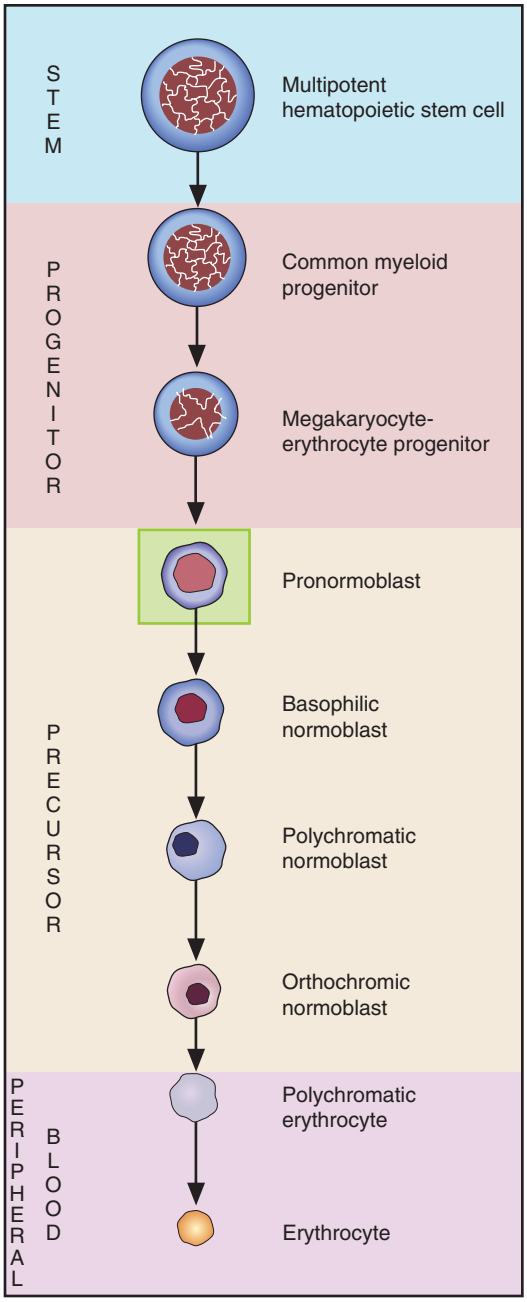


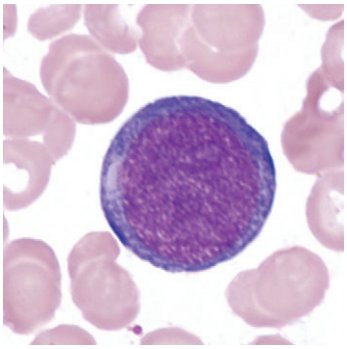
FIGURE 3–1 Erythrocyte sequence—pronormoblast.

All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

PRONORMOBLAST

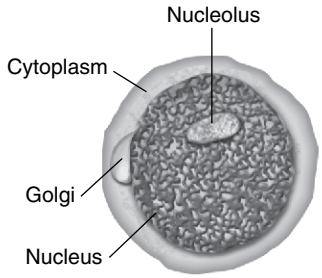
Proerythroblast

Rubriblast



A

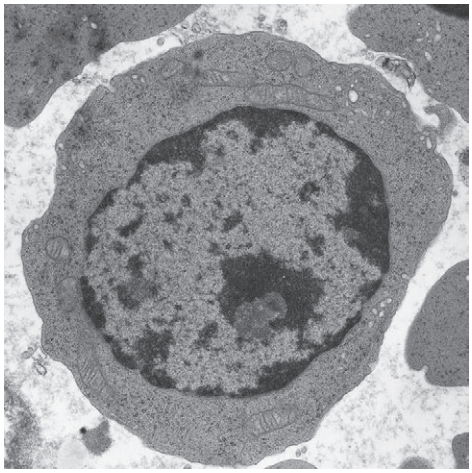
FIGURE 3–2A Pronormoblast.



B

FIGURE 3–2B Schematic of pronormoblast.

SIZE: 12-20 μm
NUCLEUS: Round to slightly oval
Nucleoli: 1-2
Chromatin: Fine
CYTOPLASM: Dark blue; may have prominent Golgi
N/C RATIO: 8:1
REFERENCE INTERVAL:
Bone Marrow: 1%
Peripheral Blood: 0%



C

FIGURE 3–2C Electron micrograph of pronormoblast (×15,575).

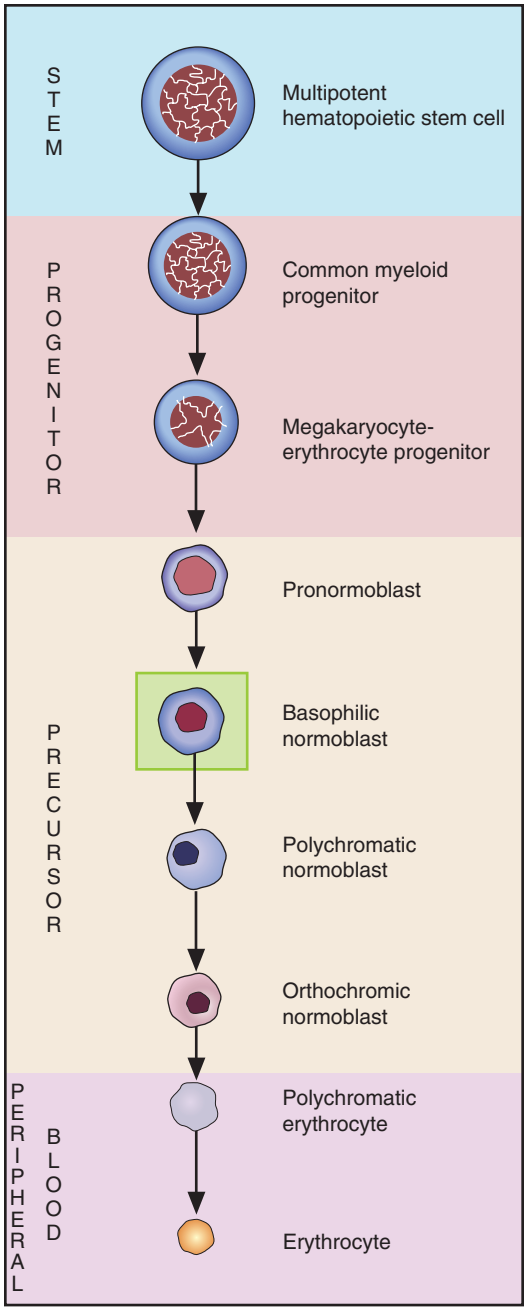
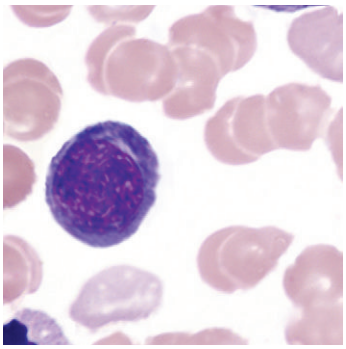


FIGURE 3–3 Erythrocyte sequence—basophilic normoblast.

BASOPHILIC NORMOBLAST

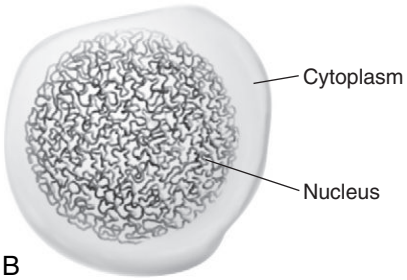
Basophilic Erythroblast

Prorubricyte



A

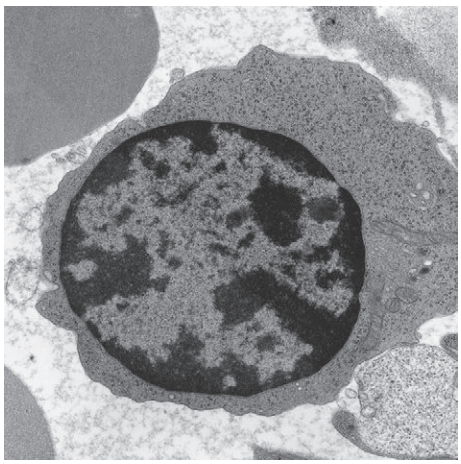
FIGURE 3–4A Basophilic normoblast.



B

FIGURE 3–4B Schematic of basophilic normoblast.

SIZE: 10-15 μm
NUCLEUS: Round to slightly oval
Nucleoli: 0-1
Chromatin: Slightly condensed
CYTOPLASM: Dark blue
N/C RATIO: 6:1
REFERENCE INTERVAL:
Bone Marrow: 1% to 4%
Peripheral Blood: 0%



C

FIGURE 3–4C Electron micrograph of basophilic normoblast (×15,575).

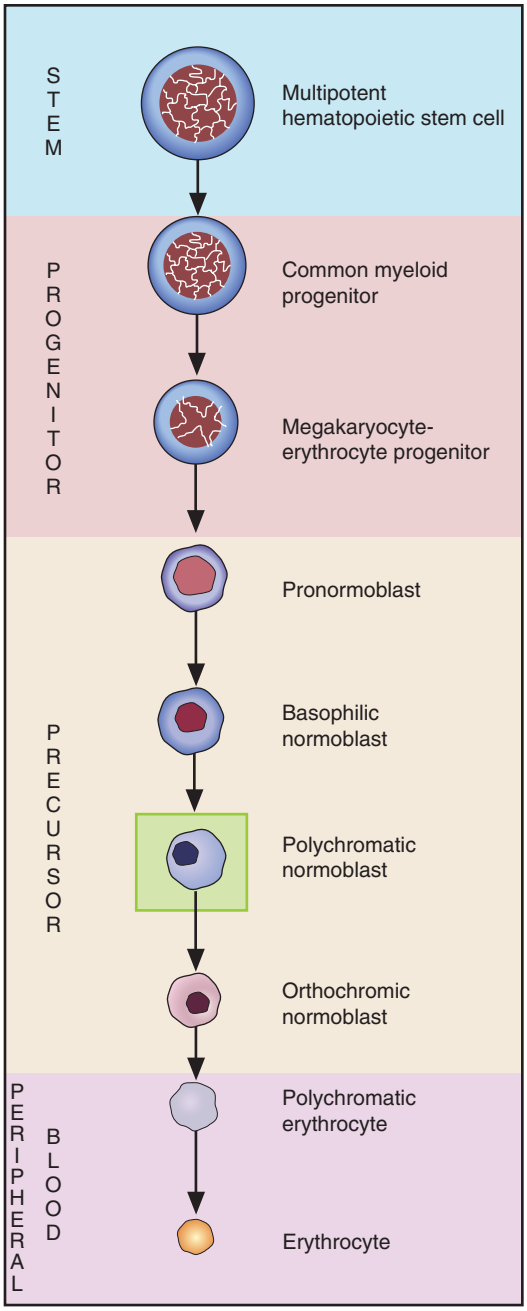
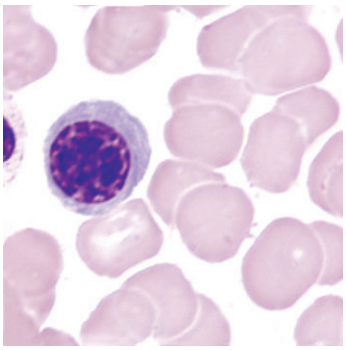


FIGURE 3–5 Erythrocyte sequence—polychromatic normoblast.

POLYCHROMATIC NORMOBLAST

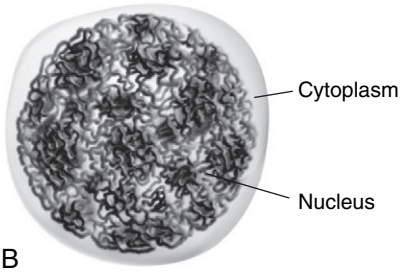
Polychromatic Erythroblast

Rubricyte



A

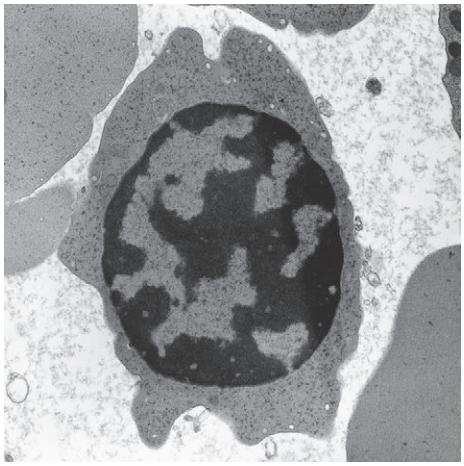
FIGURE 3–6A Polychromatic normoblast. The blue color of the cytoplasm is becoming gray-blue as hemoglobin is produced.



B

FIGURE 3–6B Schematic of polychromatic normoblast.

SIZE: 10-12 μm
NUCLEUS: Round
Nucleoli: None
Chromatin: Quite condensed
CYTOPLASM: Gray-blue as a result of hemoglobinization
N/C RATIO: 4:1
REFERENCE INTERVAL:
Bone Marrow: 10% to 20%
Peripheral Blood: 0%



C

FIGURE 3–6C Electron micrograph of polychromatic normoblast (×15,575).

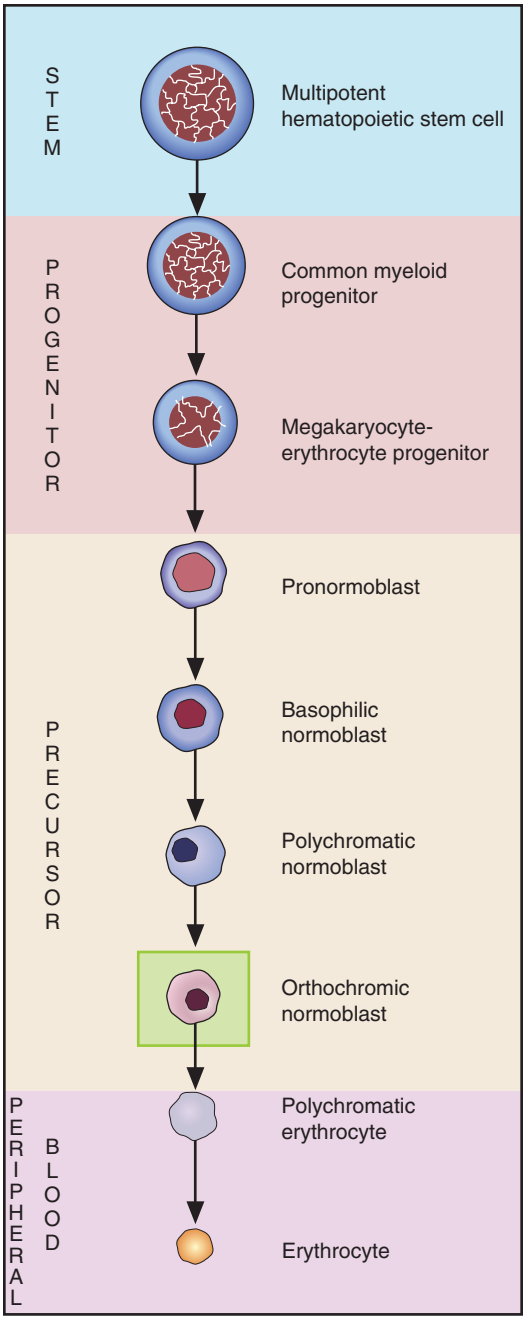
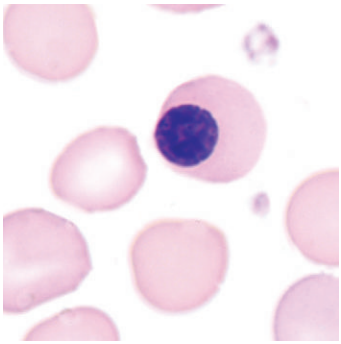


FIGURE 3–7 Erythrocyte sequence—orthochromic normoblast.

ORTHOCHROMIC NORMOBLAST

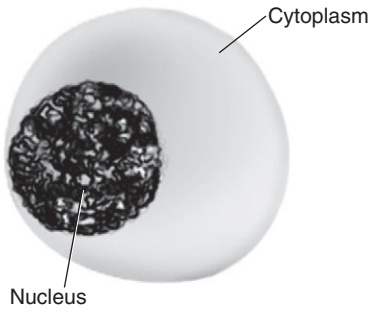
Orthochromic Erythroblast

Metarubricyte



A

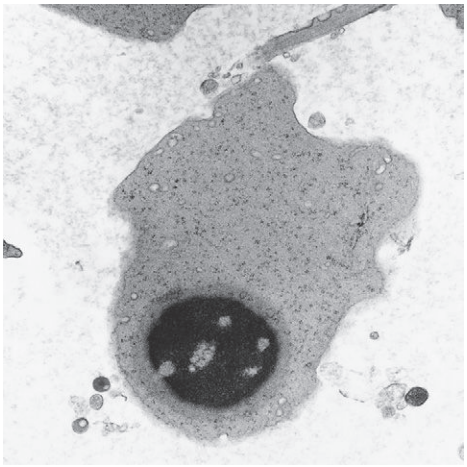
FIGURE 3–8A Orthochromic normoblast. The gray-blue color of the cytoplasm is becoming salmon as more hemoglobin is produced.



B

FIGURE 3–8B Schematic of orthochromic normoblast.

SIZE: 8-10 μm
NUCLEUS: Round
Nucleoli: 0
Chromatin: Fully condensed
CYTOPLASM: More pink or salmon than blue
N/C RATIO: 0.5:1
REFERENCE INTERVAL:
Bone Marrow: 5% to 10%
Peripheral Blood: 0%



C

FIGURE 3–8C Electron micrograph of orthochromic normoblast (×20,125).

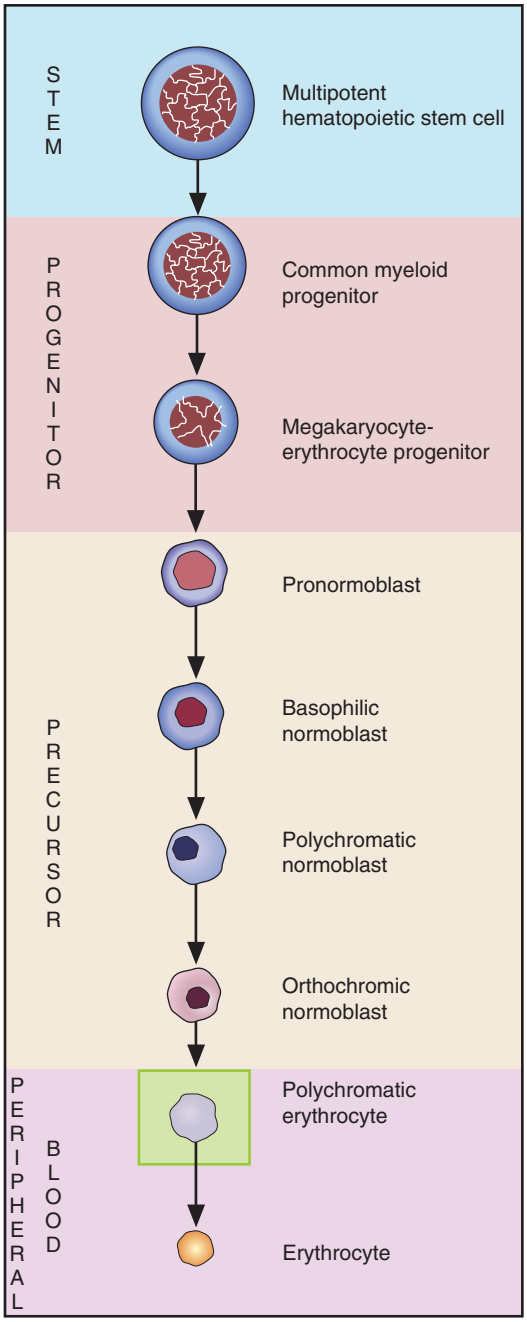
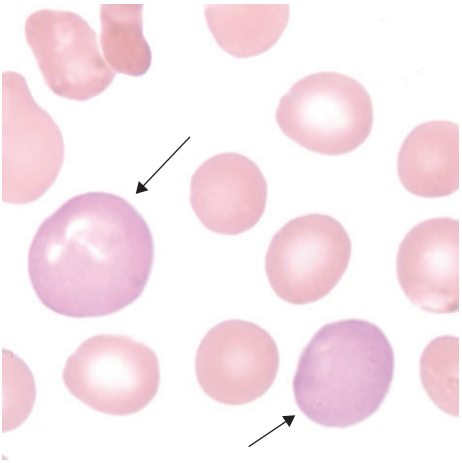


FIGURE 3–9 Erythrocyte sequence—polychromatic erythrocyte (reticulocyte).

POLYCHROMATIC ERYTHROCYTE

Diffusely Basophilic Erythrocyte

Reticulocyte



A

FIGURE 3–10A Polychromatic erythrocyte. Sometimes appears "lumpy." Slight gray-blue color persists while the cell attains full hemoglobinization.

B

FIGURE 3–10B Scanning electron micrograph of polychromatic erythrocyte (×5000). Note that the reticulocyte is a very deformable cell, giving it a "lumpy" appearance by scanning electron microscopy.

SIZE: 8-8.5 μm
NUCLEUS: Absent
Nucleoli: NA
Chromatin: NA
CYTOPLASM: Color is slightly more blue/purple than the mature erythrocyte
N/C RATIO: NA
REFERENCE INTERVAL:
Bone Marrow: 1%
Peripheral Blood: 0.5% to 2.0%
NOTE: When stained with supravital stain (e.g., new methylene blue), polychromatic erythrocytes appear as reticulocytes (contain precipitated ribosomal material; see Figure 12-5A).

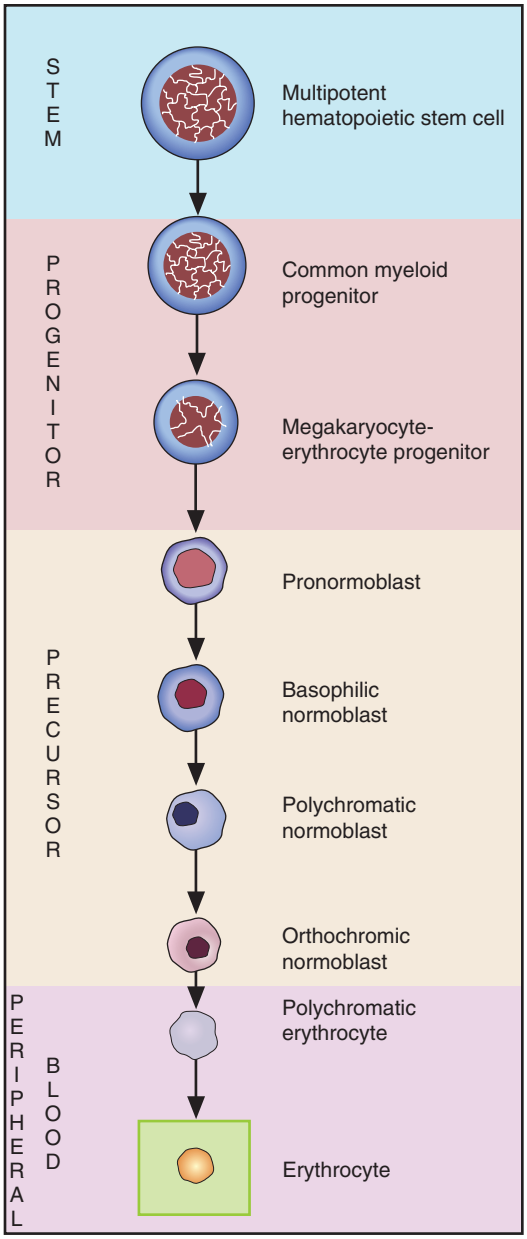
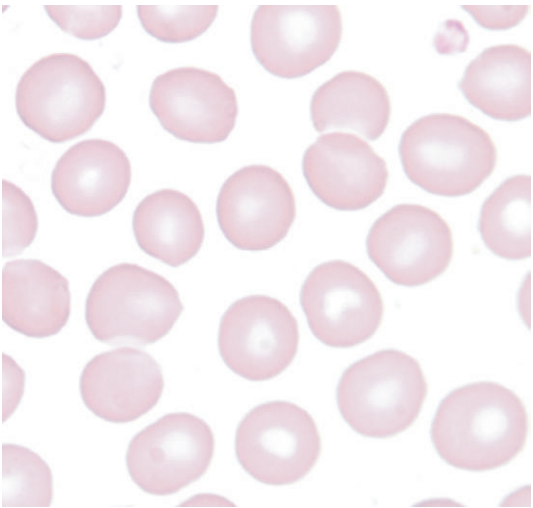


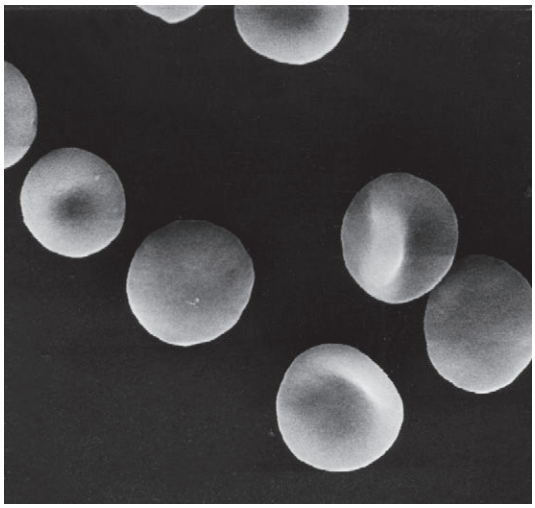
FIGURE 3–11 Erythrocyte sequence—erythrocyte.

ERYTHROCYTE



A

FIGURE 3–12A Erythrocyte. The mature erythrocyte has lost the blue-gray color and is salmon colored as hemoglobinization is complete.



B

FIGURE 3–12B Scanning electron micrograph of erythrocyte (×2500).

SIZE: 7-8 μm
NUCLEUS: Absent
Nucleoli: NA
Chromatin: NA
CYTOPLASM: Salmon with central pallor of about one-third of the diameter of the cell
N/C RATIO: NA
REFERENCE INTERVAL:
Bone Marrow: NA
Peripheral Blood: Predominant cell type

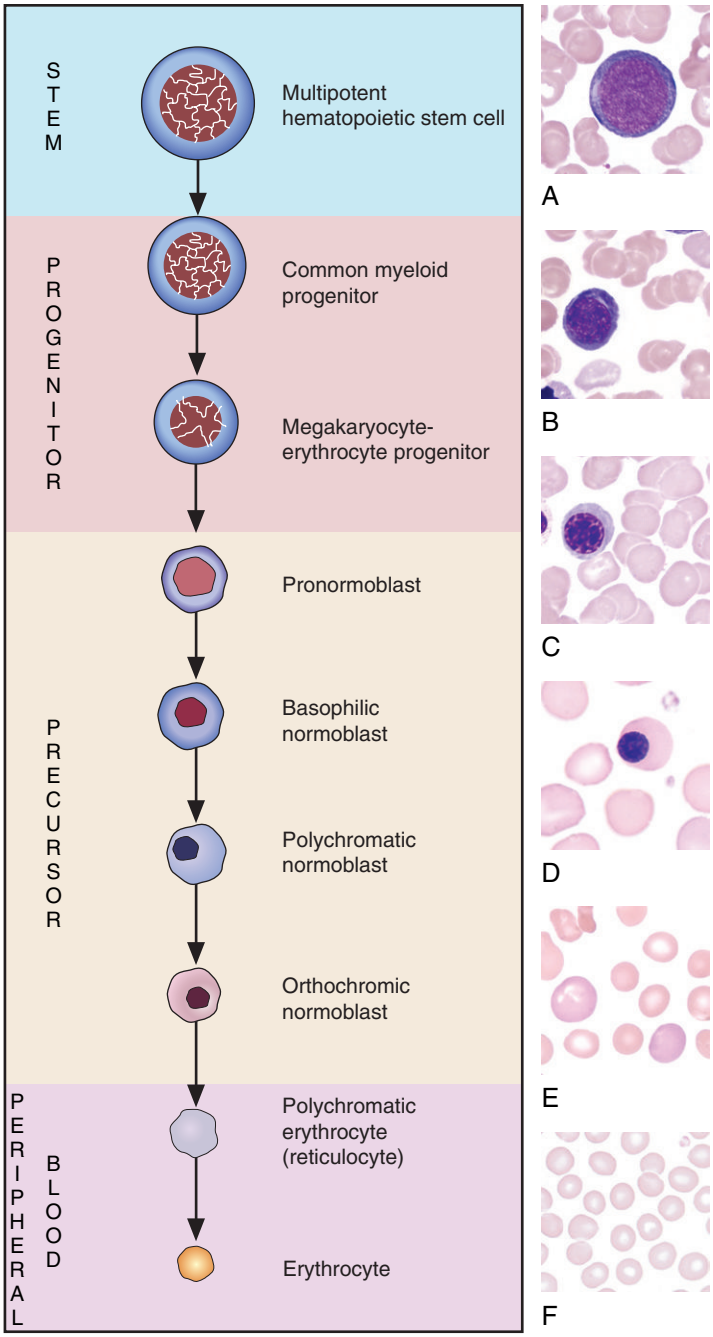
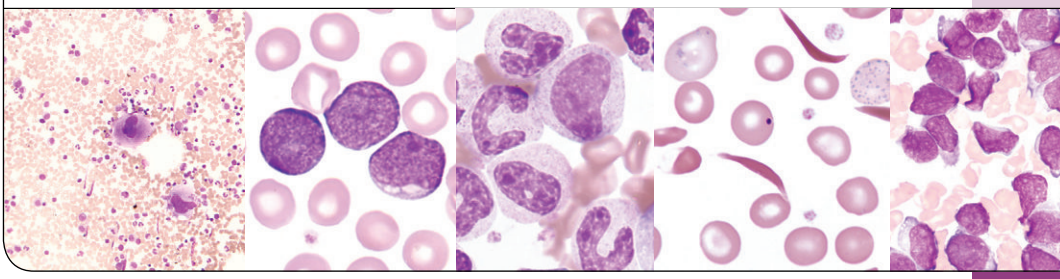


FIGURE 3–13 Erythrocyte sequence with **(A)** pronormoblast, **(B)** basophilic normoblast, **(C)** polychromatic normoblast, **(D)** orthochromic normoblast, **(E)** polychromatic erythrocyte, and **(F)** erythrocyte.

4

MEGAKARYOCYTE MATURATION



Platelets arise from the megakaryocyte. Megakaryocytes are among the largest cells in the body and mature by a unique process called *endomitosis.* In endomitosis, the nucleus is duplicated but there is no cell division, resulting in a polyploid cell. Megakaryocyte nuclei may have from 2 to 32 lobes and, in unusual cases, may have up to 64 lobes. Megakaryocytes develop copious cytoplasm, which differentiates into platelets. Platelets have several types of granules that can be visualized by electron microscopy. The granules are highly specialized. Refer to a hematology textbook for further discussion.

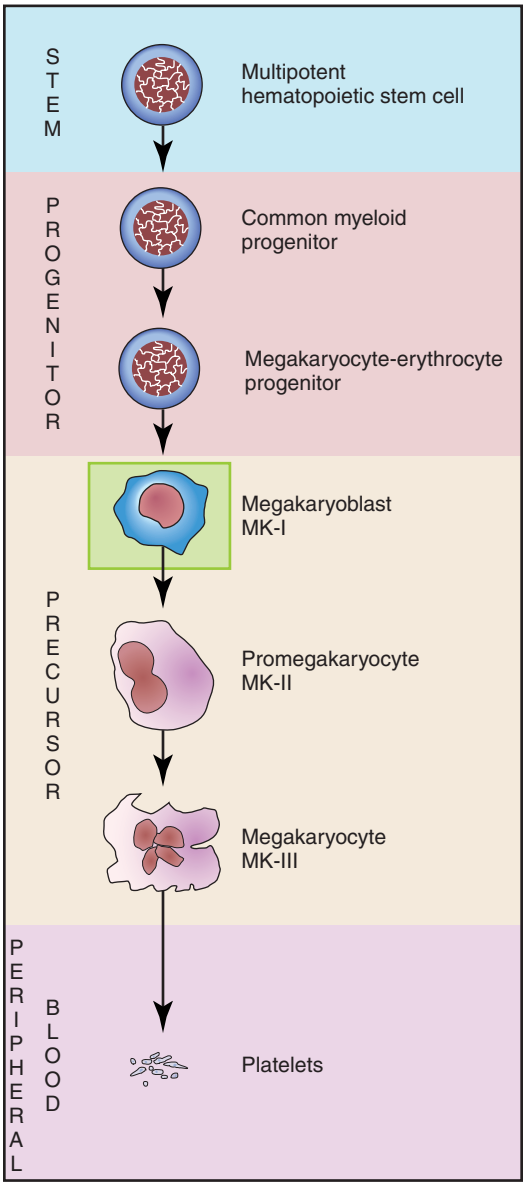
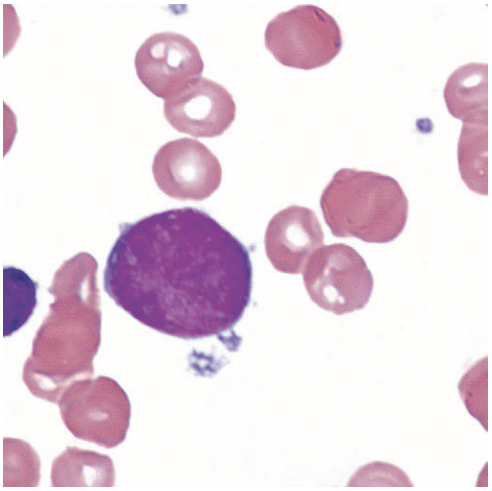


FIGURE 4–1 Megakaryocyte sequence—megakaryoblast MK-I.

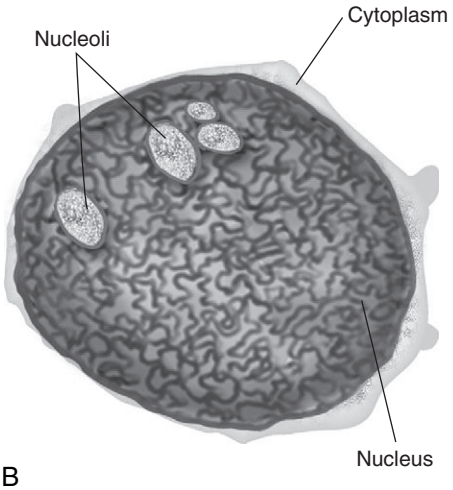
All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

MEGAKARYOBLAST (MK-I)



A

FIGURE 4–2A Megakaryoblast, MK-I—bone marrow (×1000).



B

FIGURE 4–2B Megakaryoblast, schematic.

SIZE: 10-24 μm
NUCLEUS: Round
Nucleoli: 2-6
Chromatin: Homogeneous, loosely organized
CYTOPLASM: Basophilic
Granules: Absent by Wright stain
N/C RATIO: 3:1
REFERENCE INTERVAL:
Bone Marrow: 20% of megakaryocyte precursors in bone marrow
Peripheral Blood: 0%

NOTE: The megakaryoblast appears similar to the myeloblast and pronormoblast, and identification by morphology alone is not advisable.

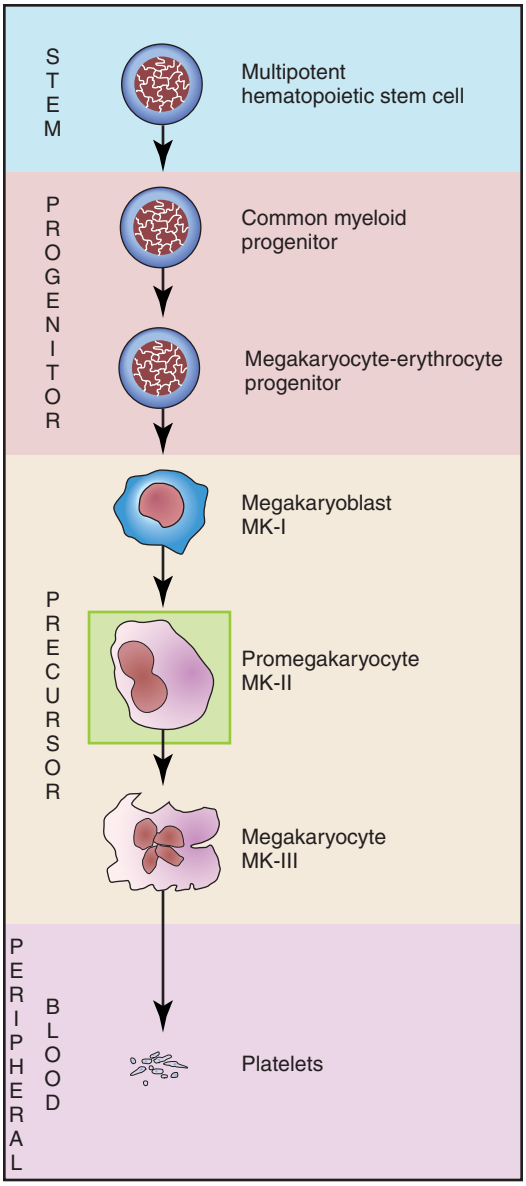
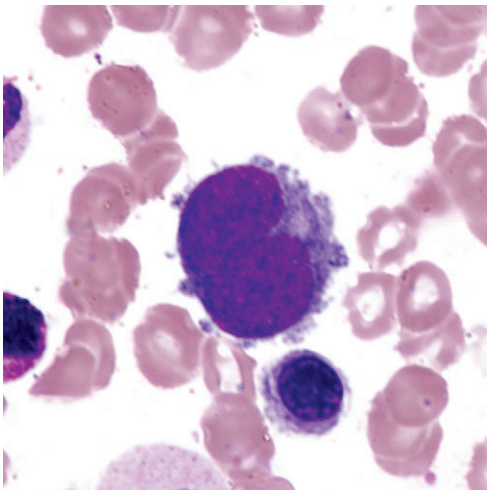


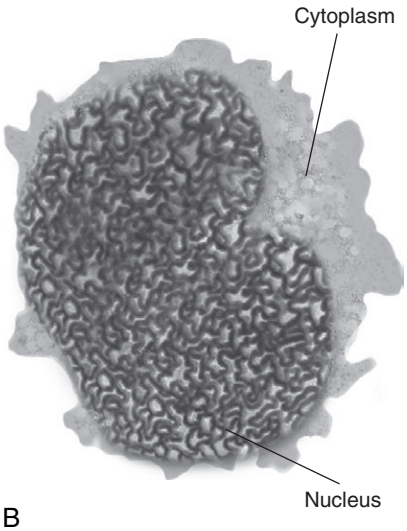
FIGURE 4–3 Megakaryocyte sequence—promegakaryocyte (MK-II).

PROMEGAKARYOCYTE (MK-II)



A

FIGURE 4–4A Promegakaryocyte, MK-II—bone marrow (×1000).



B

FIGURE 4–4B Promegakaryocyte, schematic.

SIZE: 15-40 μm
NUCLEUS: Indented
Nucleoli: Variable
Chromatin: Condensed
CYTOPLASM: Basophilic
Granules: Present
N/C RATIO: 1:2
REFERENCE INTERVAL:
Bone Marrow: 25% of megakaryocyte precursors in bone marrow
Peripheral Blood: 0%

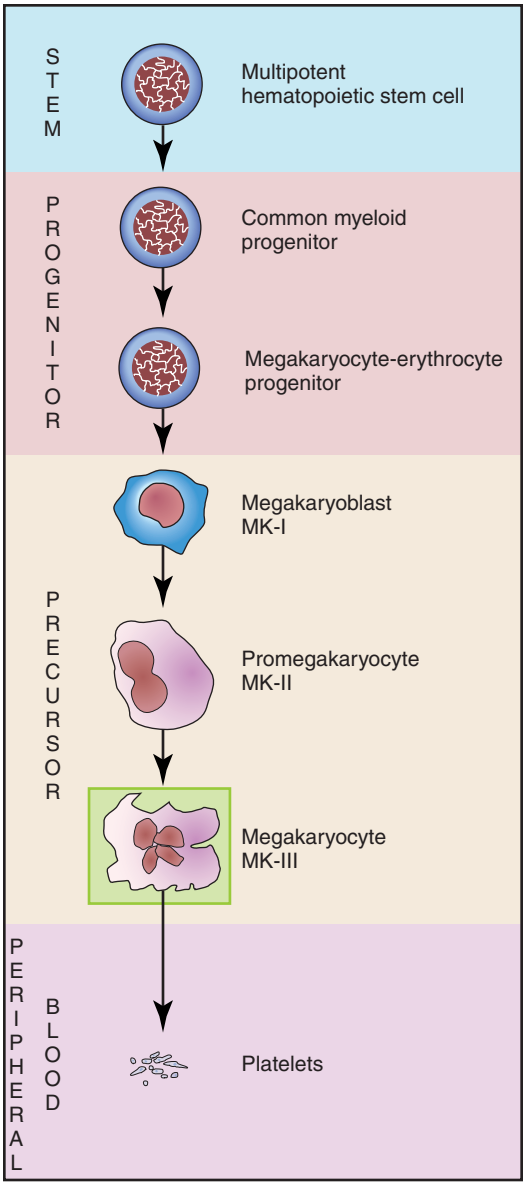
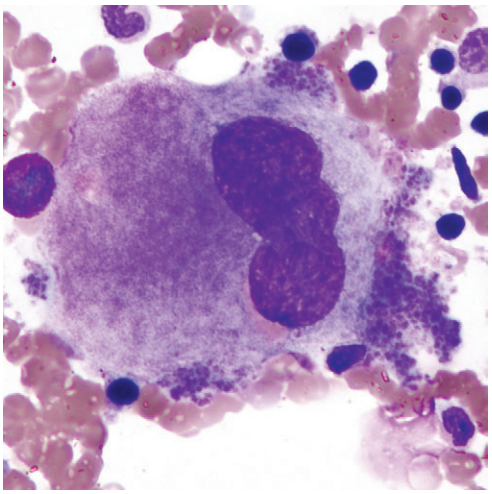


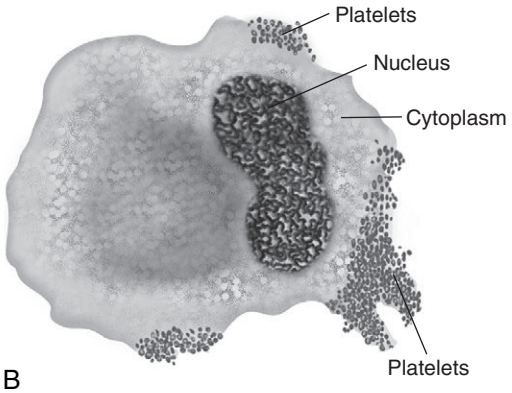
FIGURE 4–5 Megakaryocyte sequence—megakaryocyte (MK-III).

MEGAKARYOCYTE (MK-III)



A

FIGURE 4–6A Megakaryocyte, MK III—bone marrow (×500).



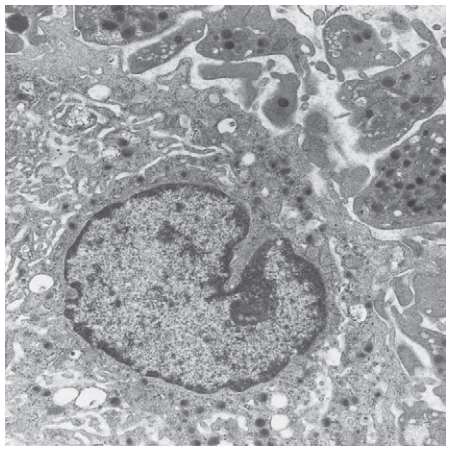
B

FIGURE 4–6B Megakaryocyte, MK-III, schematic.

SIZE: 20-90 μm
NUCLEUS: 2-32 lobes (8 lobes: most common)
NOTE: The size of the cell varies according to number of lobes present.
CYTOPLASM: Blue to pink; abundant
Granules: Reddish blue; few to abundant
N/C RATIO: Variable
REFERENCE INTERVAL:
Bone Marrow: 5-10 per 10× objective (×100 magnification)

1-2 per 50× objective (×500 magnification)

NOTE: Megakaryocytes are usually reported as adequate, increased, or decreased and not as a percentage.
Peripheral Blood: 0%



C

FIGURE 4–6C Electron micrograph of megakaryocyte (×16,500).

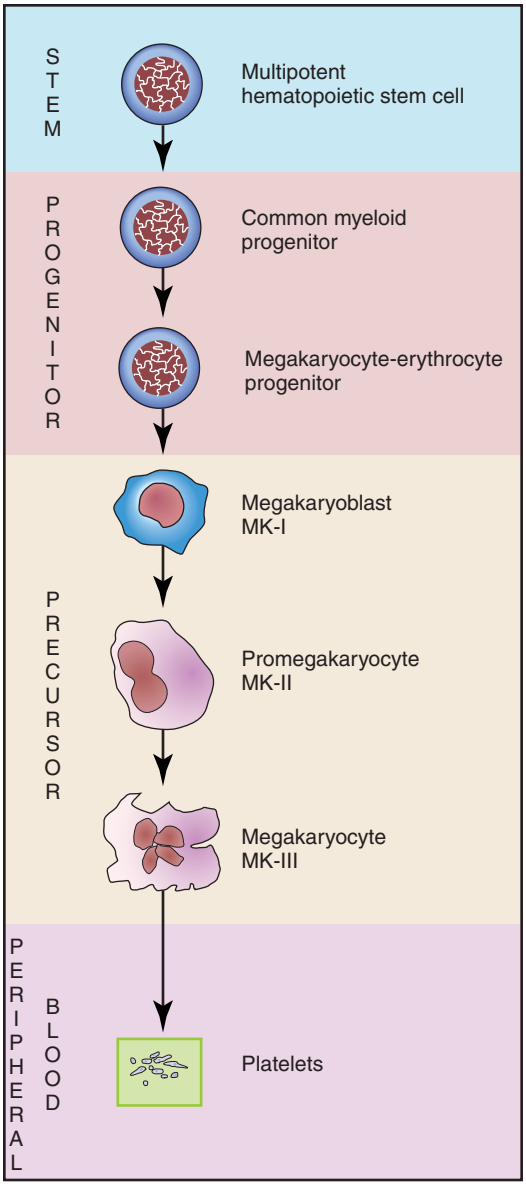
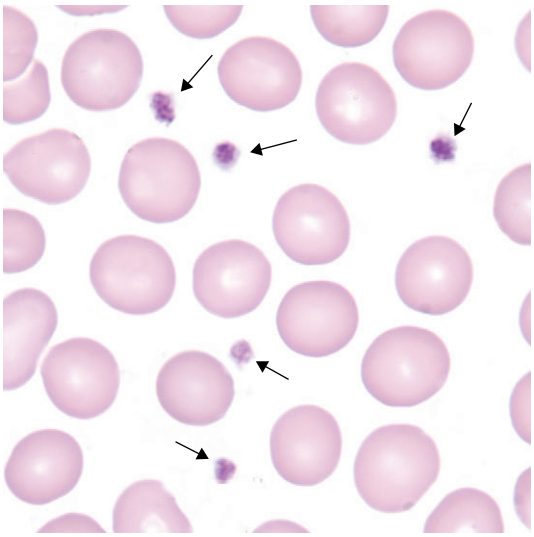


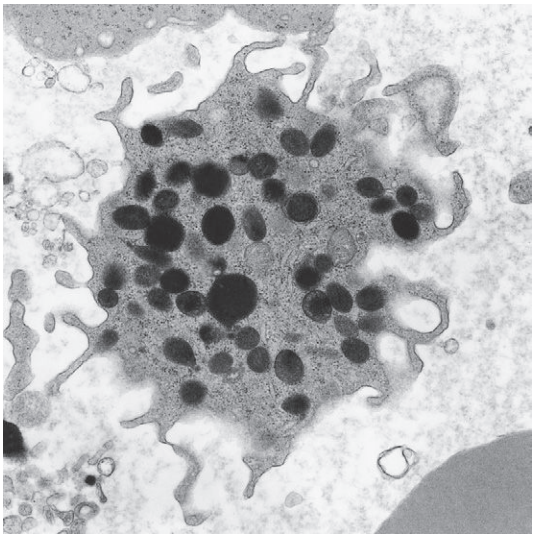
FIGURE 4–7 Megakaryocyte sequence—platelets.

PLATELET



A

FIGURE 4–8A Platelet—peripheral blood (×1000).



B

FIGURE 4–8B Electron micrograph of platelet (×28,750).

Size: 2-4 μm
Nucleus: NA
CYTOPLASM: Light blue to colorless
Granules: Red to violet, abundant
N/C RATIO: NA
REFERENCE INTERVAL:
Bone Marrow: NA
Peripheral Blood: 7-25 per 100× oil immersion field (×1000 magnification)

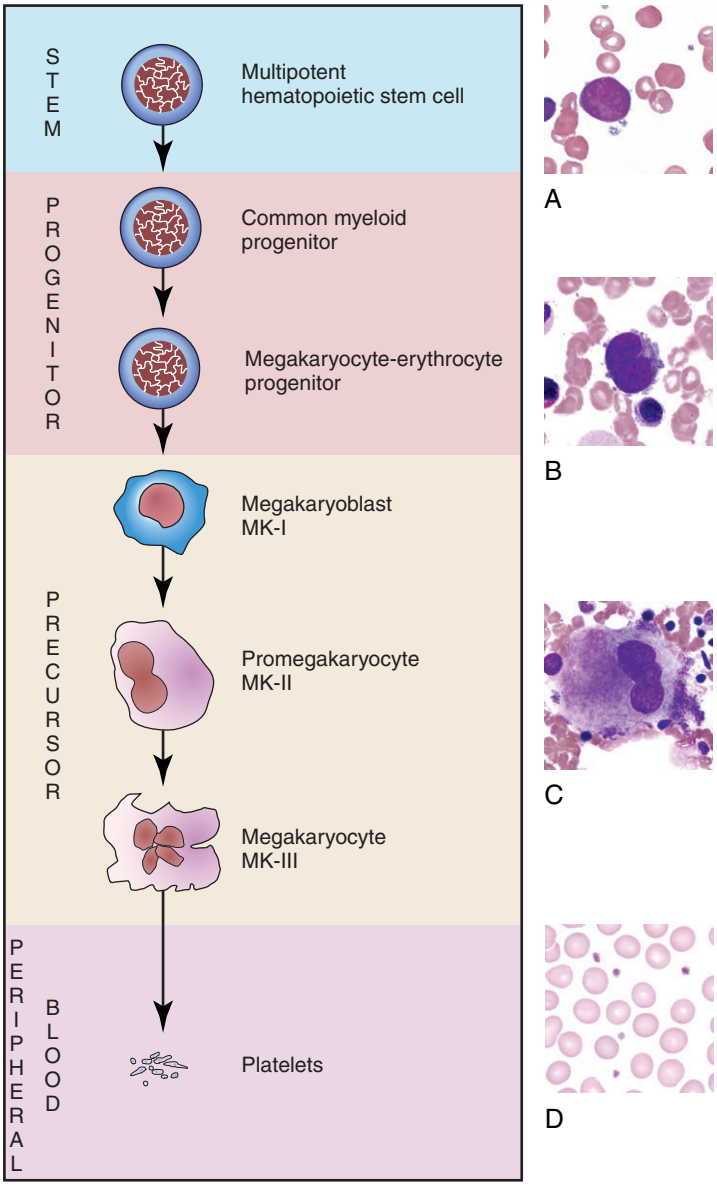
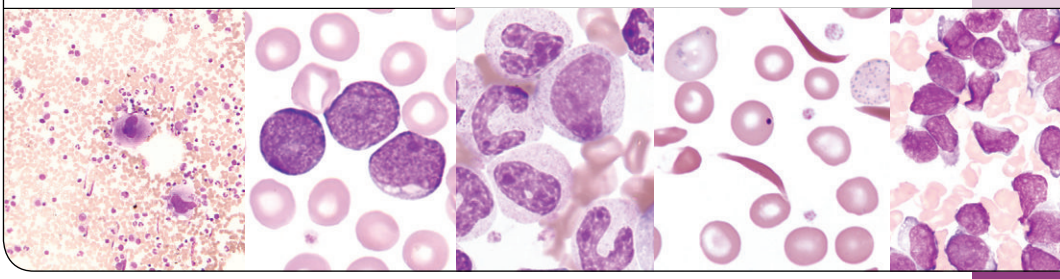


FIGURE 4–9 Megakaryocyte sequence with **(A)** megakaryoblast, **(B)** promegakaryocyte, **(C)** megakaryocyte, and (**D**) platelet.

5

NEUTROPHIL MATURATION



The common myeloid progenitor creates three types of progenitors: granulocytes/monocytes, eosinophils/basophils, and erythrocytes/megakaryocytes. Each of these divides and matures into cells known as blasts, one for each cell line. It is not possible at the light microscope level, however, to differentiate the various blasts. This chapter addresses neutrophil maturation. (See Chapter 7 for discussion of eosinophils and Chapter 8 for discussion of basophils.)

As the cells mature from the myeloblast to the promyelocyte, there is a slight increase in size, in contrast with size variation in other cell lineages. At the promyelocyte stage, the chromatin in the nucleus becomes slightly coarser than the myeloblast and primary burgundy-colored (azurophilic) granules appear in the cytoplasm. As the cell divides and matures to a myelocyte, chromatin becomes more coarse and condensed, and secondary (specific) granules appear in the cytoplasm beginning at the Golgi apparatus and spreading throughout the cytoplasm. Primary granules are still present but less visible on Wright stain because of a chemical change in their membranes. It is often possible at the myelocyte stage to see the area of the Golgi apparatus, which appears as a clearing close to the nucleus. The specific secondary granules differentiate the cell into neutrophil, eosinophil, and basophil. The nucleus then begins to indent and chromatin becomes coarser, signaling the metamyelocyte stage. In the metamyelocyte, the indentation of the nucleus is less than 50% of the hypothetical round nucleus. The cell is called a *band* when the nucleus becomes constricted without threadlike filaments and the indentation of the nucleus is more than 50% of the hypothetical round nucleus. Finally, the cell becomes a segmented neutrophil when the nucleus becomes segmented or lobated into two to five lobes. The lobes are connected by threadlike filaments with no chromatin visible in the filament. There is so much variability in the differentiation of band neutrophils from segmented neutrophils that the College of American Pathologists does not require that they be differentiated for proficiency testing.*

The dynamic nature of maturation is easily seen in the neutrophilic series; that is, cell maturation does not proceed in a stepwise fashion but occurs gradually from one stage to another. Thus morphologically, a cell may appear as a late promyelocyte or an early myelocyte. When there is a question of maturation stage, it is generally preferable to call the cell at the more mature stage.

All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

*The College of American Pathologists recommendations are available at: College of American Pathologists. Blood cell identification. In *2011 Hematology, clinical microscopy, and body fluids glossary.* Northfield, IL, 2011, College of American Pathologists. Available at: http://www.cap.org/apps/docs/proficiency_testing/2011_hematology_glossary.pdf.

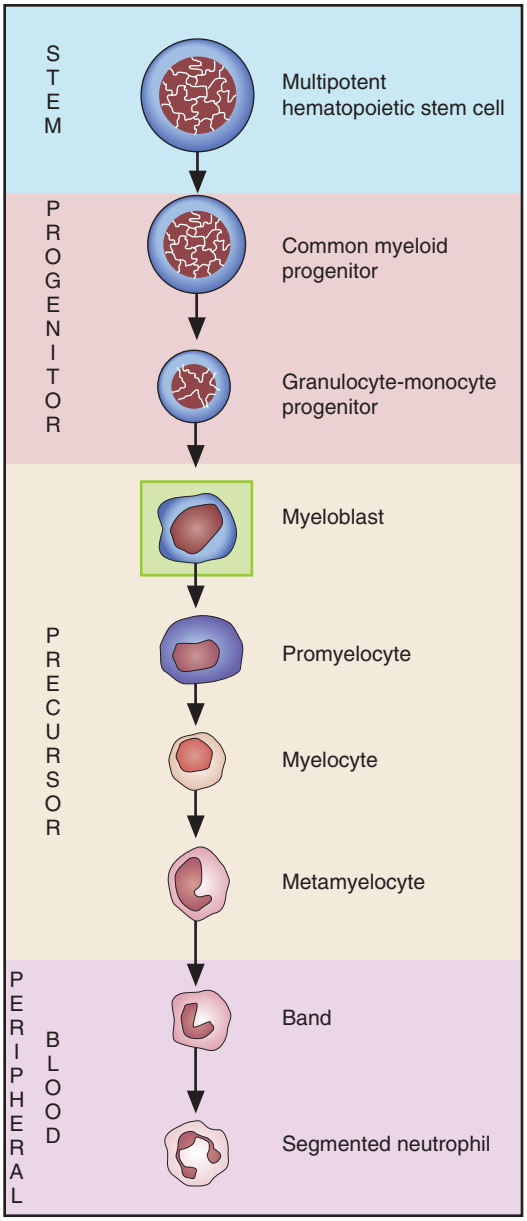
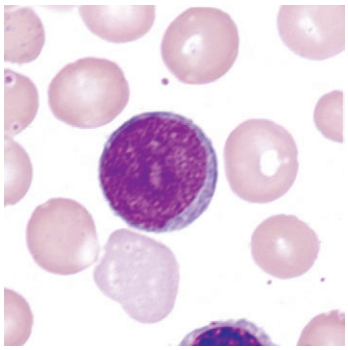


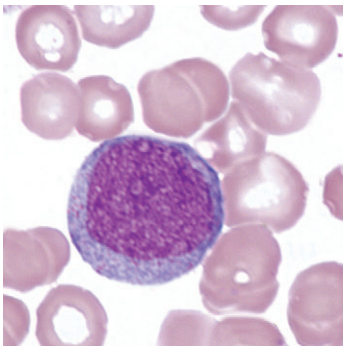
FIGURE 5–1 Neutrophilic sequence—myeloblast.

MYELOBLAST



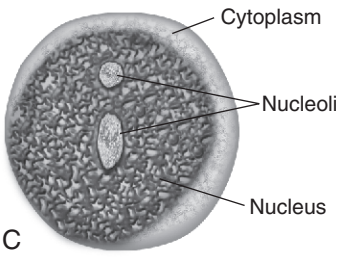
A

FIGURE 5–2A Myeloblast with no granules.



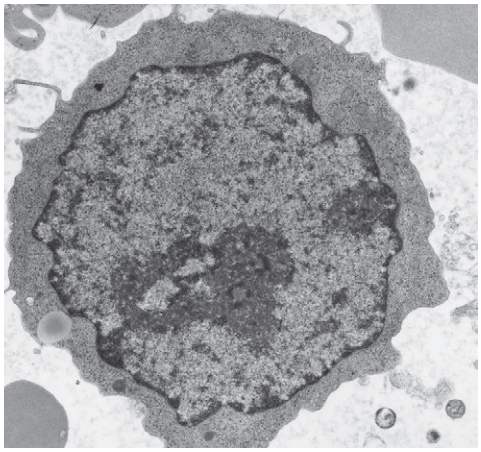
B

FIGURE 5–2B Myeloblast with up to 20 granules.



C

FIGURE 5–2C Schematic of Figure 5-2, *A*, myeloblast.



D

FIGURE 5–2D Electron micrograph of myeloblast (×16,500).

SIZE: 15-20 μm
NUCLEUS: Round to oval
Nucleoli: 2-5
Chromatin: Fine
CYTOPLASM: Moderate basophilia
Granules: Absent or up to 20
N/C RATIO: 4:1
REFERENCE INTERVAL:
Bone Marrow: 0% to 2%
Peripheral Blood: 0%

Blasts without granulation are sometimes referred to as Type I blasts and those with up to 20 granules as Type II blasts, although they are generally not separated as such in the differential count.

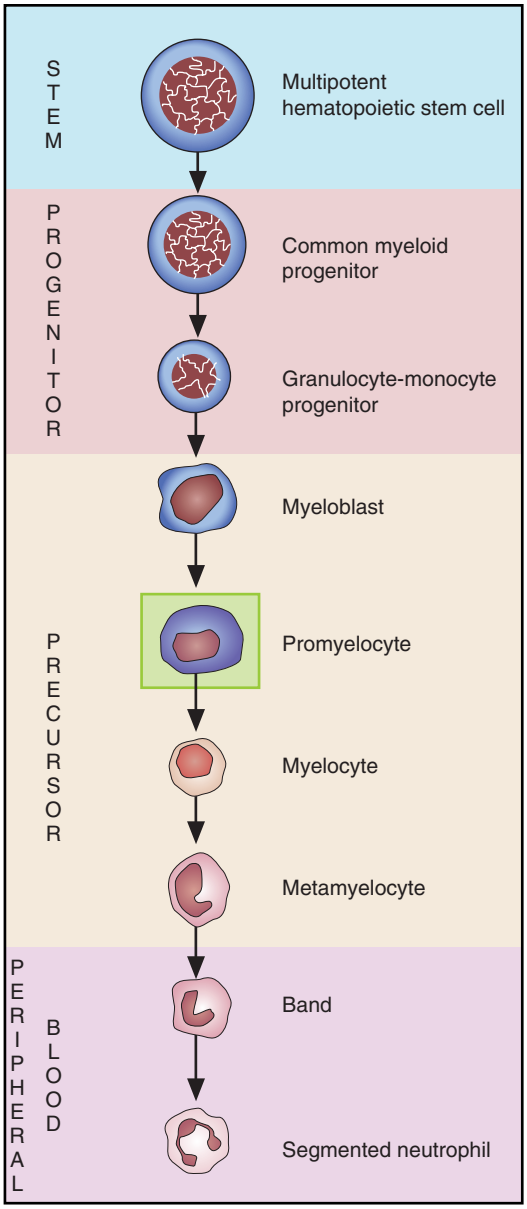
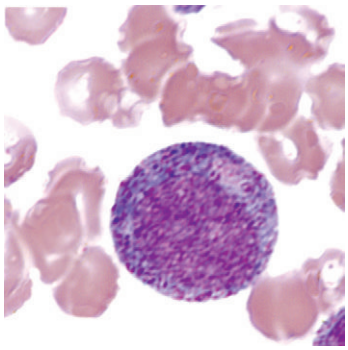


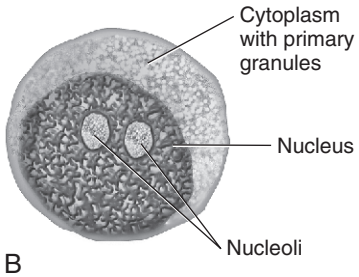
FIGURE 5–3 Neutrophilic sequence—promyelocyte.

PROMYELOCYTE (PROGRANULOCYTE)



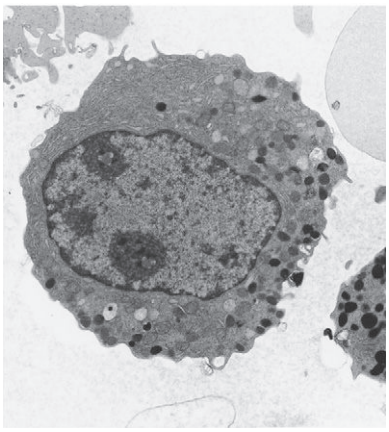
A

FIGURE 5–4A Promyelocyte.



B

FIGURE 5–4B Schematic of promyelocyte.



C

FIGURE 5–4C Electron micrograph of promyelocyte (×13,000).

SIZE: 14-24 μm (slightly larger than myeloblast)
NUCLEUS: Round to oval
Nucleoli: 1-3 or more
Chromatin: Fine, but slightly coarser than myeloblast
Cytoplasm: Basophilic
Granules:
- **Primary:** >20 to many; their color is red to purple or burgundy
- **Secondary:** None

N/C RATIO: 3:1
REFERENCE INTERVAL:
Bone Marrow: 2% to 5%
Peripheral Blood: 0%

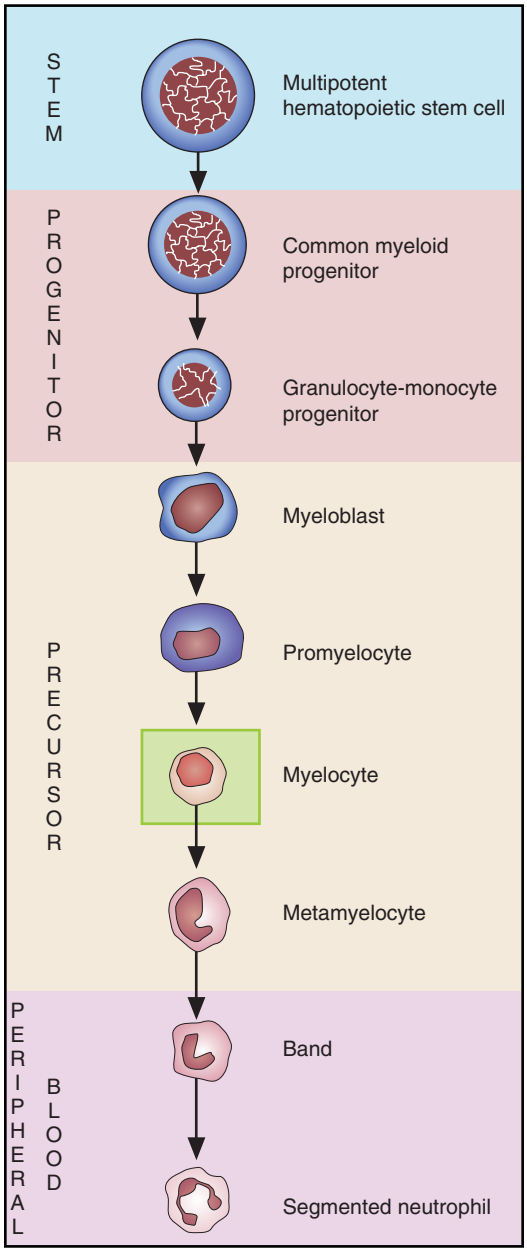
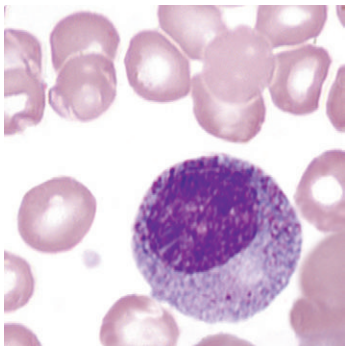


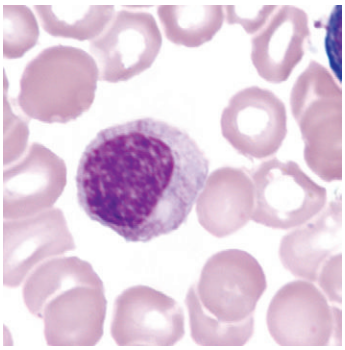
FIGURE 5–5 Neutrophilic sequence—myelocyte.

NEUTROPHILIC MYELOCYTE



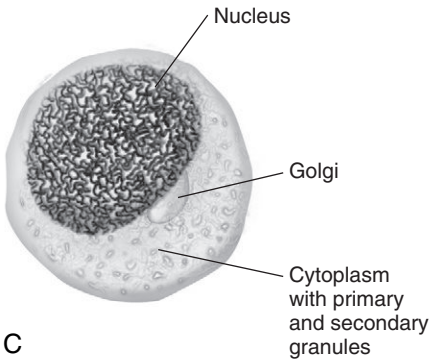
A

FIGURE 5–6A Neutrophilic myelocyte, early.



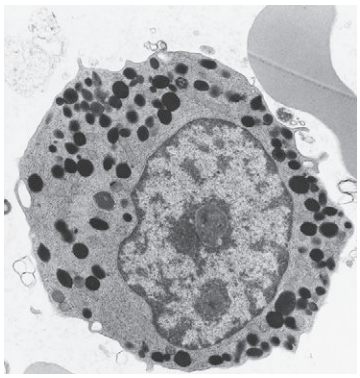
B

FIGURE 5–6B Neutrophilic myelocyte, late.



C

FIGURE 5–6C Schematic of myelocyte, late.



D

FIGURE 5–6D Electron micrograph of myelocyte (×16,500).

SIZE: 12-18 μm
NUCLEUS: Round to oval; slightly eccentric; may have one flattened side; may be a clearing next to the nucleus indicating the location of the Golgi
Nucleoli: Usually not visible
Chromatin: Coarse and more condensed than promyelocyte
CYTOPLASM: Slightly basophilic to cream-colored
Granules:
Primary: Few to moderate
Secondary: Variable number; they become predominant as cell matures
N/C RATIO: 2:1
REFERENCE INTERVAL:
Bone Marrow: 5% to 19%
Peripheral Blood: 0%
NOTE: Secondary granules in neutrophils are too small to resolve at the light microscope level. They give the cytoplasm a grainy or sandy appearance, and the overall color is lavender to pink. (See Figure 7-2 for eosinophilic myelocyte.)

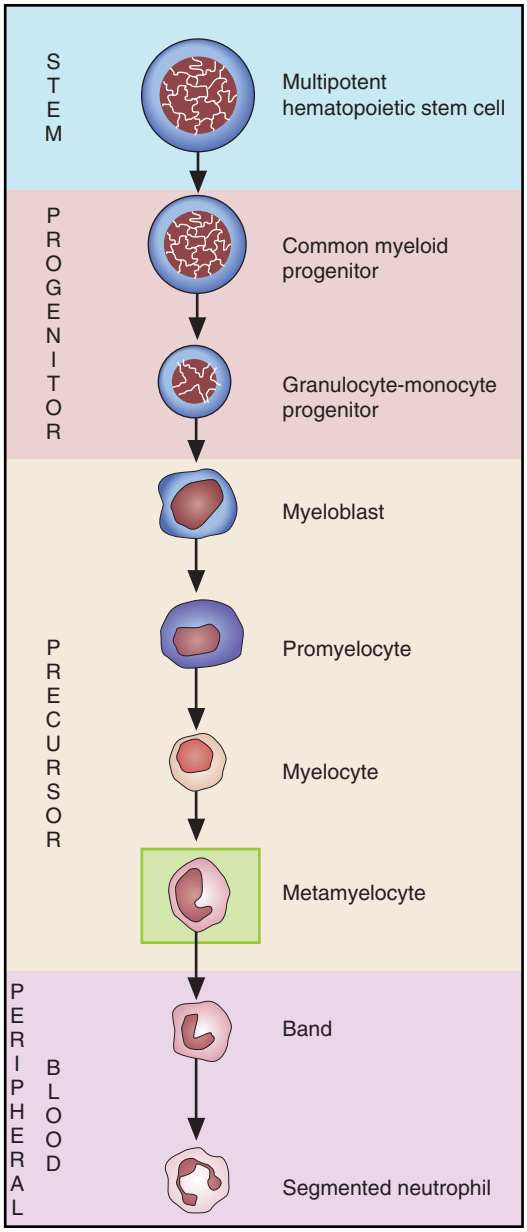
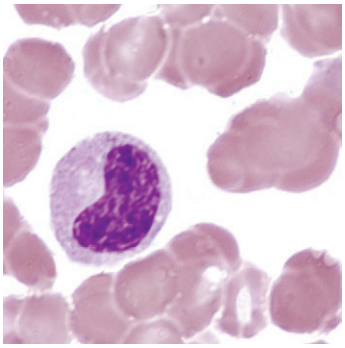


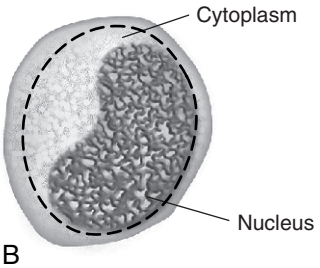
FIGURE 5–7 Neutrophilic sequence—metamyelocyte.

NEUTROPHILIC METAMYELOCYTE



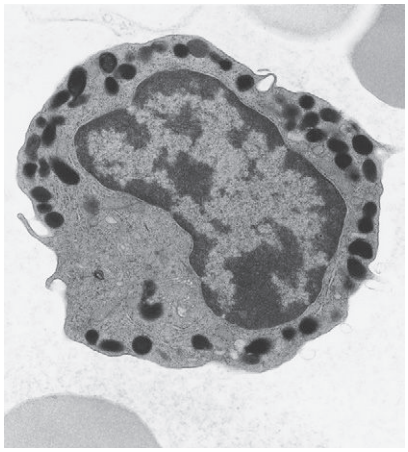
A

FIGURE 5–8A Neutrophilic metamyelocyte.



B

FIGURE 5–8B Schematic of metamyelocyte. *Dotted line* indicates hypothetical round nucleus.



C

FIGURE 5–8C Electron micrograph of metamyelocyte (×22,250).

SIZE: 10-15 μm
NUCLEUS: Indented; kidney bean shape; indentation is less than 50% of the width of a hypothetical round nucleus
Nucleoli: Not visible
Chromatin: Moderately clumped
CYTOPLASM: Pale pink to cream colored to colorless
Granules:
Primary: Few
Secondary: Many (full complement)
N/C RATIO: 1.5:1
REFERENCE INTERVAL:
Bone Marrow: 13% to 22%
Peripheral Blood: 0%

See Figure 7-4 for eosinophilic metamyelocyte.

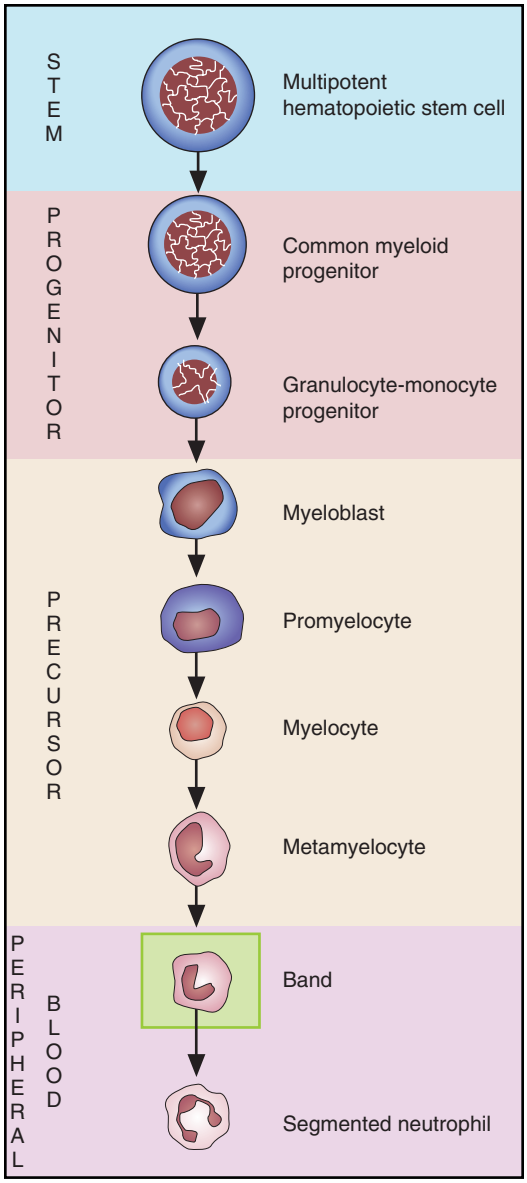
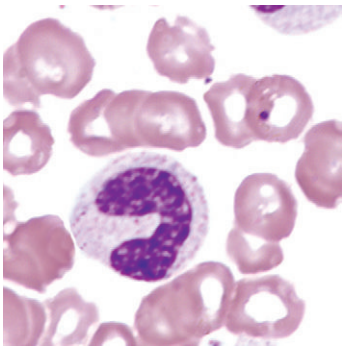


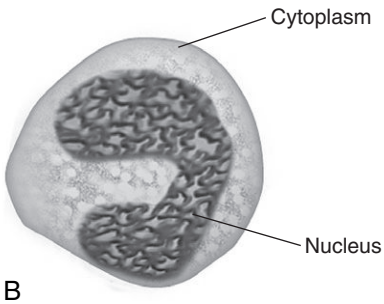
FIGURE 5–9 Neutrophilic sequence—band.

NEUTROPHILIC BAND



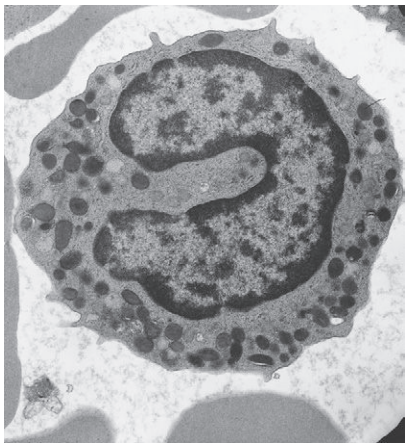
A

FIGURE 5-10A Neutrophilic band.



B

FIGURE 5-10B Schematic of band.



C

FIGURE 5-10C Electron micrograph of band (×22,250).

SIZE: 10-15 μm
NUCLEUS: Constricted but no threadlike filament; indentation is more than 50% of the width of a hypothetical round nucleus
NOTE: Chromatin must be visible in constriction; may be folded over
Nucleoli: Not visible
Chromatin: Coarse, clumped
CYTOPLASM: Pale blue to pink
Granules:
Primary: Few
Secondary: Abundant
N/C RATIO: Cytoplasm predominates
Reference Interval:
Bone Marrow: 17% to 33%
Peripheral Blood: 0% to 5%

Refer to Table 1-1 for more examples.
See Figure 7-6 for eosinophilic band.

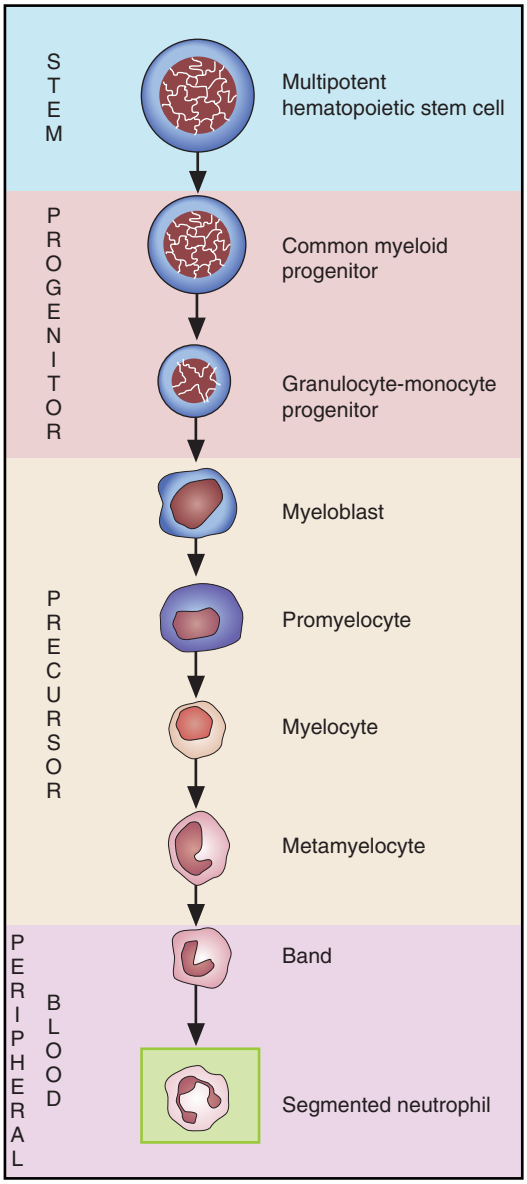
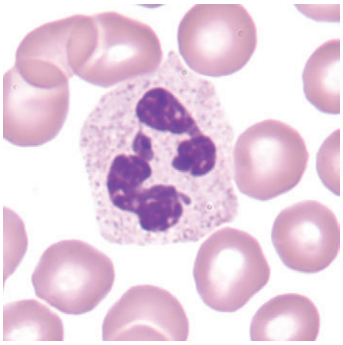


FIGURE 5-11 Neutrophilic sequence—segmented neutrophil.

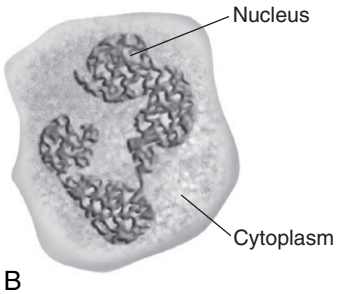
SEGMENTED NEUTROPHIL

Polymorphonuclear Neutrophil



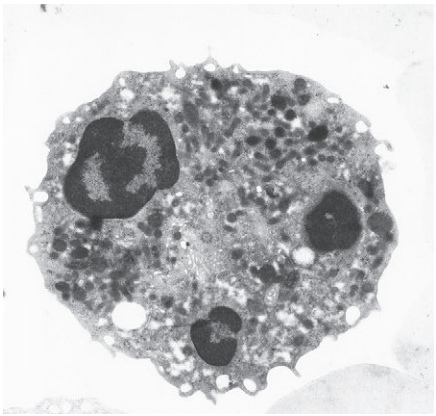
A

FIGURE 5-12A Segmented neutrophil.



B

FIGURE 5-12B Schematic of segmented neutrophil.



C

FIGURE 5-12C Electron micrograph of segmented neutrophil (×22,250).

SIZE: 10-15 μm
NUCLEUS: 2-5 lobes connected by thin filaments without visible chromatin
Nucleoli: Not visible
Chromatin: Coarse, clumped
CYTOPLASM: Pale pink, cream-colored or colorless
Granules:
Primary: Rare
Secondary: Abundant
N/C RATIO: Cytoplasm predominates
REFERENCE INTERVAL:
Bone Marrow: 3% to 11%
Peripheral Blood: 50% to 70%

Refer to Table 1-1 for more examples.

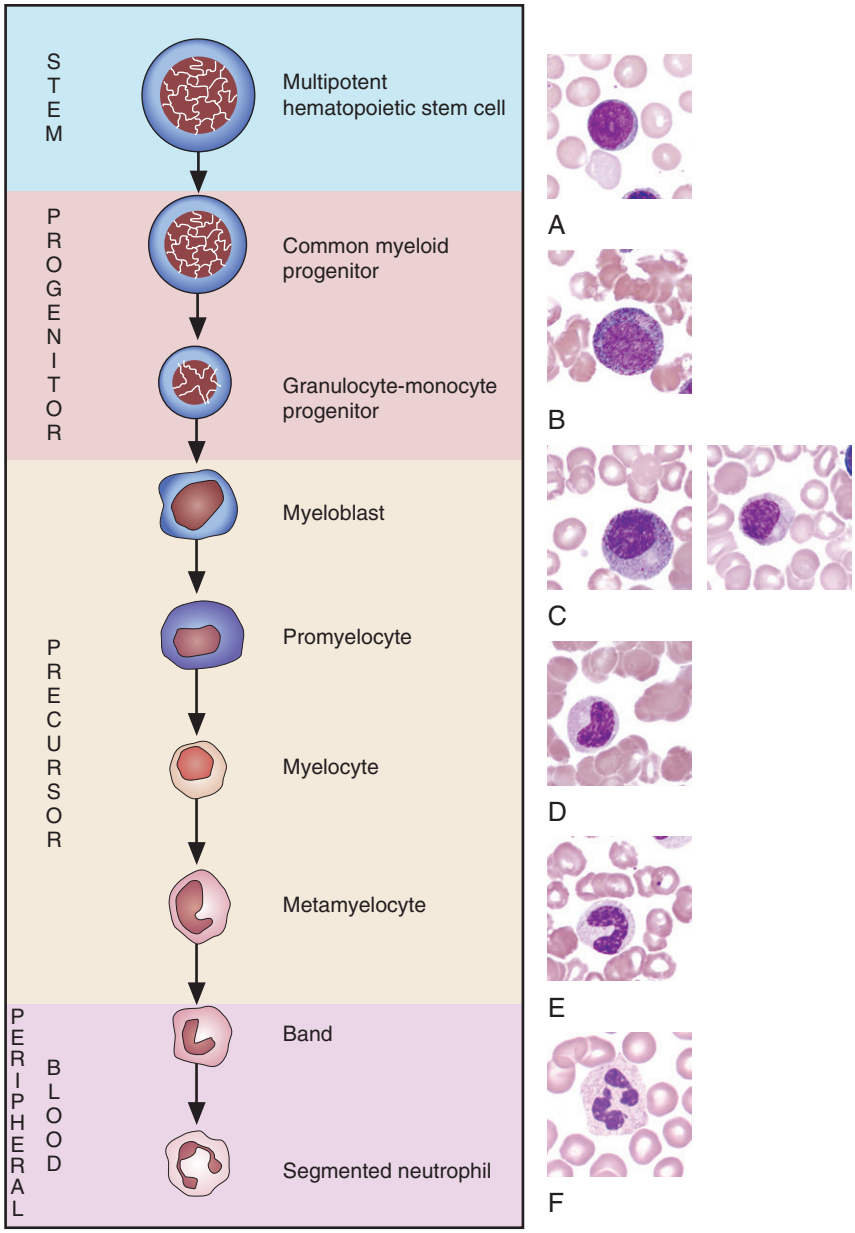


FIGURE 5-13 Neutrophilic sequence with **(A)** myeloblast, **(B)** promyelocyte, **(C)** myelocyte, **(D)** metamyelocyte, **(E)** band, and **(F)** segmented neutrophil.

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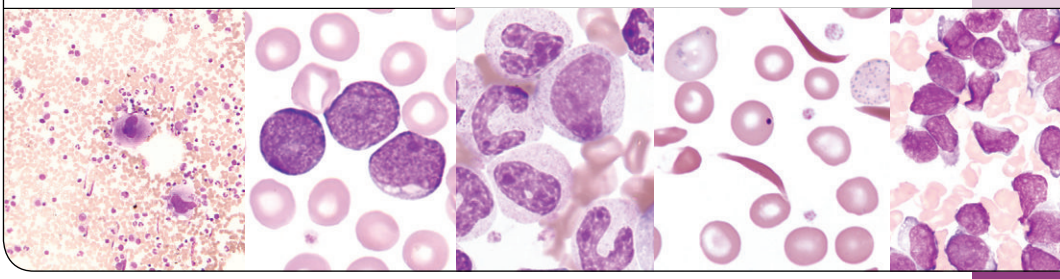
6

MONOCYTE MATURATION



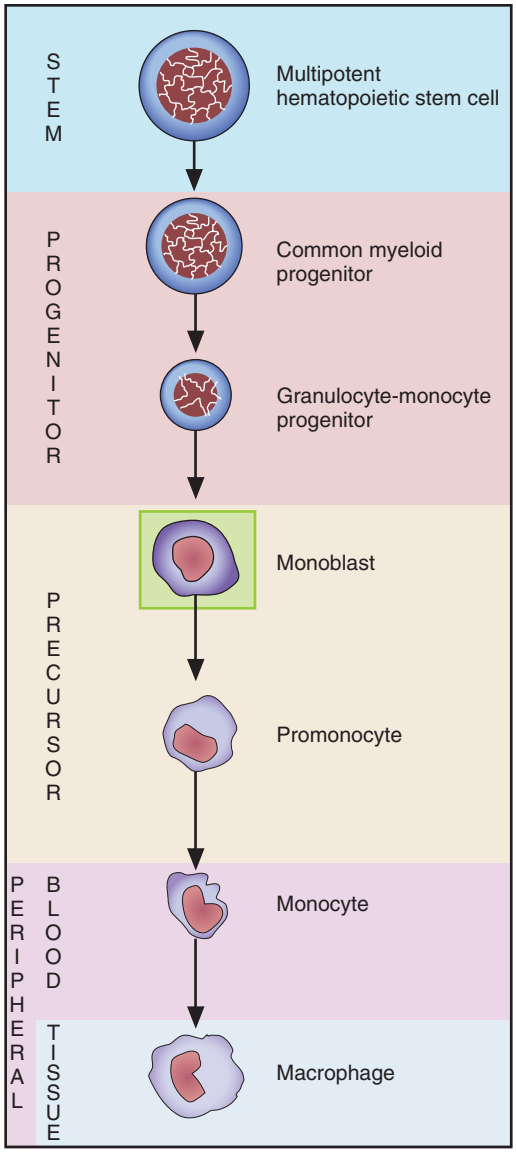
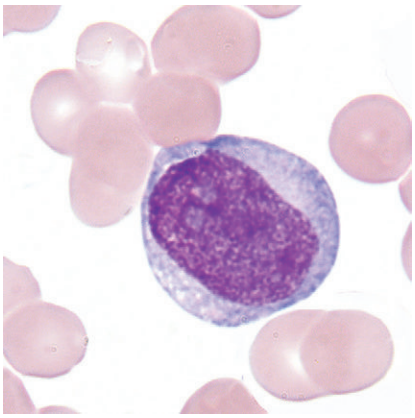


FIGURE 6–1 Monocyte sequence—monoblast.

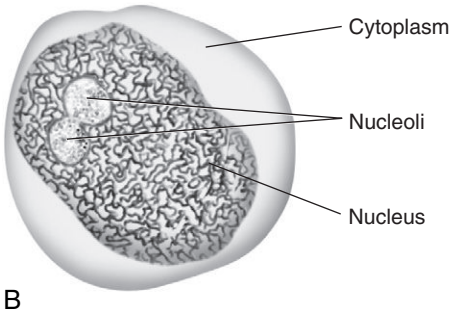
All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

MONOBLAST



A

FIGURE 6–2A Monoblast.



B

FIGURE 6–2B Schematic of monoblast.

SIZE: 12-18 μm
NUCLEUS: Round to oval; may be irregularly shaped
Nucleoli: 1-2; may not be visible
Chromatin: Fine
Cytoplasm: Light blue to gray
GRANULES: None
N/C RATIO: 4:1
REFERENCE INTERVAL:
Bone Marrow: Not defined
Peripheral Blood: None

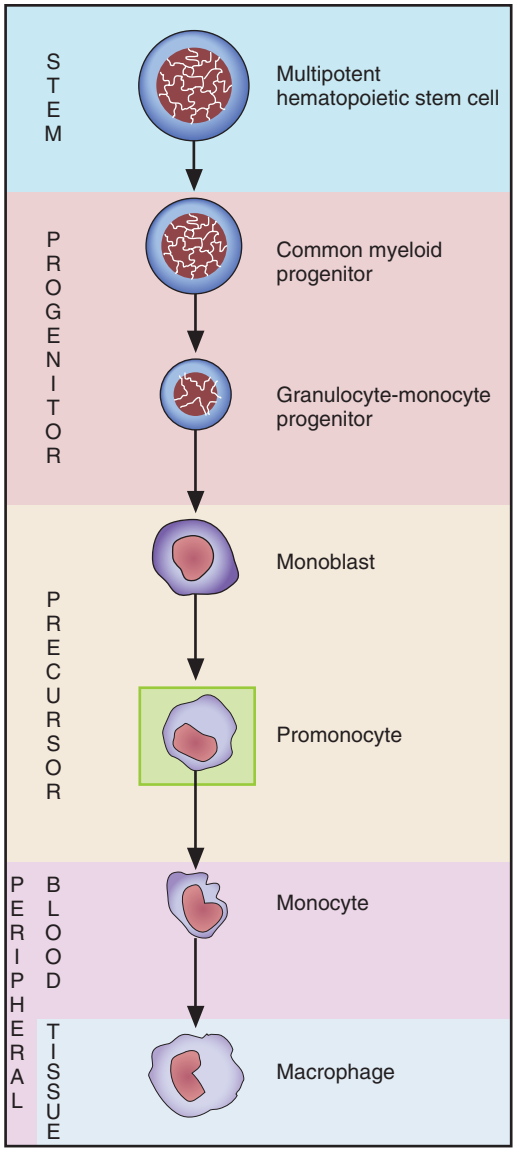
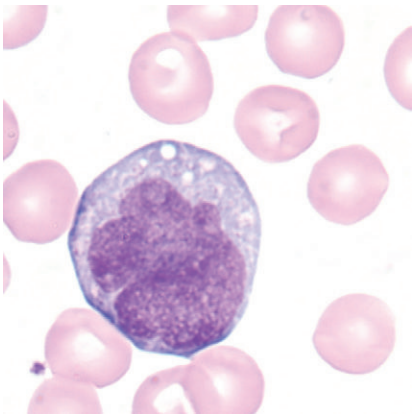


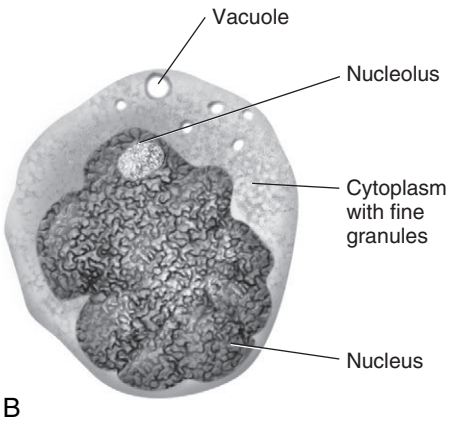
FIGURE 6–3 Monocyte sequence—promonocyte.

PROMONOCYTE



A

FIGURE 6–4A Promonocyte.



B

FIGURE 6–4B Schematic of promonocyte.

SIZE: 12-20 μm
NUCLEUS: Irregularly shaped; folded; may have brainlike convolutions
Nucleoli: May or may not be visible
Chromatin: Fine to lacy
Cytoplasm: Light blue to gray
GRANULES: Fine azurophilic (burgundy colored)
N/C RATIO: 2-3:1
REFERENCE INTERVAL:
Bone Marrow: <1%
Peripheral Blood: 0%

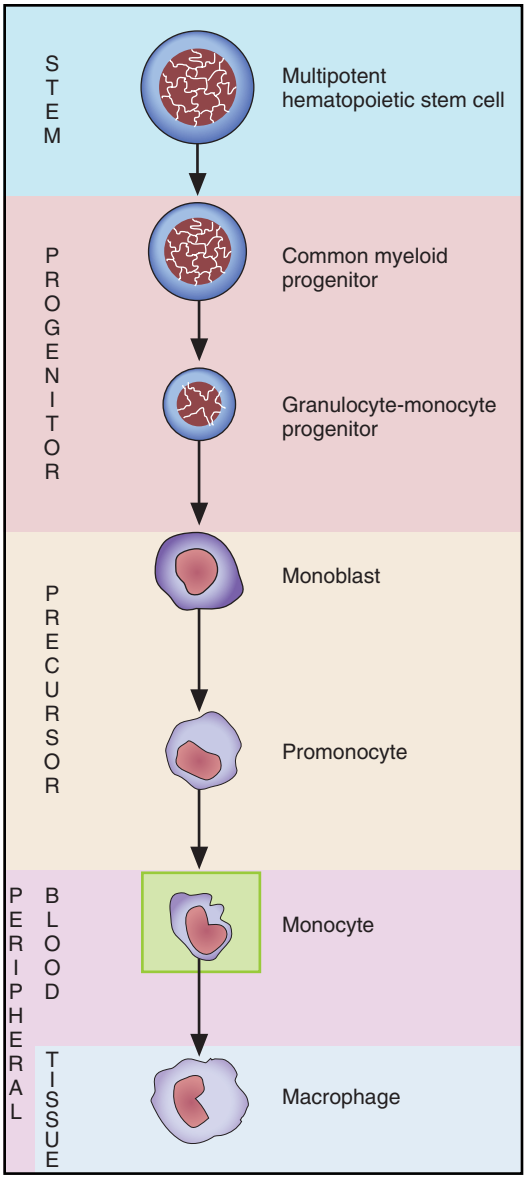
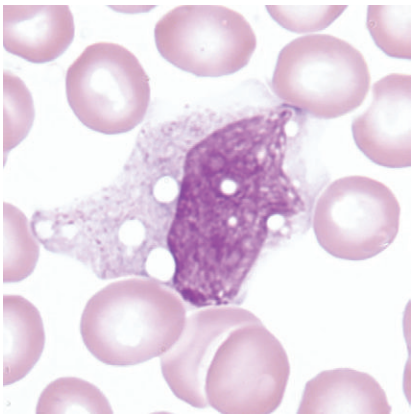


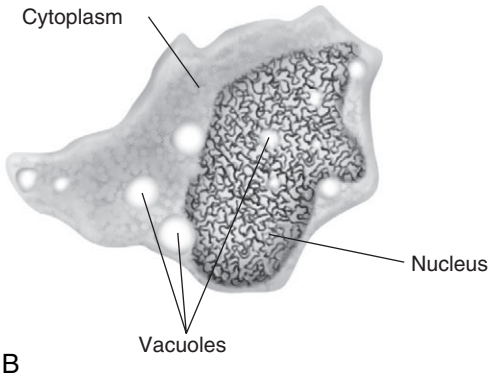
FIGURE 6–5 Monocyte sequence—monocyte.

MONOCYTE



A

FIGURE 6–6A Monocyte.

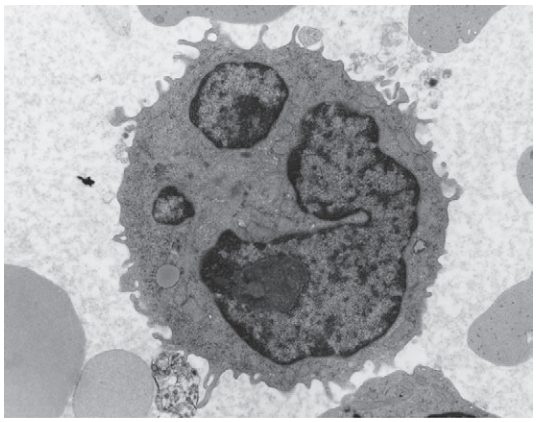


B

FIGURE 6–6B Schematic of monocyte.

SIZE: 12-20 μm
NUCLEUS: Variable; may be round, horseshoe shaped, or kidney shaped; often has folds producing "brainlike" convolutions
Nucleoli: Not visible
Chromatin: Lacy
CYTOPLASM: Blue-gray; may have pseudopods
Granules: Many fine granules frequently giving the appearance of ground glass
Vacuoles: Absent to numerous
N/C RATIO: Variable
REFERENCE INTERVAL:
Bone Marrow: 2%
Peripheral Blood: 3% to 11%

Refer to Table 1-1 for more examples.



C

FIGURE 6–6C Electron micrograph of monocyte (×16,500). Specimens for electron microscopy are prepared by embedding tissue in a suitable medium, such as resin. Ultra-thin cross-sections are then prepared. Because this image shows a cross-section, the lobes of the nucleus appear to be separate, but they are not.

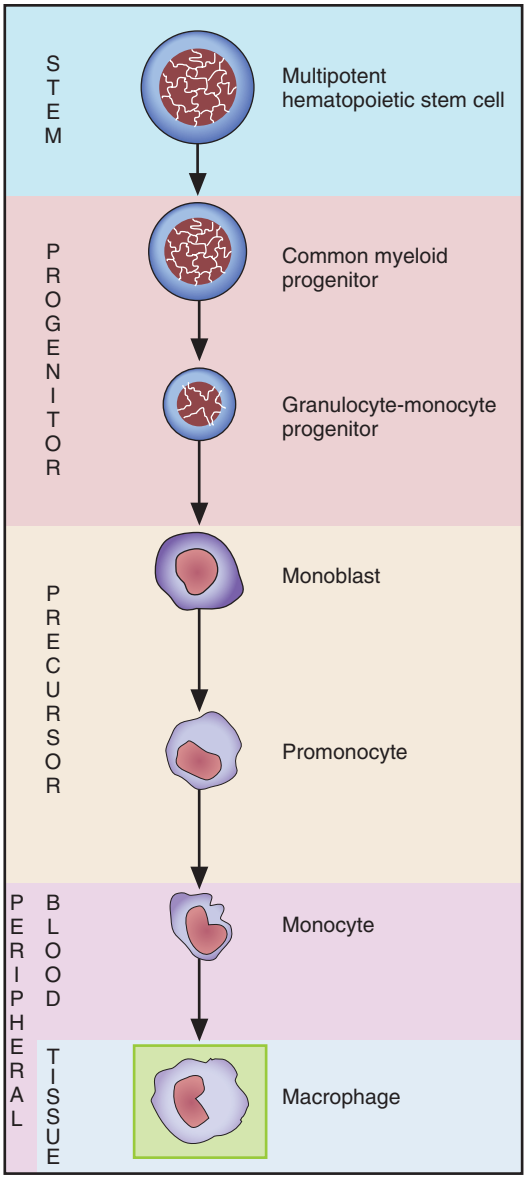
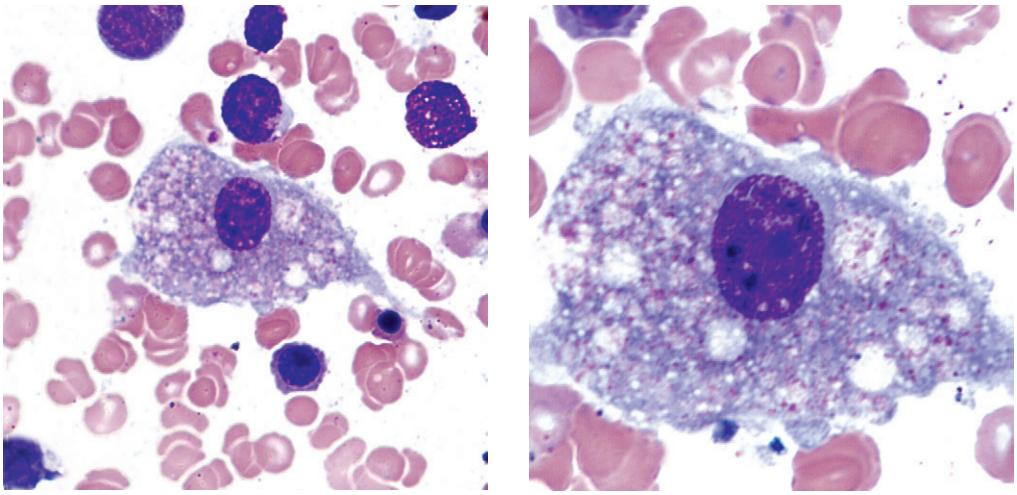


FIGURE 6–7 Monocyte sequence—macrophage.

MACROPHAGE (HISTIOCYTE)



A B

FIGURE 6–8 Macrophage. Bone marrow **(A)** (×500), **(B)** (×1000).

SIZE: 15-80 μm
NUCLEUS: Eccentric, kidney or egg-shaped, indented, or elongated
Nucleoli: 1-2
Chromatin: Fine, dispersed
CYTOPLASM: Abundant with irregular borders; may contain ingested material
Granules: Many coarse azurophilic (burgundy-colored)
Vacuoles: May be present
REFERENCE INTERVAL: Macrophages reside in tissues, such as bone marrow, spleen, liver, lungs, and others. Rarely, they are seen in the peripheral blood during severe sepsis.

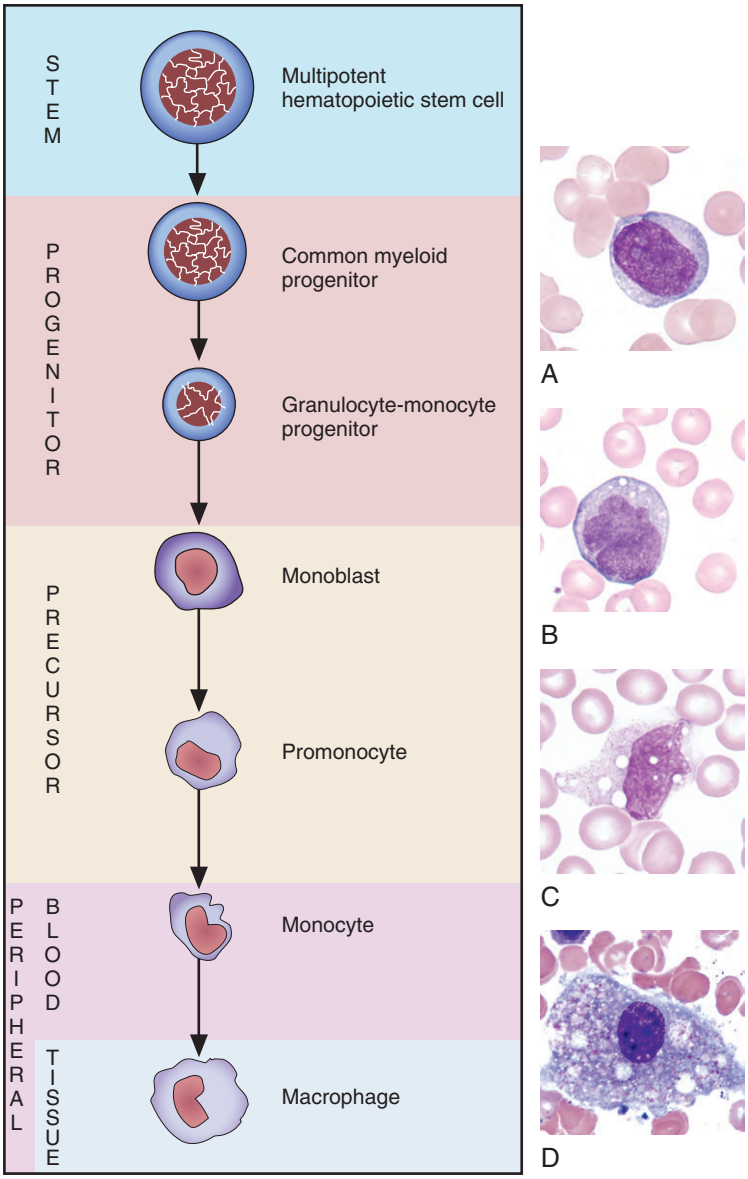
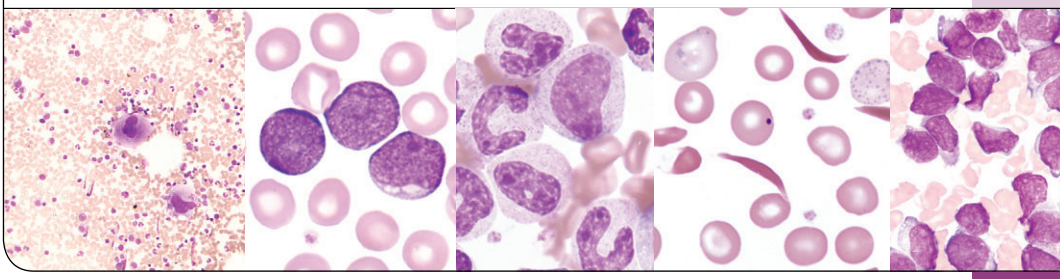


FIGURE 6–9 Monocyte sequence with **(A)** monoblast, **(B)** promonocyte, **(C)** monocyte, and **(D)** macrophage.

7

EOSINOPHIL MATURATION



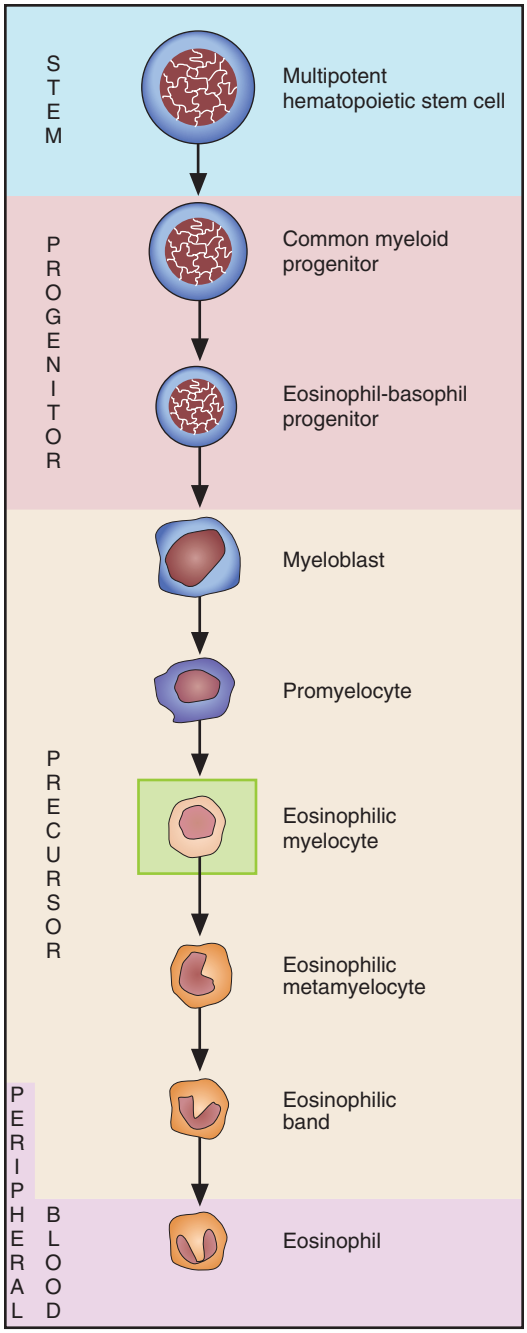
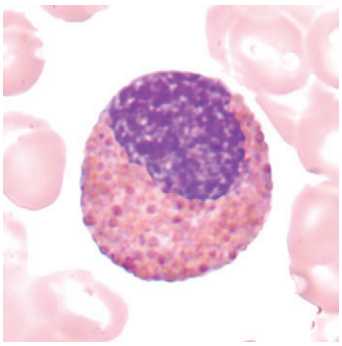


FIGURE 7–1 Eosinophilic sequence—eosinophilic myelocyte.

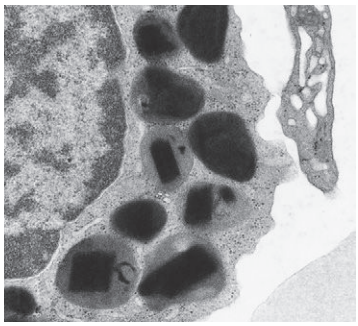
All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

EOSINOPHILIC MYELOCYTE



A

FIGURE 7–2A Eosinophilic myelocyte.



B

FIGURE 7–2B Electron micrograph of eosinophilic granules to demonstrate crystalline structure of granules.

SIZE: 12-18 μm
NUCLEUS: Round to oval; may have one flattened side
Nucleoli: Usually not visible
Chromatin: Coarse and more condensed than promyelocyte
CYTOPLASM: Colorless to pink
Granules:
Primary: Few to moderate
Secondary: Variable number; pale orange to dark orange; round; appear refractile
N/C RATIO: 2:1 to 1:1
REFERENCE INTERVAL:
Bone Marrow: 0% to 2%
Peripheral Blood: 0%
NOTE: This chapter begins with the image of the myelocyte, rather than the blast, because it is at the myelocyte stage that secondary granules, which define a cell as an eosinophil, first appear.

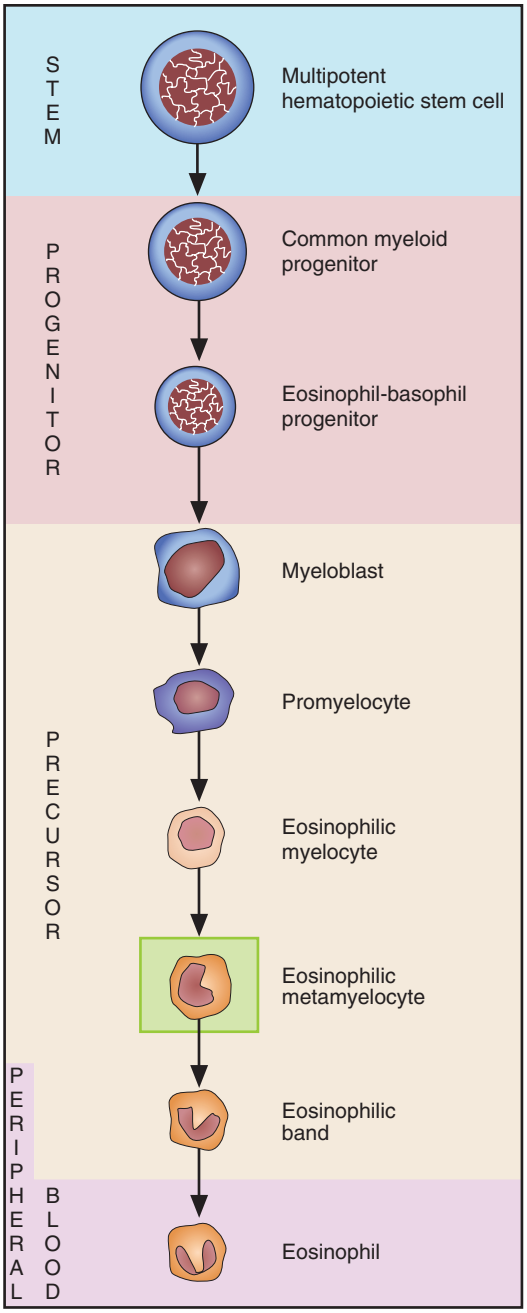


FIGURE 7–3 Eosinophilic sequence—eosinophilic metamyelocyte.

EOSINOPHILIC METAMYELOCYTE

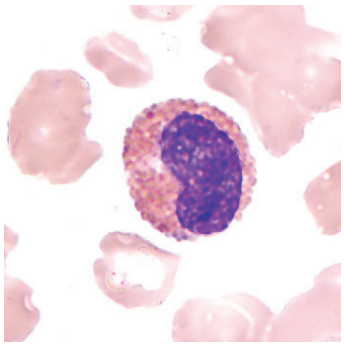


FIGURE 7–4 Eosinophilic metamyelocyte.

SIZE: 10-15 μm
NUCLEUS: Indented; kidney bean shape; indentation is less than 50% of the width of the hypothetical round nucleus
Nucleoli: Not visible
Chromatin: Coarse, clumped
CYTOPLASM: Colorless
Granules:
- **Primary:** Few
- **Secondary:** Many pale orange to dark orange; appear refractile

N/C RATIO: 1.5:1
REFERENCE INTERVAL:
Bone Marrow: 0% to 2%
Peripheral Blood: 0%

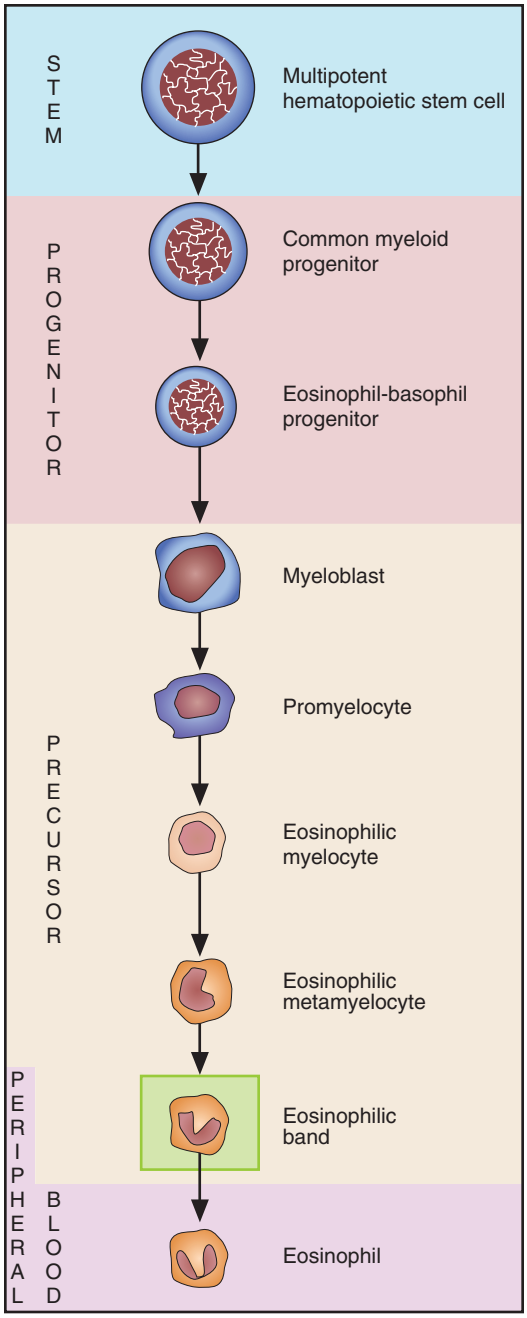


FIGURE 7–5 Eosinophilic sequence—eosinophilic band.

EOSINOPHILIC BAND

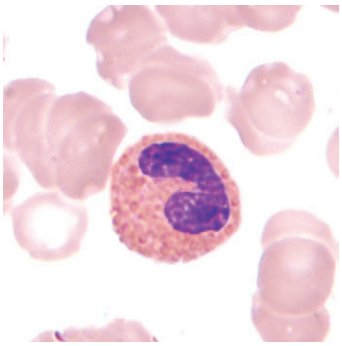


FIGURE 7–6 Eosinophilic band.

SIZE: 10-15 μm
NUCLEUS: Constricted but no threadlike filament: indentation is more than 50% of the width of a hypothetical round nucleus
NOTE: Chromatin must be visible in constriction
Nucleoli: Not visible
Chromatin: Coarse, clumped
CYTOPLASM: Colorless, cream-colored
Granules:
Primary: Few
Secondary: Abundant pale to dark orange; appear refractile
N/C RATIO: Cytoplasm predominates
REFERENCE INTERVAL:
Bone Marrow: 0% to 2%
Peripheral Blood: Rarely seen

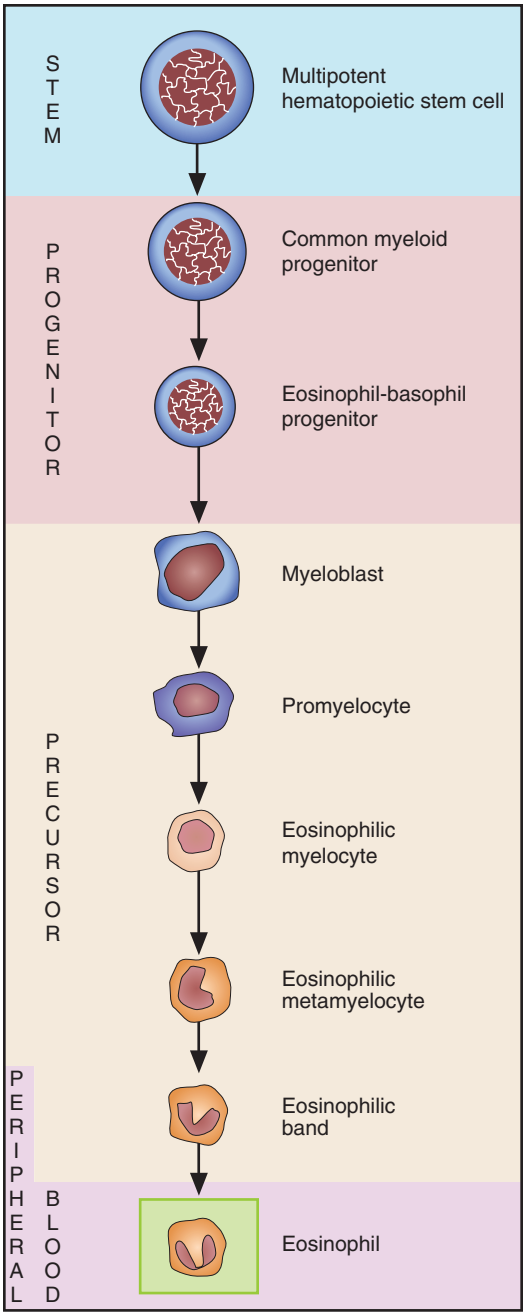
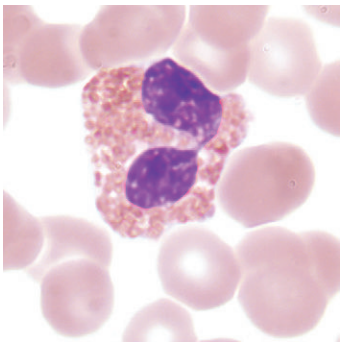


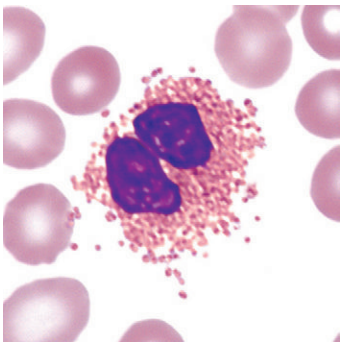
FIGURE 7–7 Eosinophilic sequence—eosinophil.

EOSINOPHIL



A

FIGURE 7–8A Eosinophil.



B

FIGURE 7–8B Fractured eosinophil.

SIZE: 12-17 μm
NUCLEUS: 2-5 lobes connected by thin filaments without visible chromatin; majority of mature cells have 2-3 lobes
Nucleoli: Not visible
Chromatin: Coarse, clumped
CYTOPLASM: Cream-colored; may have irregular borders
Granules:
Primary: Rare
Secondary: Abundant pale orange to dark orange; round
N/C RATIO: Cytoplasm predominates
REFERENCE INTERVAL:
Bone Marrow: 0% to 3%
Peripheral Blood: 0% to 5%

Refer to Table 1-1 for more examples.

NOTE: Eosinophils are fragile and may easily fracture when preparing blood film.

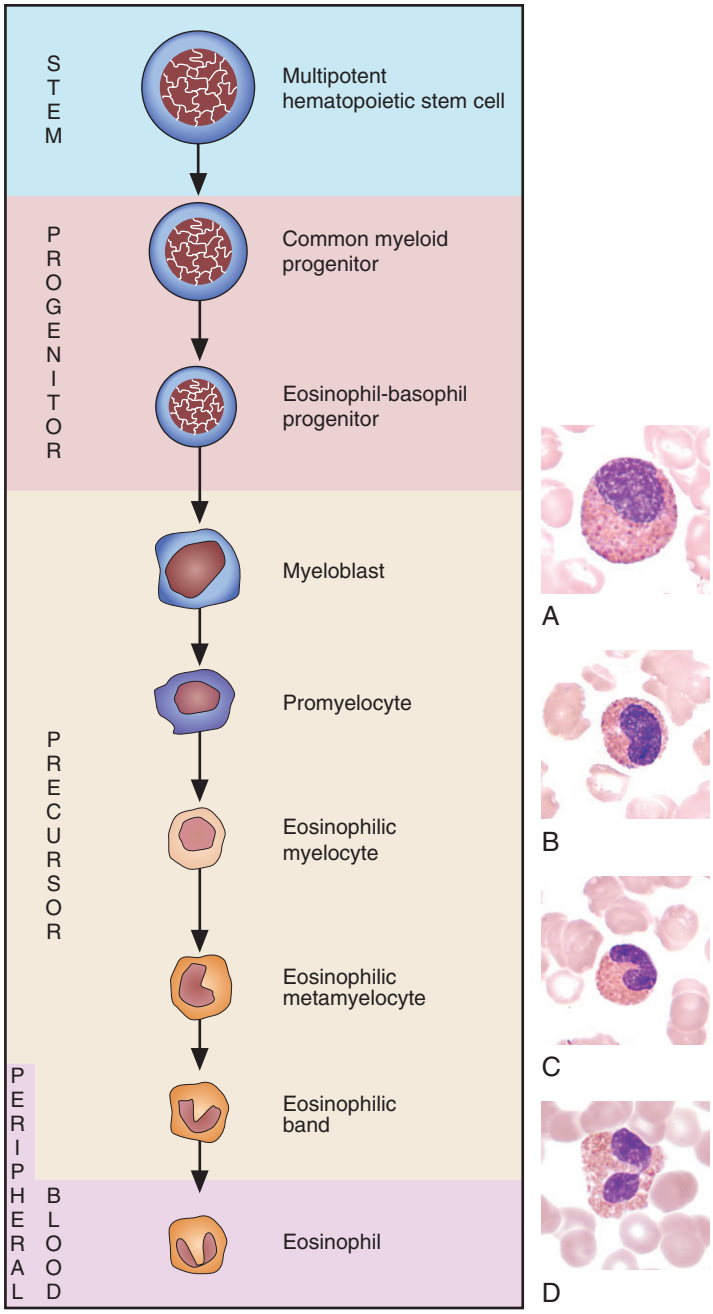
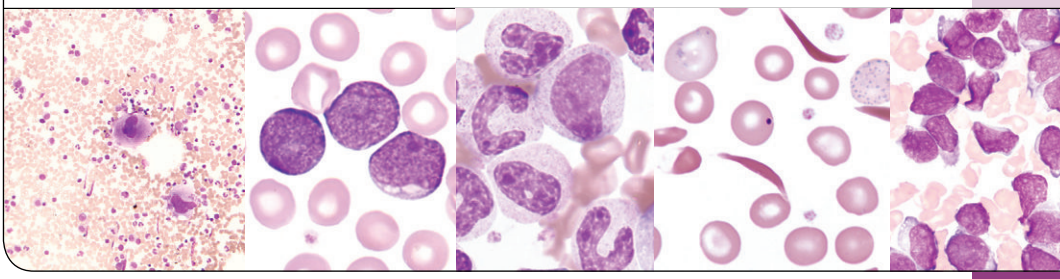


FIGURE 7–9 Eosinophilic sequence with **(A)** eosinophilic myelocyte, **(B)** eosinophilic metamyelocyte, **(C)** eosinophilic band, and **(D)** eosinophil.

8

BASOPHIL MATURATION



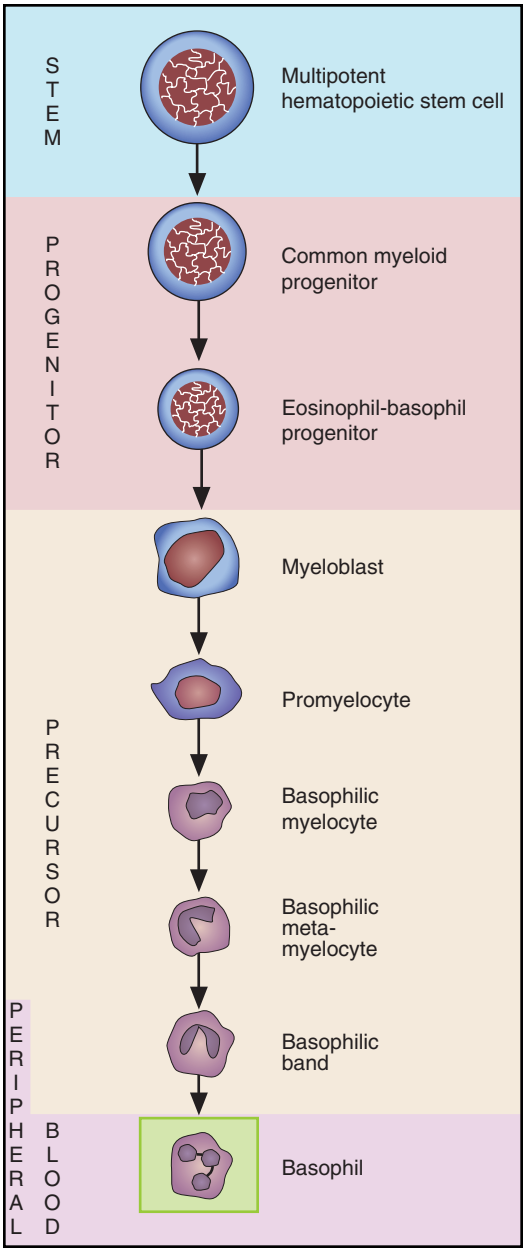
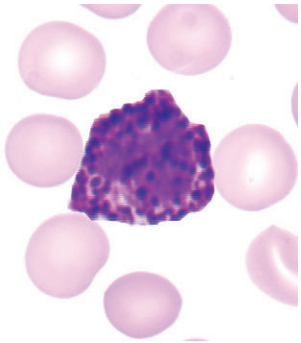


FIGURE 8–1 Basophilic sequence—Basophil. Maturation parallels that of the neutrophil; however, immature stages are very rare and generally seen only in basophil proliferative disorders.

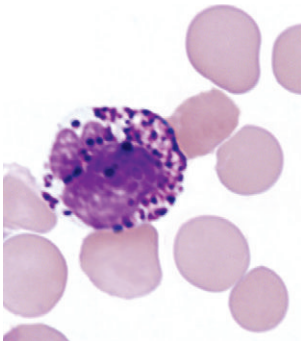
All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

BASOPHIL



A

FIGURE 8–2A Basophil.

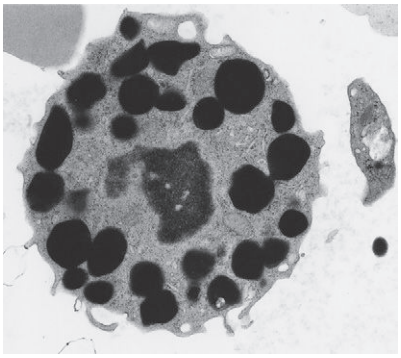


B

FIGURE 8–2B Basophil. Note that granules are water-soluble and may be dissolved during the staining process, leaving clear area in the cytoplasm.

SIZE: 10-14 μm
NUCLEUS: Usually two lobes connected by thin filaments without visible chromatin
Nucleoli: Not visible
Chromatin: Coarse, clumped
CYTOPLASM: Lavender to colorless
Granules:
Primary: Rare
Secondary: Variable in number with uneven distribution, may obscure nucleus (Figure 8-2A); deep purple to black; irregularly shaped. Granules are water-soluble and may be washed out during staining; thus they appear as empty areas in the cytoplasm (Figure 8-2B).
N/C RATIO: Cytoplasm predominates
REFERENCE INTERVAL:
Bone Marrow: <1%
Peripheral Blood: 0% to 1%

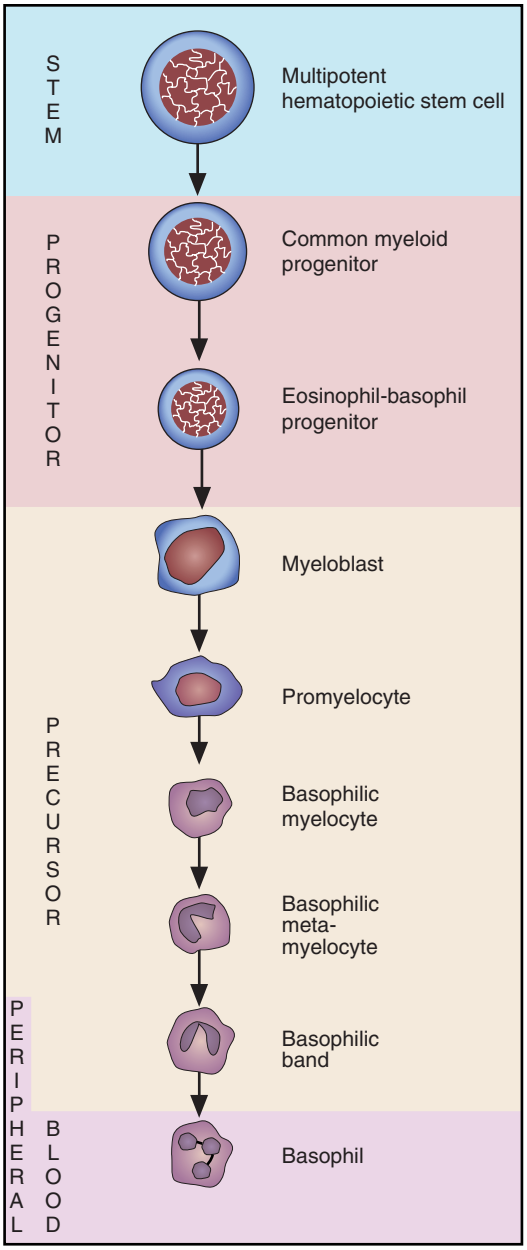
Refer to Table 1-1 for more examples.

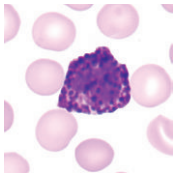


C

FIGURE 8–2C Electron micrograph of basophil (×28,750).

All photomicrographs are ×1000 with Wright-Giemsa stain unless stated otherwise.



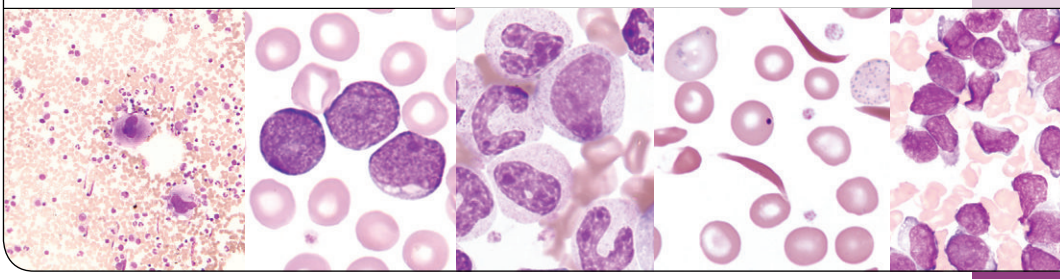


A

FIGURE 8–3 Maturation parallels that of the neutrophil; however, immature stages are very rare and generally seen only in basophil proliferative disorders. **(A)** Basophil.

9

LYMPHOCYTE MATURATION



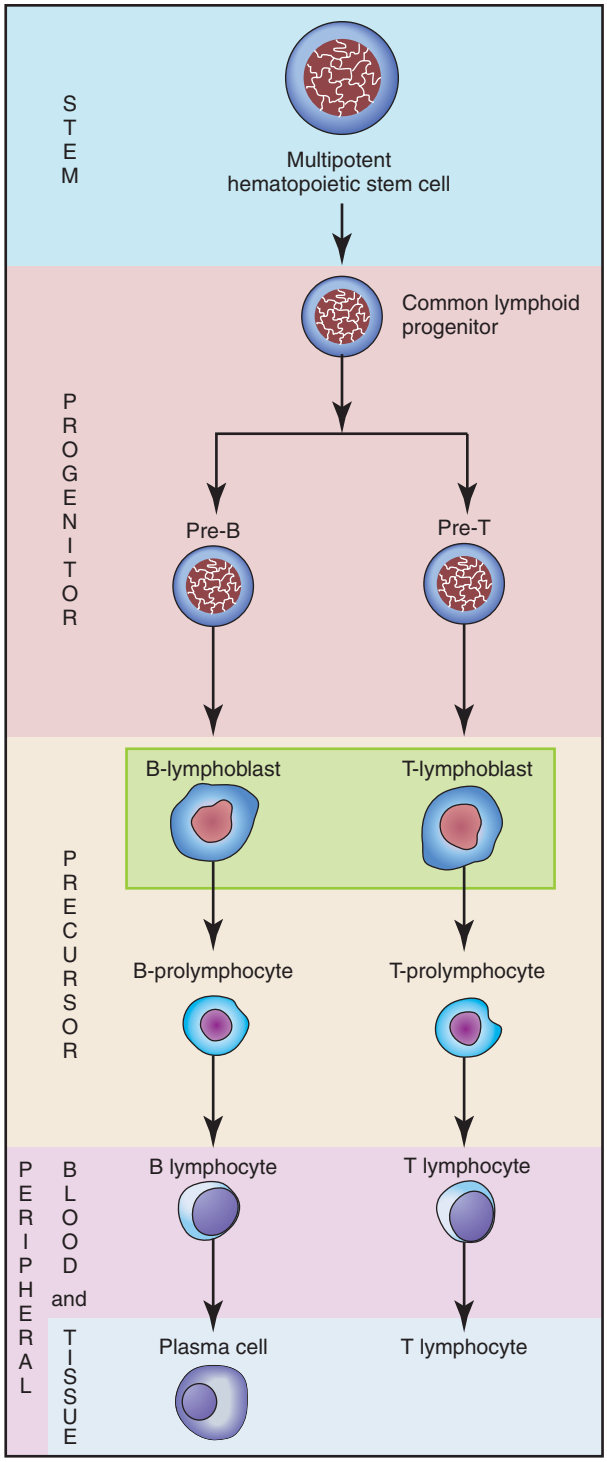
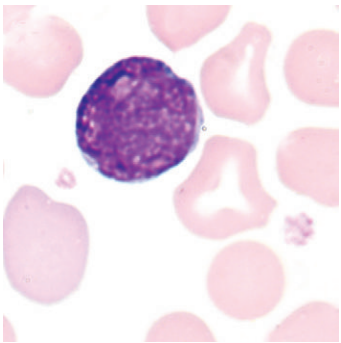


FIGURE 9–1 Lymphocyte sequence—B and T lymphoblasts.

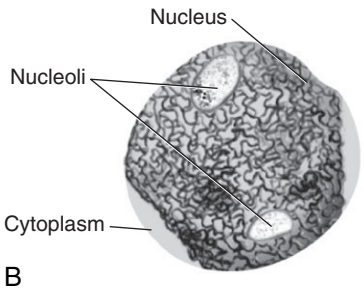
All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

LYMPHOBLAST



A

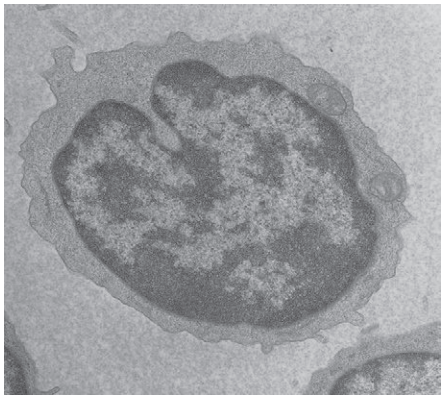
FIGURE 9–2A Lymphoblast.



B

FIGURE 9–2B Schematic of lymphoblast.

SIZE: 10-20 μm
NUCLEUS: Round to oval
Nucleoli: ≥1
Chromatin: Fine, evenly stained
CYTOPLASM: Scant; slightly to moderately basophilic
Granules: None
N/C RATIO: 7:1 to 4:1
REFERENCE INTERVAL:
Bone Marrow: Not defined
Peripheral Blood: 0%



C

FIGURE 9–2C Electron micrograph of lymphoblast (×28,750). Lymphoblasts are difficult to distinguish morphologically in normal bone marrow.

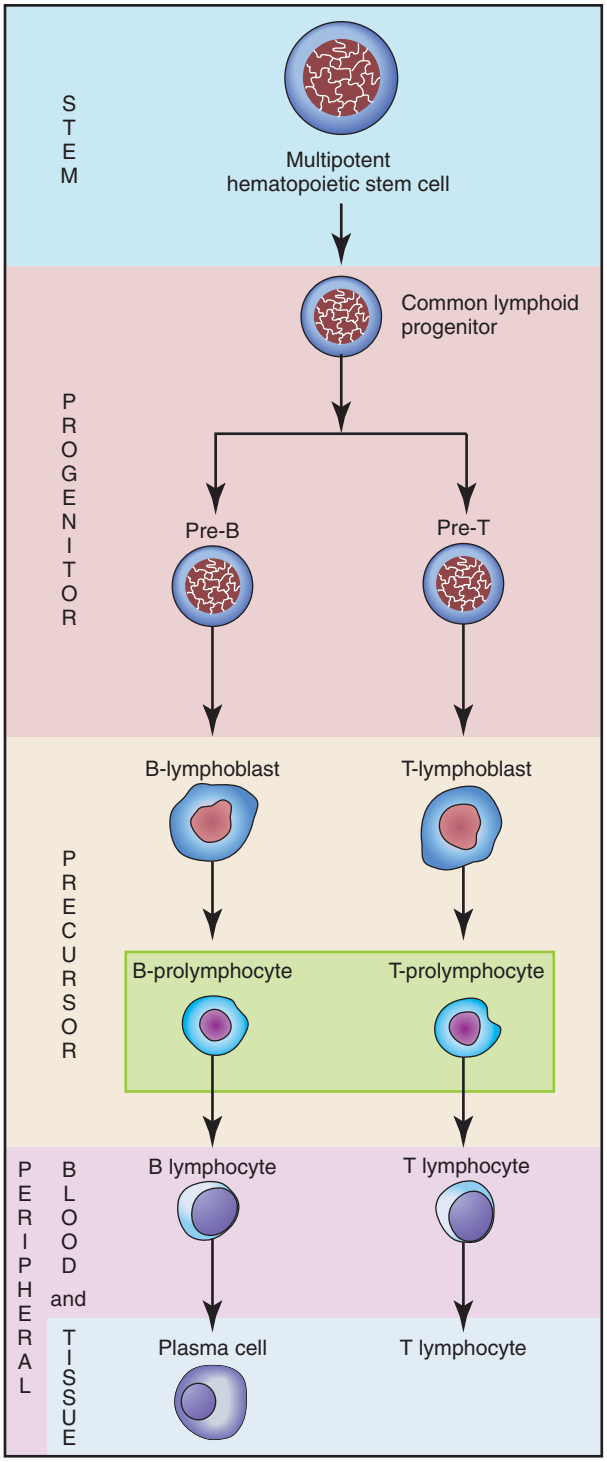
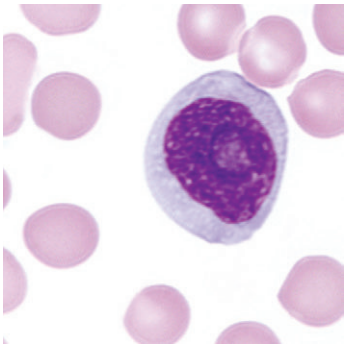


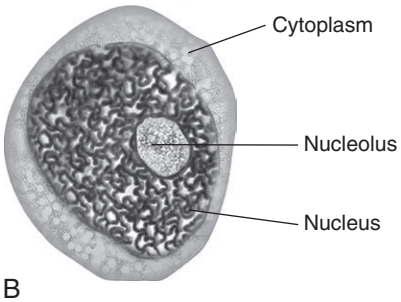
FIGURE 9–3 Lymphocyte sequence—B and T prolymphocytes.

PROLYMPHOCYTE



A

FIGURE 9–4A Prolymphocyte.



B

FIGURE 9–4B Schematic of prolymphocyte. Prolymphocytes are difficult to distinguish morphologically in normal bone marrow.

SIZE: 9-18 μm
NUCLEUS: Round or indented
Nucleoli: 0-1; usually single, prominent, large nucleolus
Chromatin: Slightly clumped; intermediate between lymphoblast and mature lymphocyte
CYTOPLASM: Light blue
Granules: None
N/C RATIO: 3-4:1
REFERENCE INTERVAL:
Bone Marrow: Not defined
Peripheral Blood: None

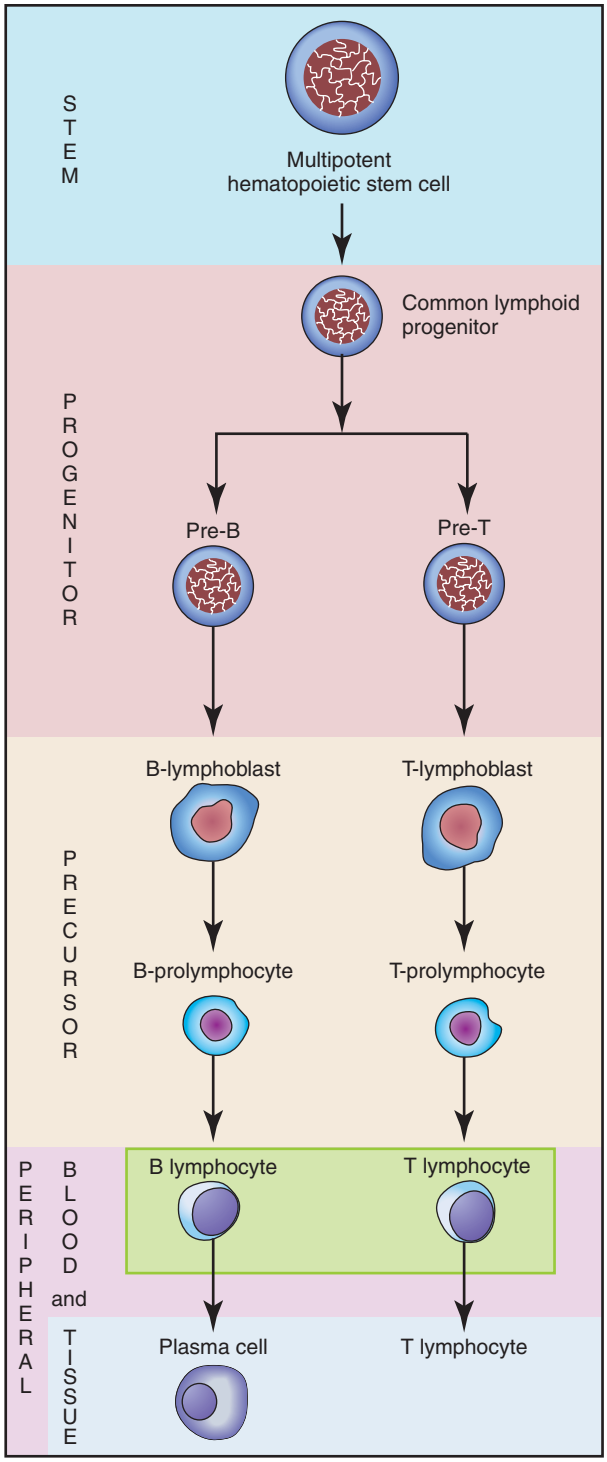
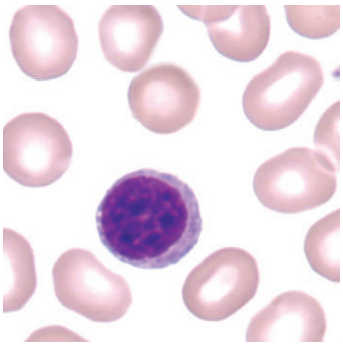


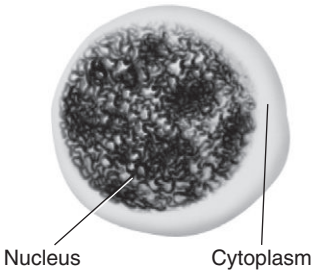
FIGURE 9–5 Lymphocyte sequence—B and T lymphocytes. (NOTE: T lymphocytes cannot be distinguished from B lymphocytes with Wright stain.)

LYMPHOCYTE



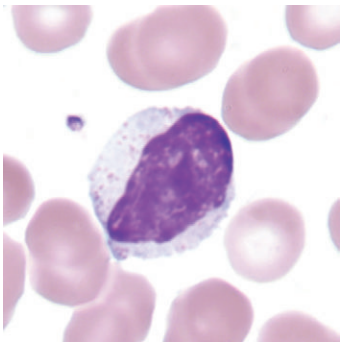
A

FIGURE 9–6A Small lymphocyte.



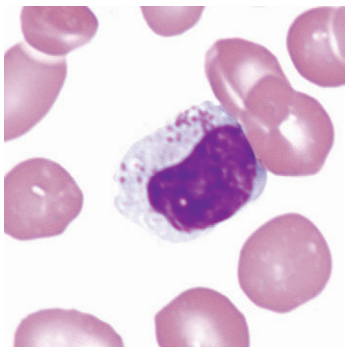
B

FIGURE 9–6B Schematic of lymphocyte.



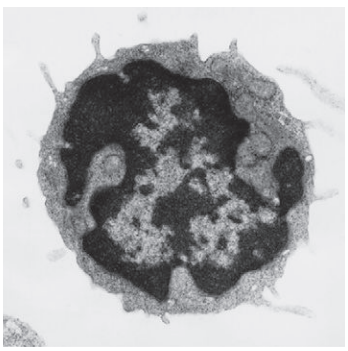
C

FIGURE 9–6C Large lymphocyte. Note irregular nucleus and more abundant cytoplasm than small lymphocyte.



D

FIGURE 9–6D Large granular lymphocyte. Note prominent azurophilic granules in cytoplasm.



E

FIGURE 9–6E Electron micrograph of lymphocyte (×30,000).

SIZE: 7-18 μm
NUCLEUS: Round to oval; may be slightly indented
Nucleoli: Occasional
Chromatin: Condensed; clumped; blocky, smudged
CYTOPLASM: Scant to moderate; sky blue; vacuoles may be present
Granules: None in small lymphocyte; may be a few azurophilic in larger lymphocytes; if granules are prominent, the cell is called a large granular lymphocyte.
N/C RATIO: 5:1 to 2:1
Reference Interval (for combined small and large lymphocytes):
Bone Marrow: 5% to 15%
Peripheral Blood: 20% to 40%

Refer to Table 1-1 for more examples.

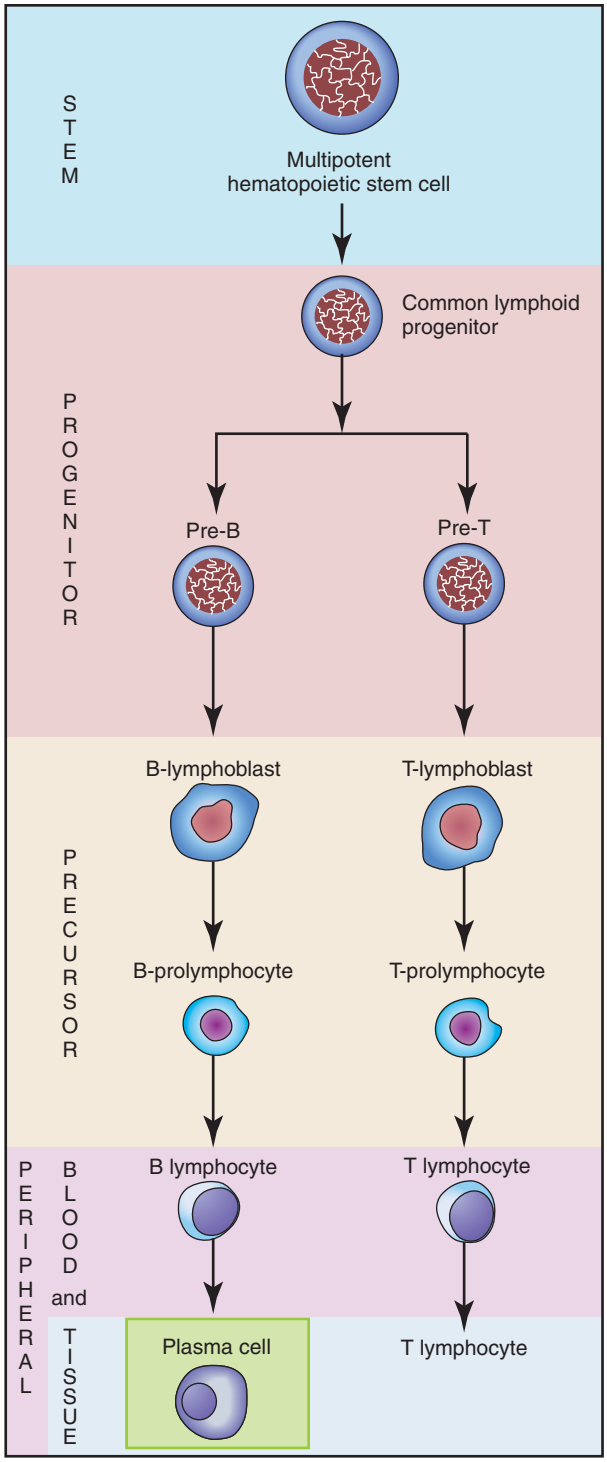
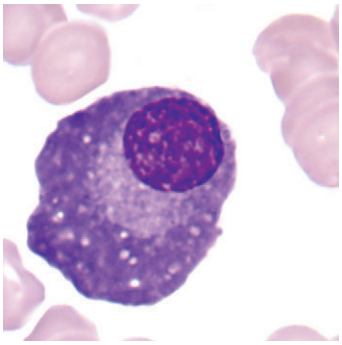


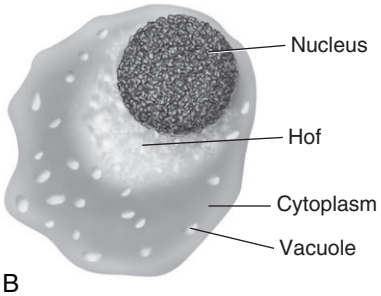
FIGURE 9–7 Lymphocyte sequence—plasma cell.

PLASMA CELL



A

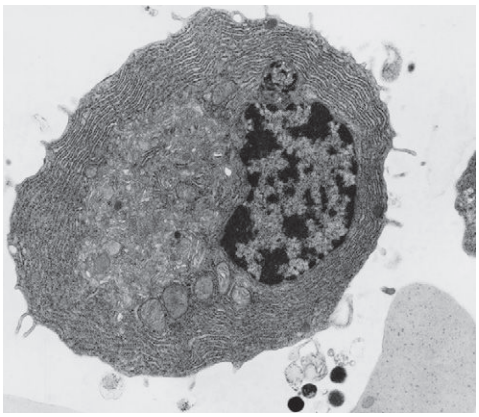
FIGURE 9–8A Plasma cell.



B

FIGURE 9–8B Schematic of plasma cell.

SIZE: 8-20 μm
NUCLEUS: Round or oval; eccentric
Nucleoli: None
Chromatin: Coarse
CYTOPLASM: Deeply basophilic, often with perinuclear clear zone (hof)
Granules: None
Vacuoles: None to several
N/C RATIO: 2:1 to 1:1
REFERENCE INTERVAL:
Bone Marrow: 0% to 1%
Peripheral Blood: 0%



C

FIGURE 9–8C Electron micrograph of plasma cell (×17,500).

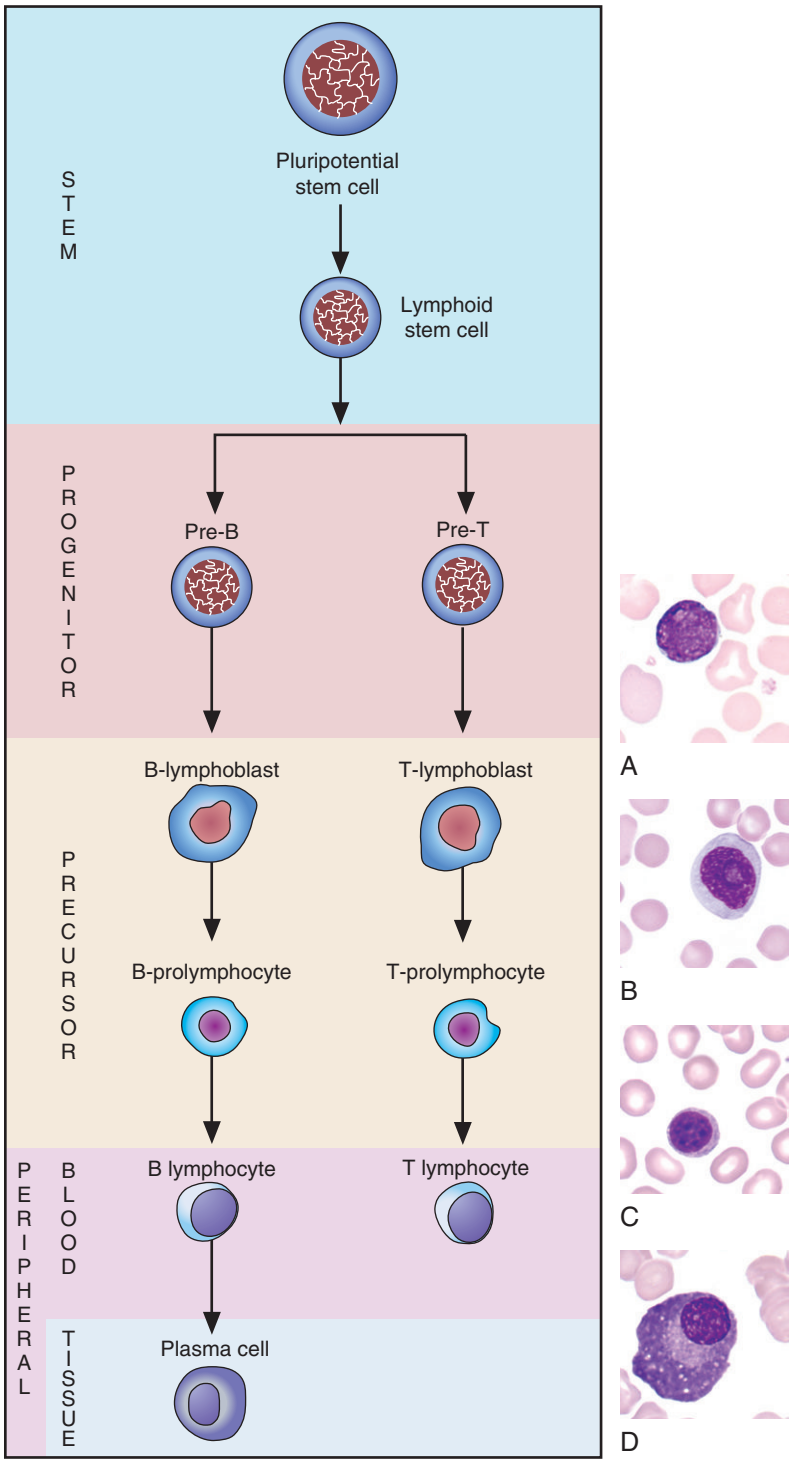
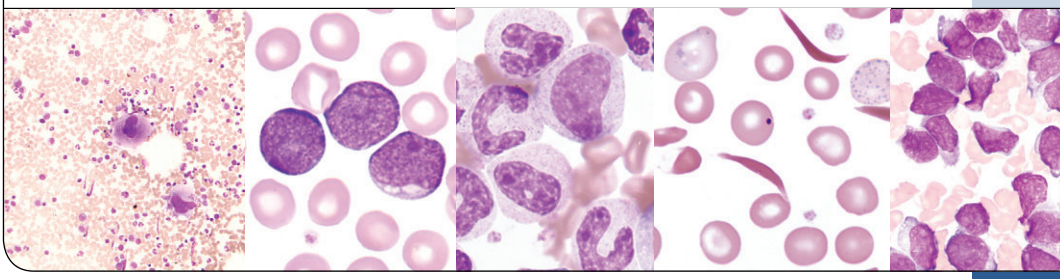


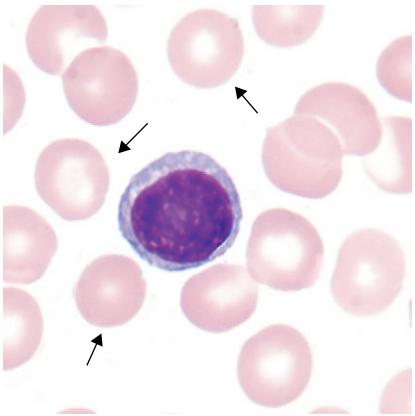
FIGURE 9–9 Lymphocyte sequence with **(A)** lymphoblast, **(B)** prolymphocyte, **(C)** lymphocyte, and **(D)** plasma cell.

10

VARIATIONS IN SIZE AND COLOR OF ERYTHROCYTES



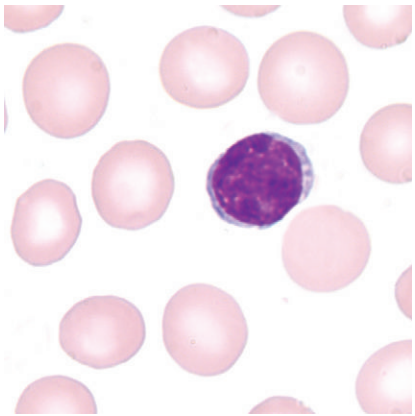
VARIATIONS IN SIZE



A

FIGURE 10–1A Microcytes (MCV < 80 fL.).

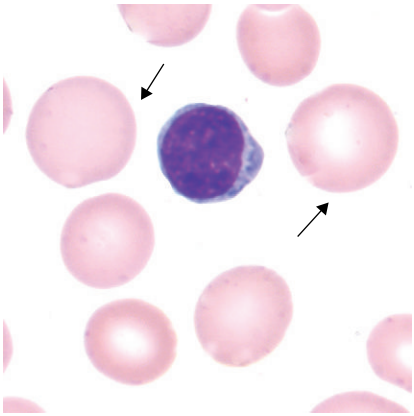
Associated with: Iron deficiency anemia, thalassemia minor, chronic inflammation (some cases), lead poisoning, hemoglobinopathies (some), sideroblastic anemia



B

FIGURE 10–1B Normocytes (MCV 80-100 fL.).

Normal erythrocytes are approximately the same size as the nucleus of a small lymphocyte.



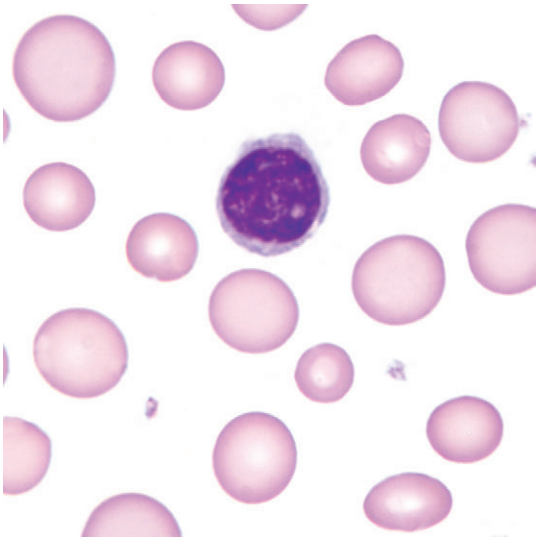
C

FIGURE 10–1C Macrocytes (MCV > 100 fL.).

Associated with: Liver disease, vitamin B_{12} deficiency, folate deficiency, neonates, reticulocytosis

Anisocytosis is the variation in red blood cell (RBC) diameter (or RBC volume) on a blood film. This variation correlates with the electronically determined red blood cell distribution width (RDW). An RDW greater than 14.5% indicates a heterogenous population of RBCs and a variety of sizes of RBCs should be seen. A low RDW is of no significance.

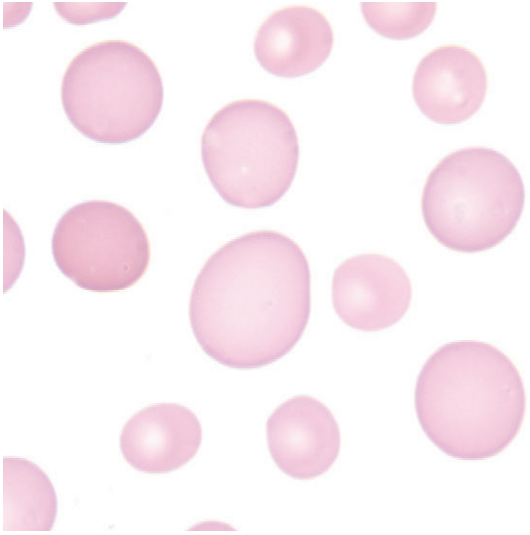
ANISOCYTOSIS



A

FIGURE 10–2A Heterogeneous population of erythrocytes, indicating anisocytosis (RDW > 14.5%).

Associated with: Anemias, especially iron deficiency, megaloblastic and hemolytic

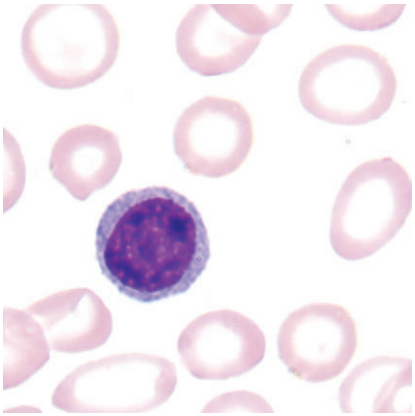


B

FIGURE 10–2B When two distinct populations of RBCs are seen, it is termed a dimorphic population (RDW > 14.5%).

Associated with: Transfusion, myelodysplastic syndromes, vitamin B_{12}, folate, or iron deficiencies—early in treatment process

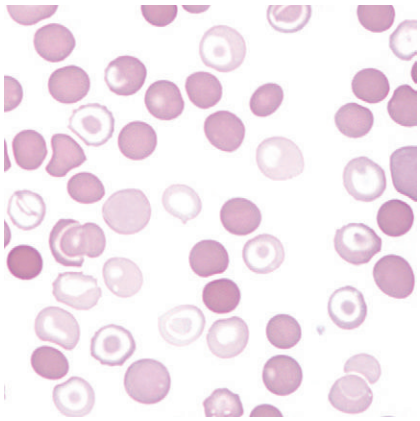
VARIATION IN COLOR OF ERYTHROCYTES



A

FIGURE 10–3A Hypochromia. The central pallor zone of the erythrocyte must be greater than one-third of the diameter of the cell before it is classified as hypochromic. (Note: the MCHC, not the MCH, should be used as a gauge of hypochromia; however, the MCHC is not always decreased when few hypochromic cells are seen.)

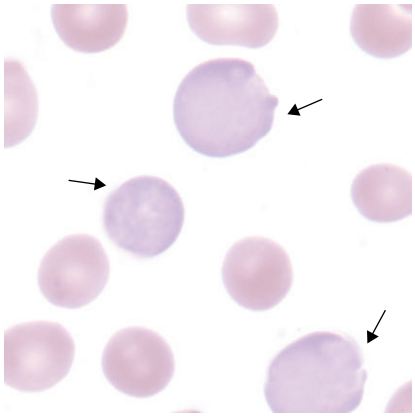
Associated with: Iron deficiency anemia, thalassemias, sideroblastic anemia, lead poisoning, some cases of anemia of chronic inflammation.



B

FIGURE 10–3B Dichromic population of erythrocytes. (Two populations of RBCs are shown: one normochromic and one hypochromic.)

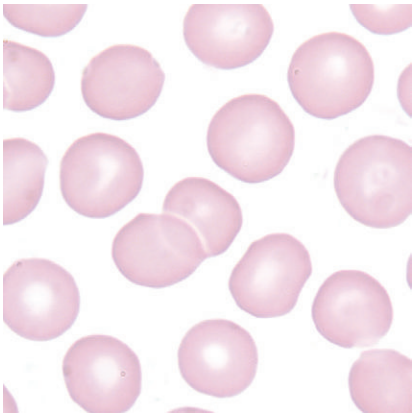
Associated with: Transfusions, sideroblastic anemia.



C

FIGURE 10–3C Polychromasia; retained RNA in RBCs.

Associated with: Acute and chronic hemorrhage, hemolysis, effective treatment for anemia, neonates.

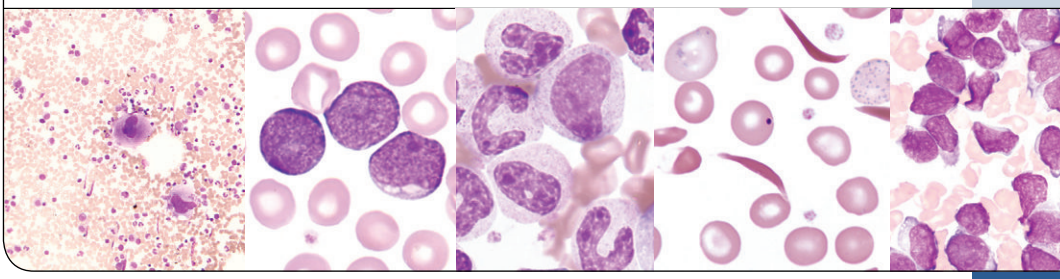


D

FIGURE 10–3D Normochromic erythrocytes. (MCHC 32-36 g/dL or 32%-36%.) For comparison with hypochromic and polychromatic erythrocytes.

11

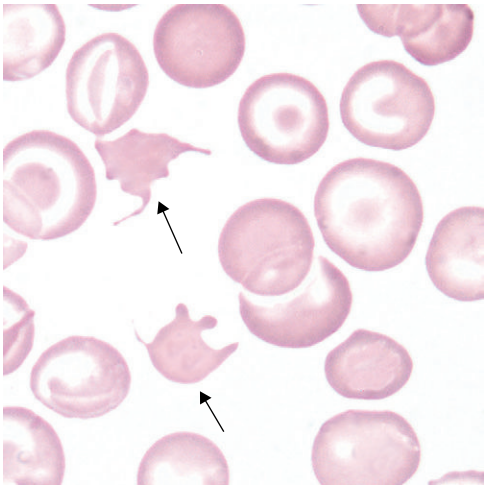
VARIATIONS IN SHAPE AND DISTRIBUTION OF ERYTHROCYTES



Poikilocytosis is a general term for the presence of abnormally shaped red blood cells. In most cases, we have opted to use the more specific name for each abnormally shaped red blood cell in place of the term *poikilocytosis.*

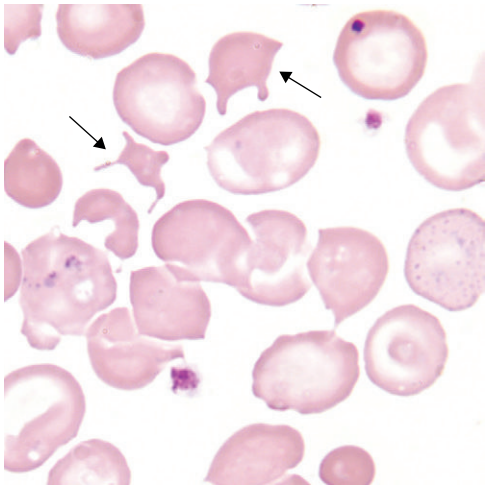
ACANTHOCYTE

Spur Cell



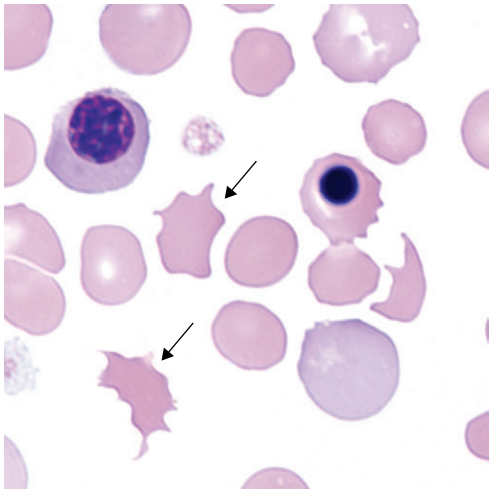
A

FIGURE 11–1A Acanthocytes.



B

FIGURE 11–1B Acanthocytes.



C

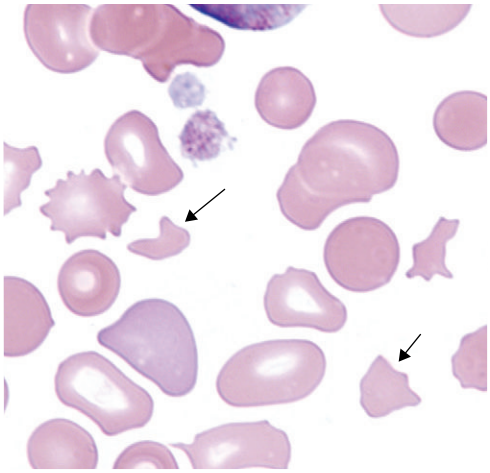
FIGURE 11–1C Acanthocytes; two nucleated red blood cells in field.

DESCRIPTION: Erythrocyte with irregularly spaced projections that vary in width, length, and number; usually dense, lacking central pallor

Associated with: Severe liver disease, splenectomy, malabsorption, hypothyroidism, vitamin E deficiency, abetalipoproteinemia

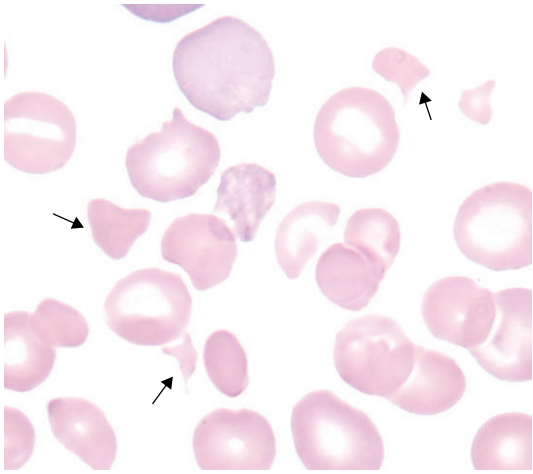
SCHISTOCYTE

Schizocyte



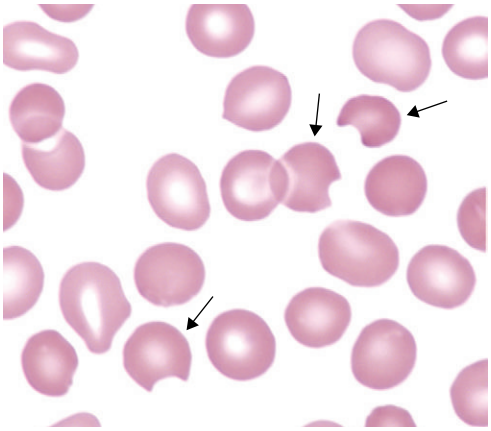
A

FIGURE 11–2A Schistocytes.



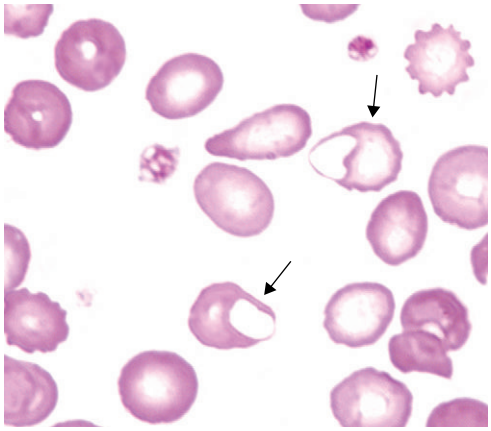
B

FIGURE 11–2B Schistocytes.



C

FIGURE 11–2C Bite cells.



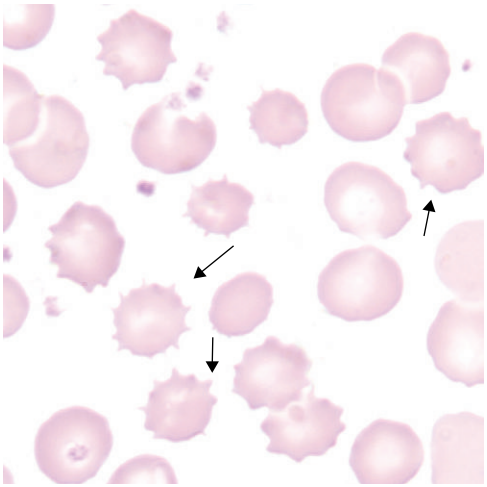
D

FIGURE 11–2D Blister cells.

COLOR: Red to salmon
SHAPE: Fragmented erythrocytes; many sizes and shapes may be present on a smear; often display pointed extremities
Associated with: Microangiopathic hemolytic anemia (hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation), severe burns, renal graft rejection
NOTE: Bite and blister cells are the result of splenic pitting of Heinz bodies (see Figure 12-5, *B*). These cells are often included in the schistocyte category.

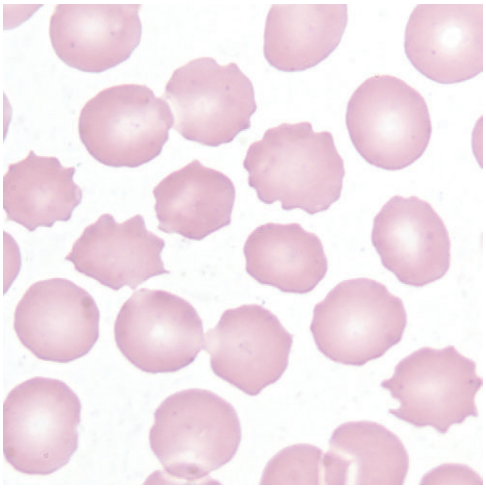
ECHINOCYTE

Burr Cell



A

FIGURE 11–3A Echinocytes/burr cells.

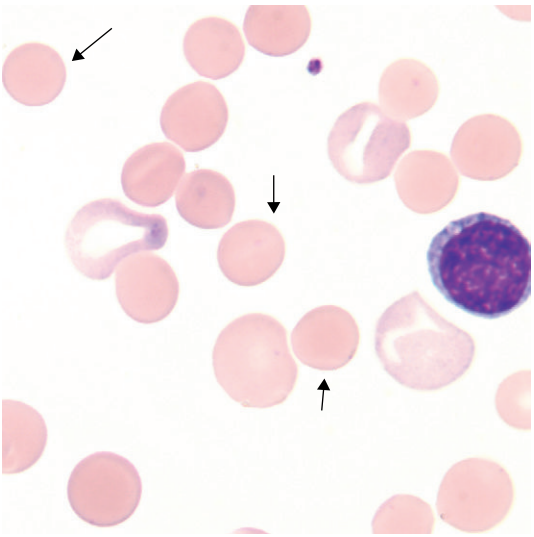


B

FIGURE 11–3B Echinocytes/burr cells.

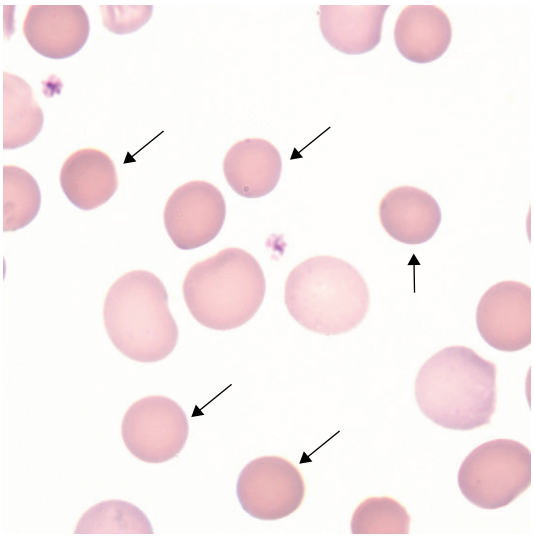
DESCRIPTION: Erythrocyte with short, evenly spaced projections usually with central pallor
Associated with: Uremia, pyruvate kinase deficiency, microangiopathic hemolytic anemia, neonates (especially premature), artifact

SPHEROCYTE



A

FIGURE 11–4A Spherocytes.



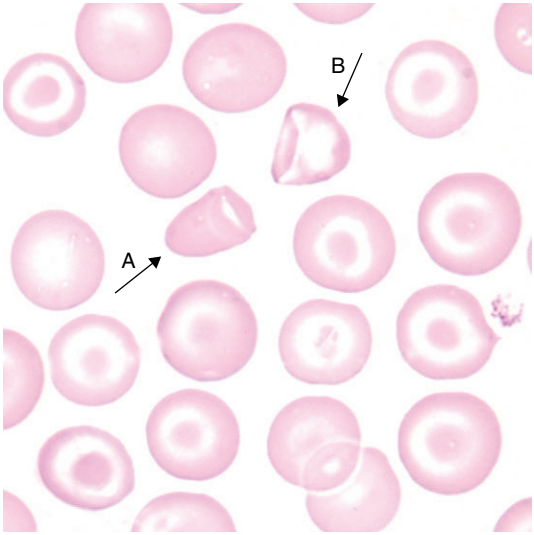
B

FIGURE 11–4B Spherocytes.

COLOR: Darker than surrounding red blood cells
SHAPE: Round; no central pallor zone
Associated with: Hereditary spherocytosis, some hemolytic anemias, transfused cells, severe burns

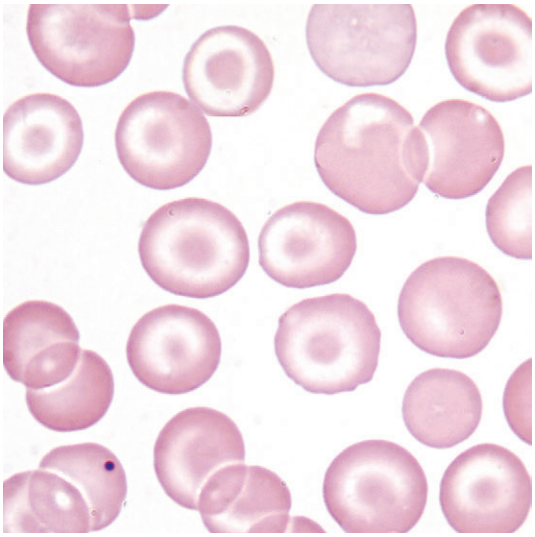
TARGET CELL

Codocyte



A

FIGURE 11–5A Target cells.



B

FIGURE 11–5B Target cells.

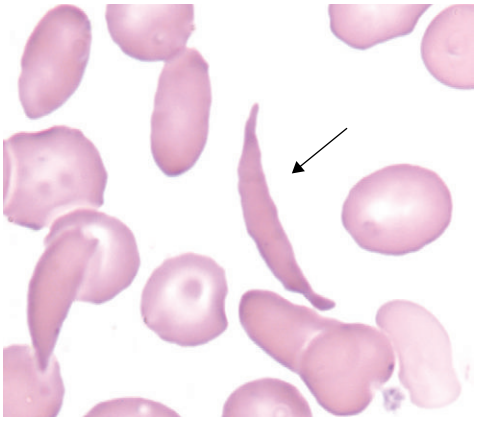
COLOR: Red to salmon

SHAPE: Bull's eye; central concentration of hemoglobin surrounded by colorless area with peripheral ring of hemoglobin resembling bull's eye; may be bell (Figure 11-5, *A*, *arrow A*) or cup (see Figure 11-5, *A*, *arrow B*) shaped.

Associated with: Hemoglobinopathies, thalassemia, iron deficiency anemia, splenectomy, obstructive liver disease

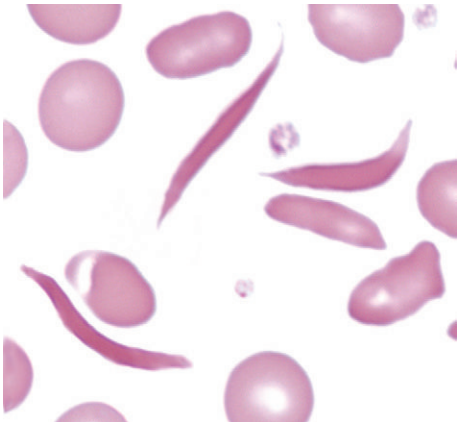
SICKLE CELL

Drepanocyte



A

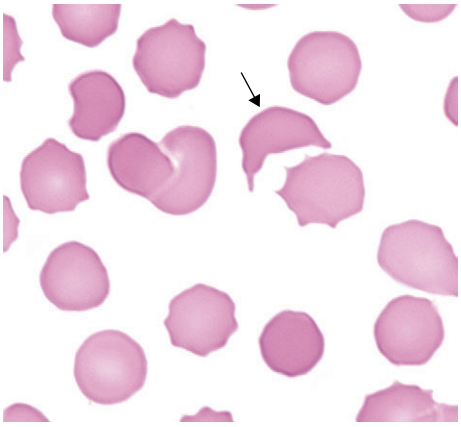
FIGURE 11–6A Sickle cells.



B

FIGURE 11–6B Sickle cells.

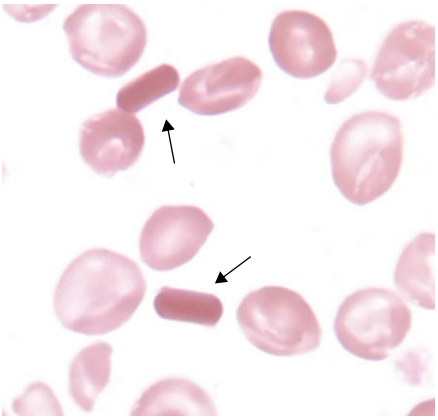
COLOR: Dark red to salmon, lacks central pallor
SHAPE: Elongated cell with point on each end; may be curved or S-shaped
COMPOSITION: Hemoglobin S
Associated with: Homozygous hemoglobin S disease, sometimes hemoglobin SC



C

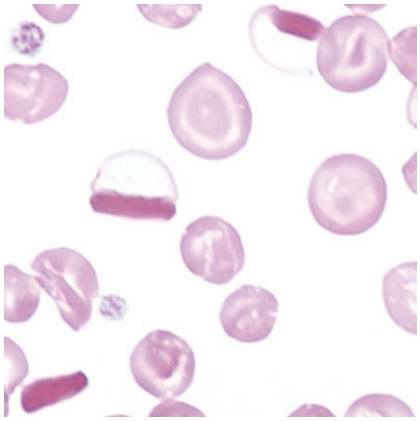
FIGURE 11–6C Schistocyte resembling sickle cell. (Note: Central area is markedly thicker than the ends.)

HEMOGLOBIN C CRYSTAL



A

FIGURE 11–7A Hemoglobin CC crystals.

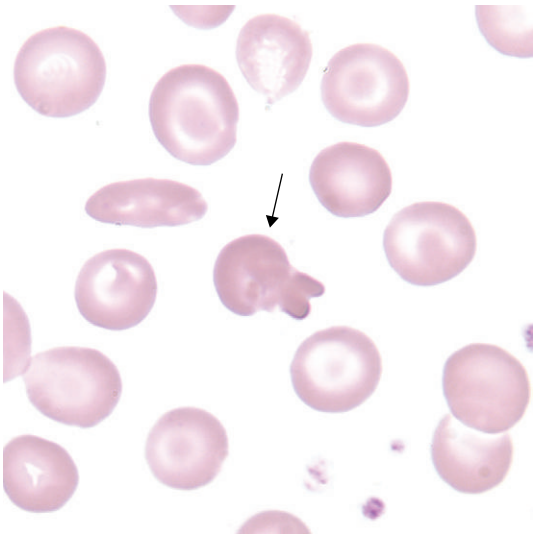


B

FIGURE 11–7B Hemoglobin CC crystals with visible red blood cell membrane.

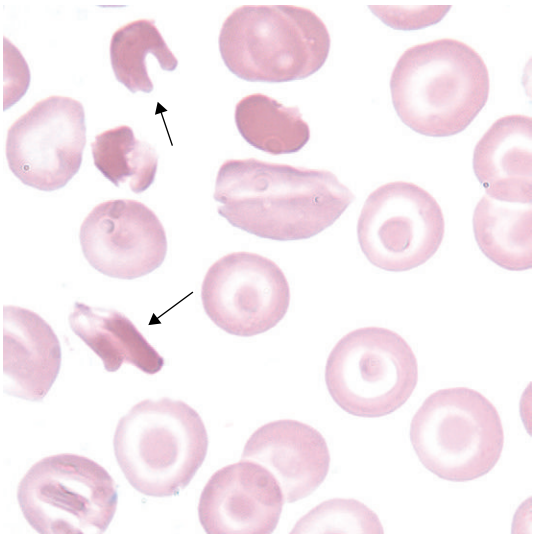
COLOR: Dark red
SHAPE: Hexagonal
NUMBER PER CELL: 1
COMPOSITION: Hemoglobin C
Associated with: Homozygous hemoglobin C disease

HEMOGLOBIN SC CRYSTAL



A

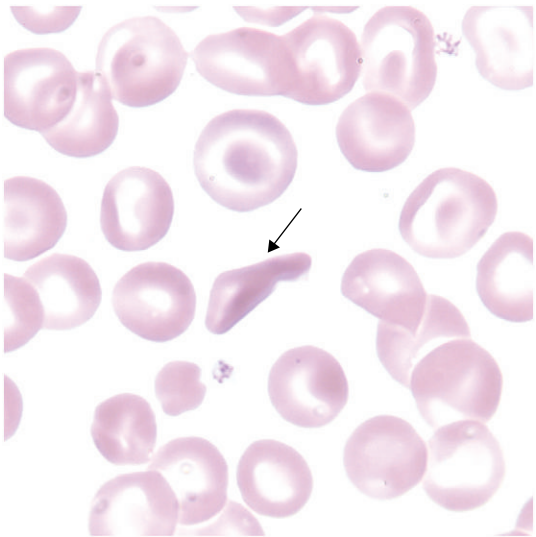
FIGURE 11–8A Hemoglobin SC.



B

FIGURE 11–8B Hemoglobin SC.

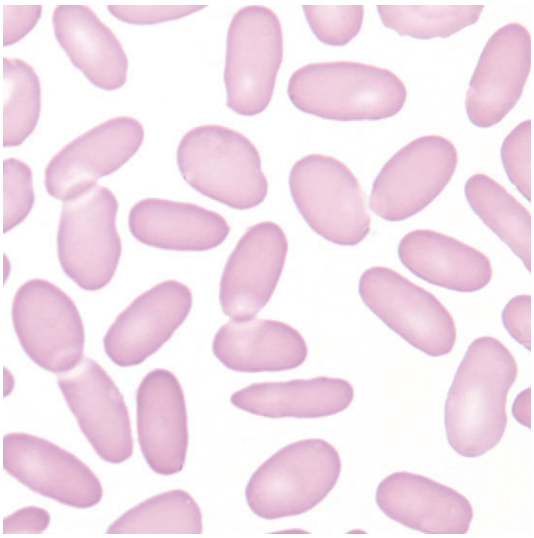
COLOR: Dark red
SHAPE: 1-2 fingerlike projections; may look like a mitten or the Washington Monument (obelisk); cell may appear folded
NUMBER PER CELL: 1-2
COMPOSITION: Hemoglobin SC
Associated with: Hemoglobin SC disease



C

FIGURE 11–8C Hemoglobin SC.

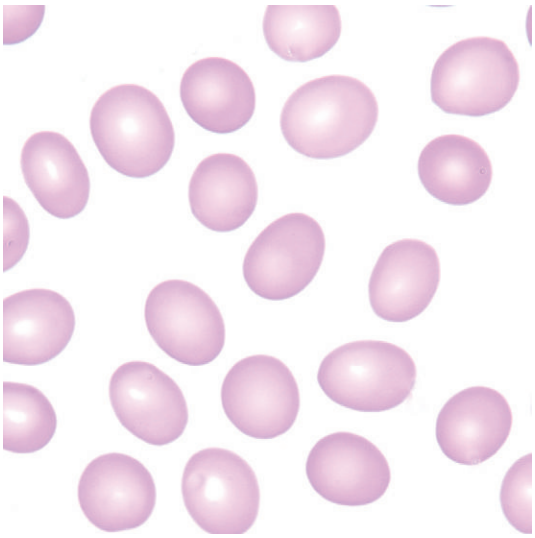
ELLIPTOCYTE/OVALOCYTE



A

FIGURE 11–9A Elliptocytes.

DESCRIPTION: Elliptocyte—cigar-shaped erythrocyte



B

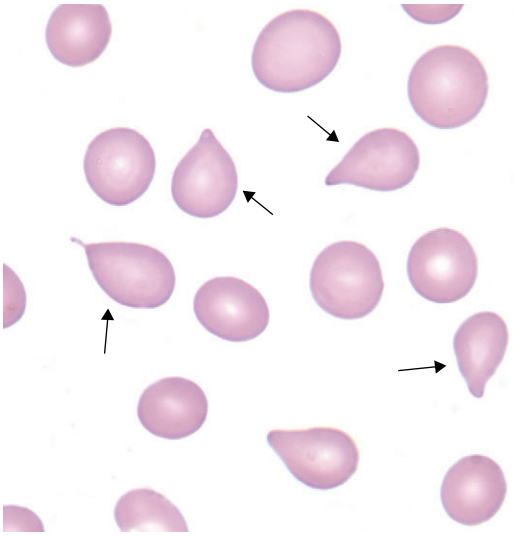
FIGURE 11–9B Ovalocytes.

DESCRIPTION: Ovalocyte—egg-shaped erythrocyte

Associated with: Hereditary elliptocytosis or ovalocytosis, thalassemia major, iron deficiency anemia, megaloblastic anemias (macro-ovalocytes), myelophthisic anemias

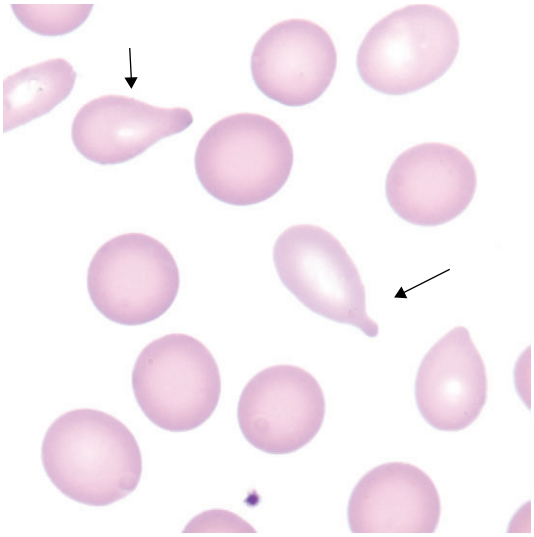
TEAR DROP CELL

Dacryocyte



A

FIGURE 11–10A Tear drop cells.



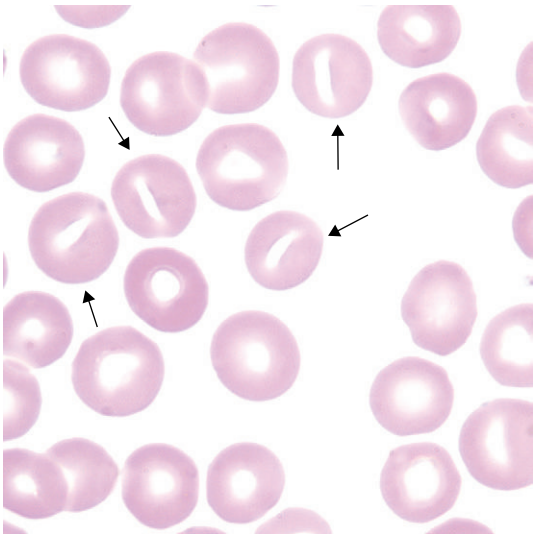
B

FIGURE 11–10B Tear drop cells.

DESCRIPTION: Erythrocyte shaped like a tear drop or pear; may have one blunt projection

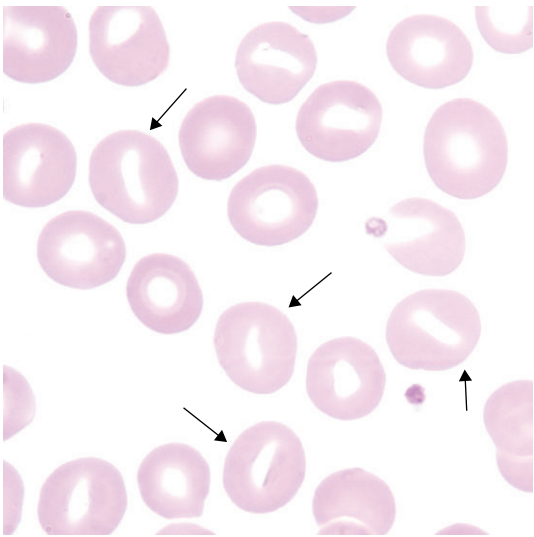
Associated with: Primary myelofibrosis, thalassemia, myelophthisic anemia, other causes of extramedullary hematopoiesis

STOMATOCYTE



A

FIGURE 11–11A Stomatocytes.

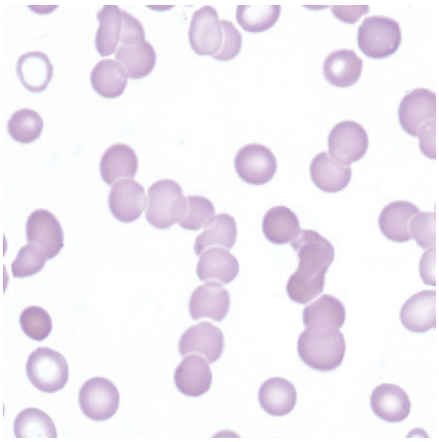


B

FIGURE 11–11B Stomatocytes.

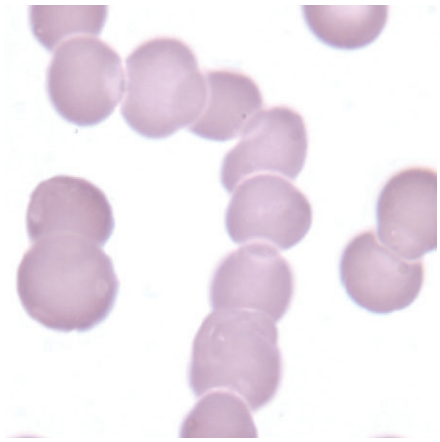
DESCRIPTION: Erythrocyte with slitlike area of central pallor (similar to a mouth or stoma)
Associated with: Hereditary stomatocytosis, alcoholism, liver disease, Rh null phenotype, artifact

ROULEAUX VERSUS AUTOAGGLUTINATION



A

FIGURE 11–12A Rouleaux (×500).

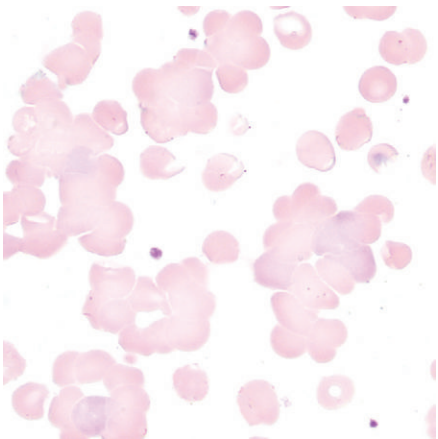


B

FIGURE 11–12B Rouleaux (×1000).

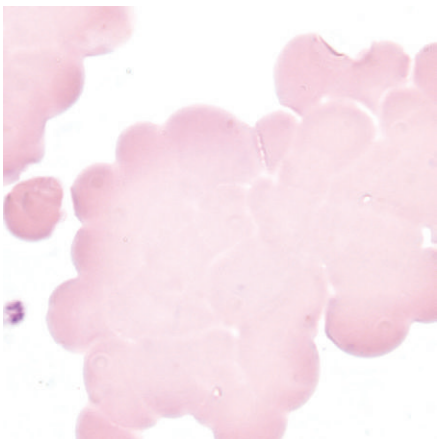
ROULEAUX

DESCRIPTION: Erythrocytes arranged in rows like stacks of coins; increased proteins in patients with rouleaux may make the background of the slide appear blue
Associated with: Acute and chronic inflammatory disorders, plasma cell myeloma, lymphoplasmacytic lymphoma
NOTE: These aggregates will disperse with saline.



C

FIGURE 11–12C Autoagglutination (×500).



D

FIGURE 11–12D Autoagglutination (×1000).

AUTOAGGLUTINATION

DESCRIPTION: Clumping of erythrocytes; outlines of individual cells may not be evident
Associated with: Antigen-antibody reactions
NOTE: Aggregate will not disperse with saline.

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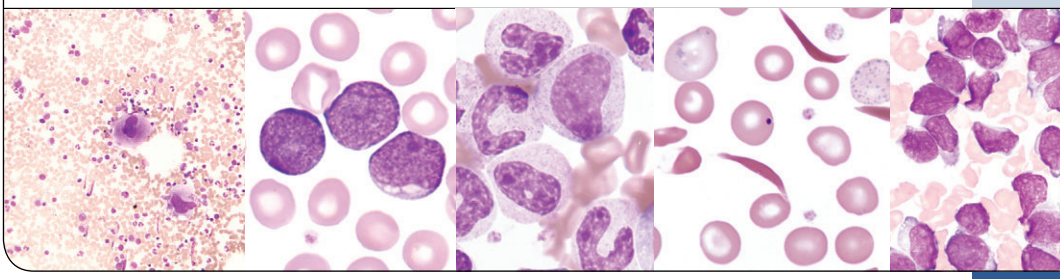
12

INCLUSIONS IN ERYTHROCYTES



HOWELL-JOLLY BODIES

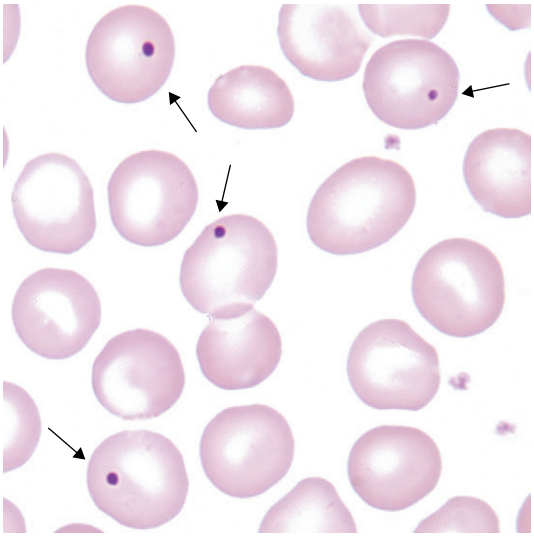
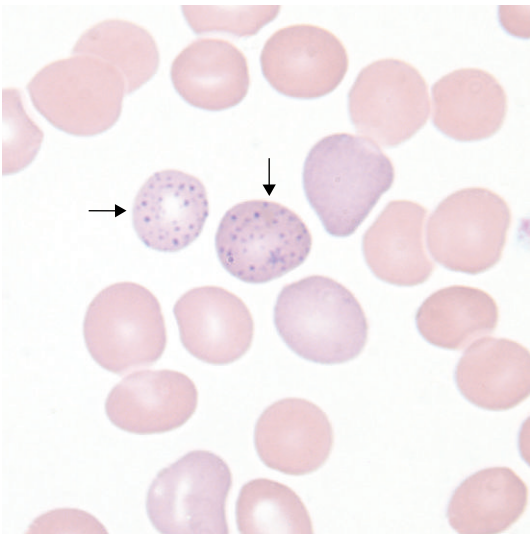


FIGURE 12–1 Howell-Jolly bodies.

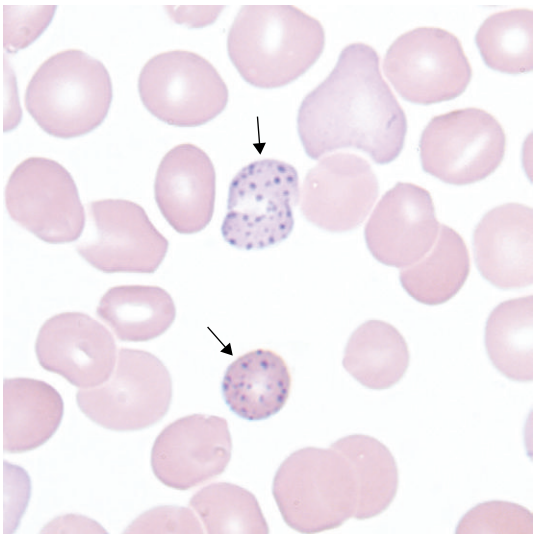
COLOR: Dark blue to purple
SHAPE: Round to oval
SIZE: 0.5-1.5 μm
NUMBER PER CELL: Usually 1; may be multiple
COMPOSITION: DNA
Associated with: Splenectomy, hyposplenism, megaloblastic anemia, hemolytic anemia

BASOPHILIC STIPPLING



A

FIGURE 12–2A Basophilic stippling.



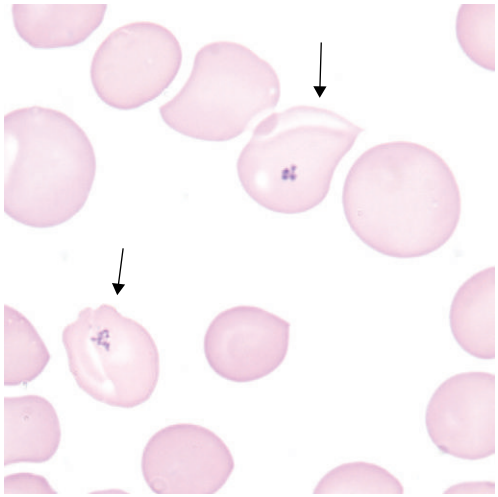
B

FIGURE 12–2B Basophilic stippling.

COLOR: Dark blue to purple
SHAPE: Fine or coarse punctate granules
NUMBER PER CELL: Numerous with fairly even distribution
COMPOSITION: RNA
Associated with: Lead intoxication, thalassemia, abnormal heme synthesis

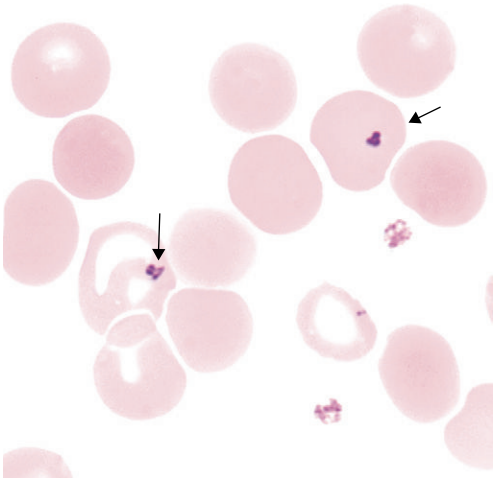
PAPPENHEIMER BODIES

Siderotic Granules



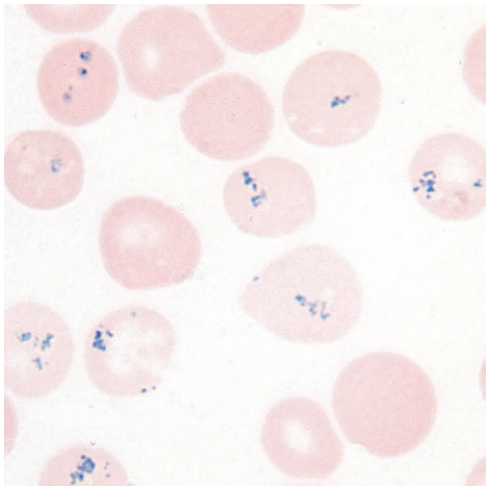
A

FIGURE 12–3A Pappenheimer bodies (Wright stain).



B

FIGURE 12–3B Pappenheimer bodies (Wright stain).

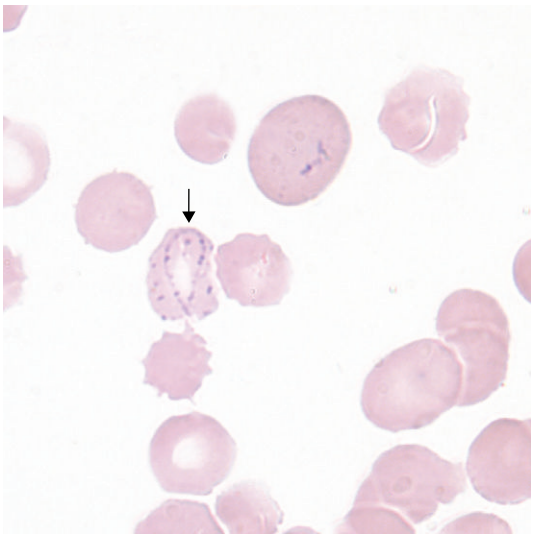


C

FIGURE 12–3C Siderotic granules (iron stain).

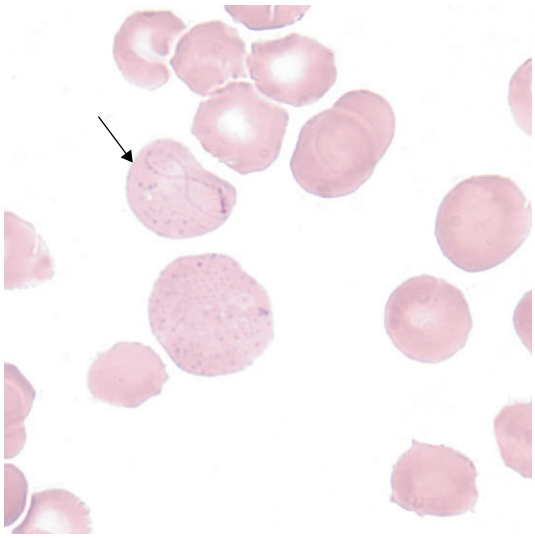
COLOR: Light blue
SHAPE: Fine irregular granules in clusters
NUMBER PER CELL: Usually one cluster; may be multiples; often at periphery of cell
COMPOSITION: Iron
Associated with: Splenectomy, hemolytic anemia, sideroblastic anemia, megaloblastic anemia, hemoglobinopathies

CABOT RINGS



A

FIGURE 12–4A Cabot ring.



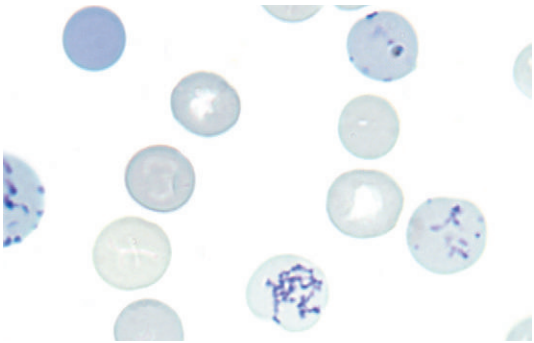
B

FIGURE 12–4B Cabot ring—figure eight.

COLOR: Dark blue to purple
SHAPE: Loop, ring, or figure eight; may look like beads on a string
NUMBER PER CELL: 1-2
COMPOSITION: Thought to be remnants of mitotic spindle
Associated with: Myelodysplastic syndrome, megaloblastic anemia
NOTE: This is a rare finding. Do not confuse with malaria (see Figure 21-1).

INCLUSIONS WITH SUPRAVITAL STAIN

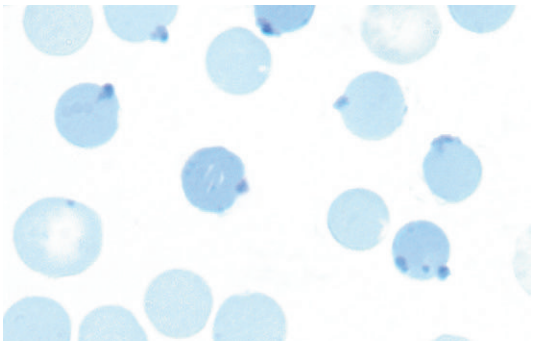
Stained with New Methylene Blue



A

FIGURE 12–5A Reticulocytes.

CELL: Anuclear immature erythrocyte
COMPOSITION: Precipitated RNA
NUMBER: ≥2 per cell
COLOR: Dark blue
Associated with: Erythrocyte maturation
NOTE: Supravital stains are taken up by living cells.



B

FIGURE 12–5B Heinz bodies.

CELL: Mature erythrocyte
COMPOSITION: Precipitated hemoglobin
NUMBER: Single or multiple, generally membrane-bound
COLOR: Dark blue to purple
Associated with: Unstable hemoglobin, some hemoglobinopathies, some erythrocyte enzyme deficiencies (e.g., glucose-6-phosphate dehydrogenase)

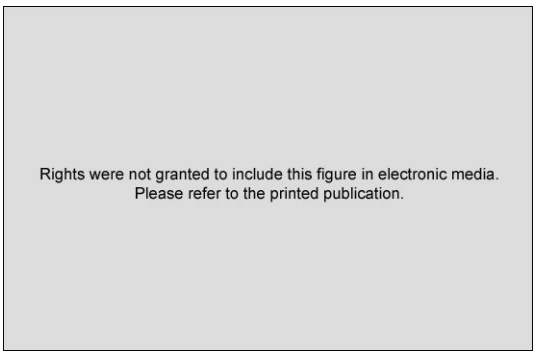


FIGURE 12–5C Hemoglobin H. (From the American Society for Hematology slide bank.)

CELL: Mature erythrocyte
COMPOSITION: Hemoglobin β chains
NUMBER: Multiple evenly dispersed granules described as “golf balls” or “raspberries”
COLOR: Dark blue

TABLE 12-1 Staining Qualities of Erythrocyte Inclusion Bodies

Inclusion	Composition	Wright- Giemsa Stain	New Methylene Blue (or Other Supravital Stain)	Prussian Blue (Iron)
Howell-Jolly body	DNA	+	+	0
Basophilic stippling	RNA	+	+	0
Pappenheimer body	Iron	+	+	+
Cabot ring	Remnant of mitotic spindle	+	+	0
Heinz body	Unstable hemoglobin	0	+	0
Hemoglobin H	β chains	0	+	0

+, Positive; 0, negative.

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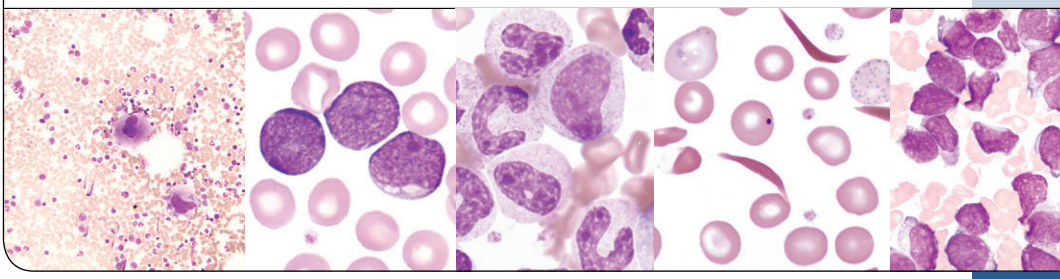
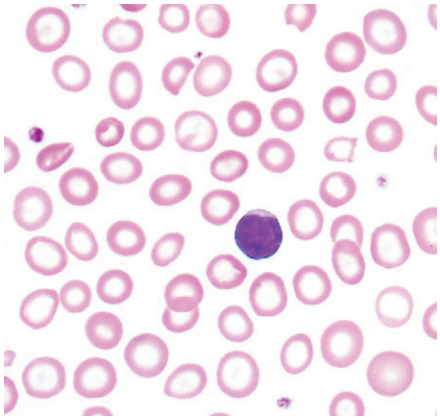
13

DISEASES AFFECTING ERYTHROCYTES



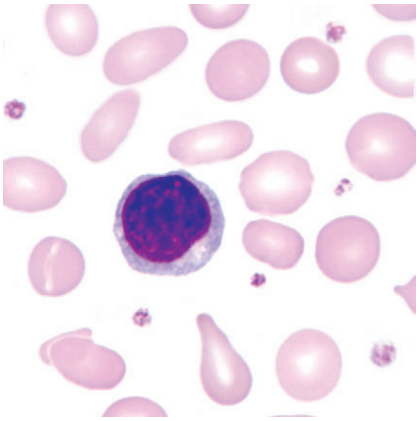
MICROCYTIC/HYPOCHROMIC ANEMIA

Iron Deficiency Anemia



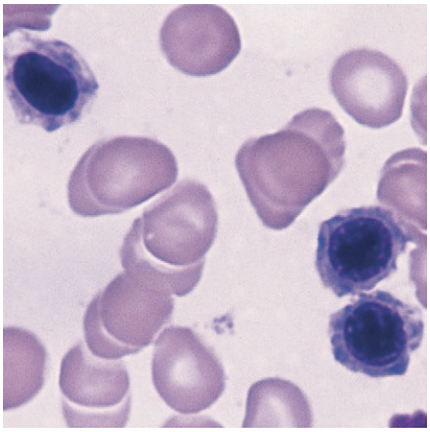
A

FIGURE 13–1A Severe iron deficiency anemia (peripheral blood [PB] ×500).



B

FIGURE 13–1B Iron deficiency anemia (PB ×1000).



C

FIGURE 13–1C Iron deficiency anemia (bone marrow [BM] ×1000; showing shaggy cytoplasm).

Peripheral Blood: Erythrocytes are hypochromic and microcytic; large variation in size; possible thrombocytosis

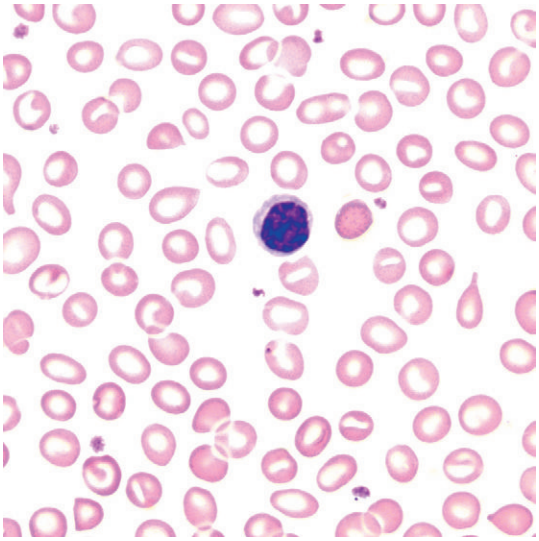
NOTE: Small lymphocyte depicted for size comparison.

Bone Marrow: Erythrocyte precursors are smaller and more numerous than normal and have shaggy cytoplasm. There is nuclear cytoplasmic asynchrony, with cytoplasmic maturation lagging behind that of the nucleus.

Although characteristic findings for disease states are listed, not all may be present in one patient. The most common ones are depicted.

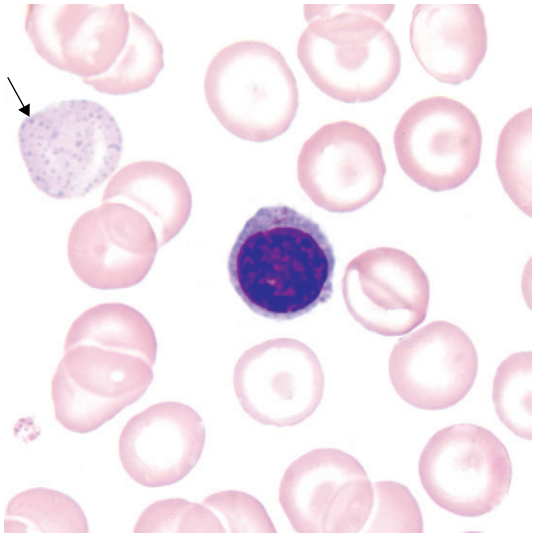
β-THALASSEMIA MINOR

β/β^{+} β/β^{o} $\beta/\delta\beta^{o}$ $\beta/\delta\beta^{Lepore}$



A

FIGURE 13–2A β-Thalassemia minor (PB ×500).



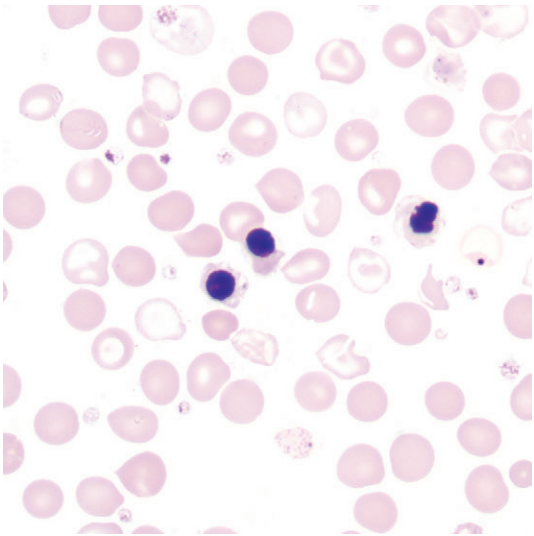
B

FIGURE 13–2B β-Thalassemia minor (PB ×1000). The presence of basophilic stippling (*arrow*) is common in thalassemia minor but not in iron deficiency anemia.

Peripheral Blood: Microcytosis, slight hypochromia, target cells, basophilic stippling

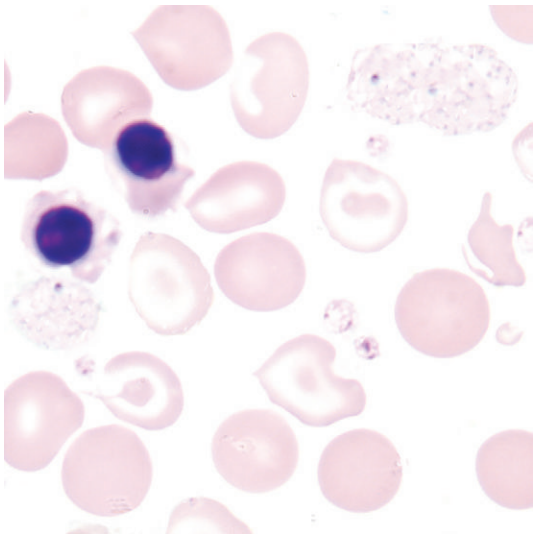
β-THALASSEMIA MAJOR

$\beta^o\beta^o$ $\beta^+\beta^+$ $\beta^o\beta^+$ $\delta\beta^{Lepore}/\delta\beta^{Lepore}$



A

FIGURE 13–3A β-Thalassemia major (PB ×500).



B

FIGURE 13–3B β-Thalassemia major (PB ×1000).

Peripheral Blood: Marked variation in size and shape, numerous nucleated erythrocytes, microcytes, hypochromia, target cells, basophilic stippling, tear drop cells, schistocytes, polychromasia

α-THALASSEMIA*

Hemoglobin H

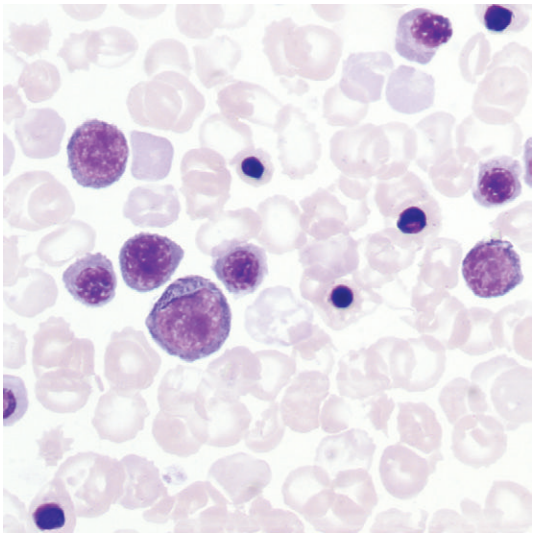
$--/-\alpha$

Peripheral Blood: Microcytes, hypochromia, marked poikilocytosis, target cells, polychromasia (see Figure 12-5, *C*).

*α-Thalassemia minor ($--/\alpha\alpha, -\alpha/-\alpha$) has red cell morphology similar to β-thalassemia minor and as such is not represented here.

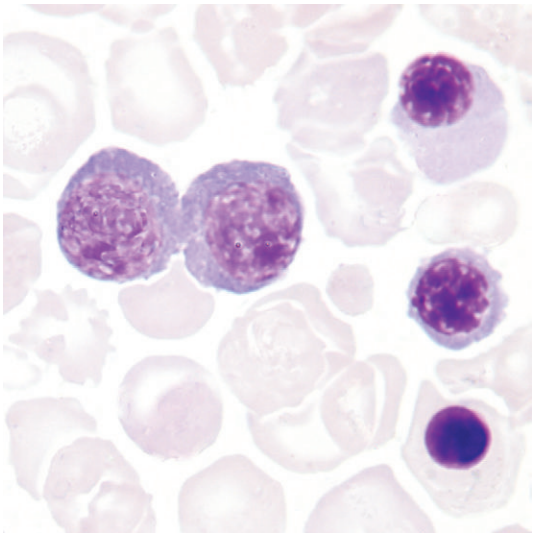
HEMOGLOBIN BART HYDROPS FETALIS SYNDROME

––/––



A

FIGURE 13–4A Bart hemoglobin (PB ×500).



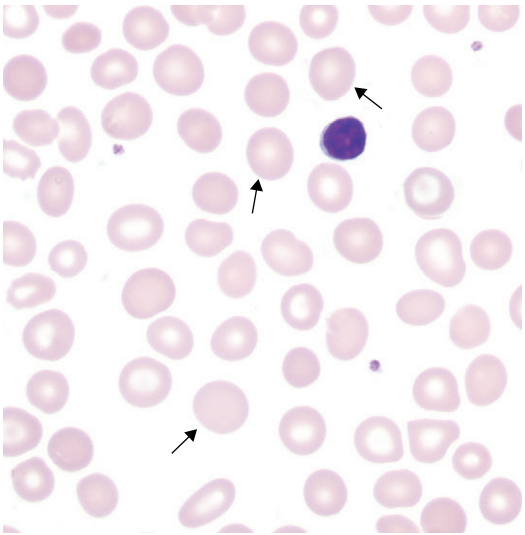
B

FIGURE 13–4B Bart hemoglobin (PB ×1000).

Peripheral Blood: Numerous nucleated erythrocytes, marked variation in size, hypochromia, variable polychromasia, macrocytes

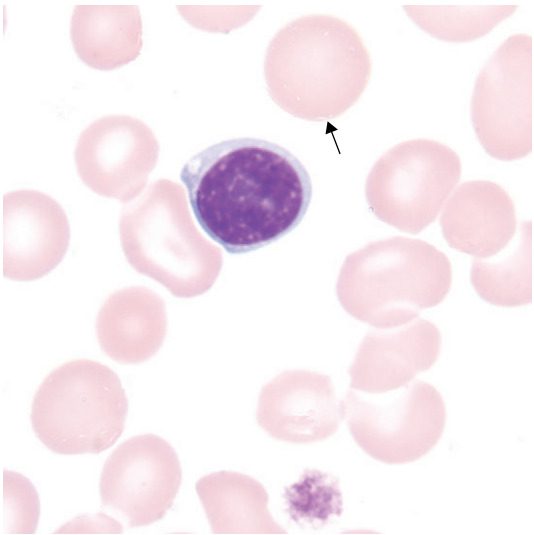
MACROCYTOSIS

Nonmegaloblastic



A

FIGURE 13–5A Macrocytic (nonmegaloblastic) (PB ×500).

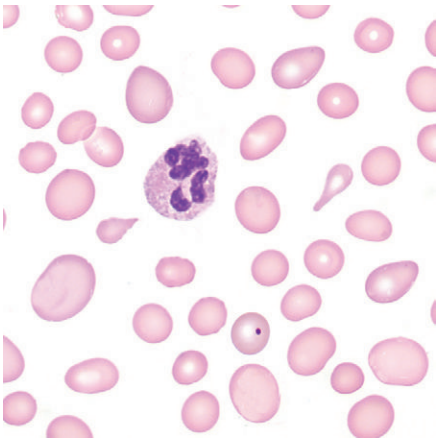


B

FIGURE 13–5B Macrocytic (nonmegaloblastic) (PB ×1000).

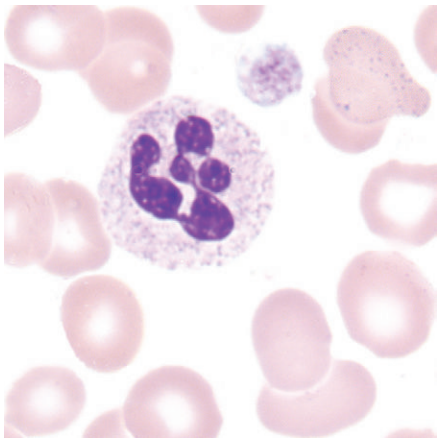
Peripheral Blood: Round macrocytes, leukocyte and platelet counts usually normal
Bone Marrow: No megaloblastic changes
Associated with: Normal newborn, liver disease, chronic alcoholism

MEGALOBLASTIC ANEMIA



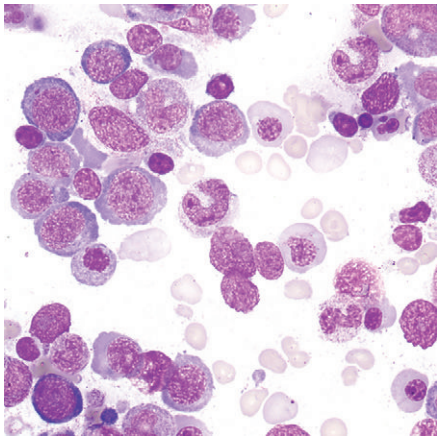
A

FIGURE 13–6A Megaloblastic anemia (PB ×500).



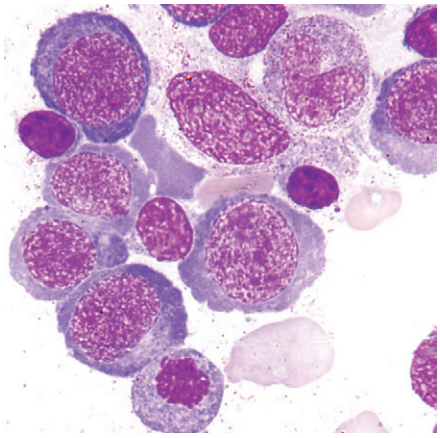
B

FIGURE 13–6B Megaloblastic anemia (PB ×1000).



C

FIGURE 13–6C Megaloblastic anemia (BM original ×500).



D

FIGURE 13–6D Megaloblastic anemia (BM original ×1000).

Peripheral Blood: Pancytopenia, hypersegmentation of neutrophils, oval macrocytes, Howell-Jolly bodies, nucleated erythrocytes, basophilic stippling, schistocytes, spherocytes, tear drop cells, target cells, giant platelets

NOTE: Characteristic triad of abnormalities: oval macrocytes, hypersegmented neutrophils, and Howell-Jolly bodies

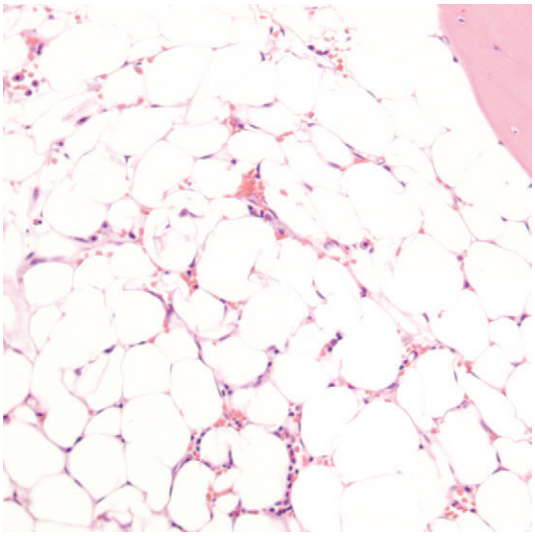
Bone Marrow: Hypercellular, asynchrony (trilineage) with nuclear maturation lagging behind cytoplasmic maturation, giant bands, giant metamyelocytes, hypersegmented neutrophils

Associated with: Vitamin B_{12} deficiency, folate deficiency, myelodysplastic syndrome

APLASTIC ANEMIA

A

FIGURE 13–7A Aplastic anemia (PB ×1000).

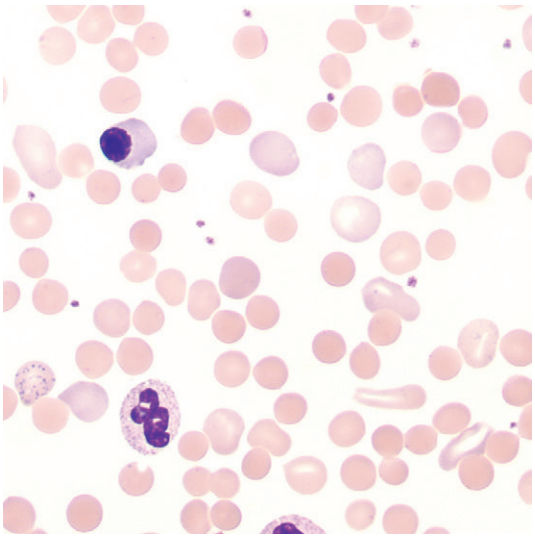


B

FIGURE 13–7B Aplastic anemia (BM biopsy ×1000).

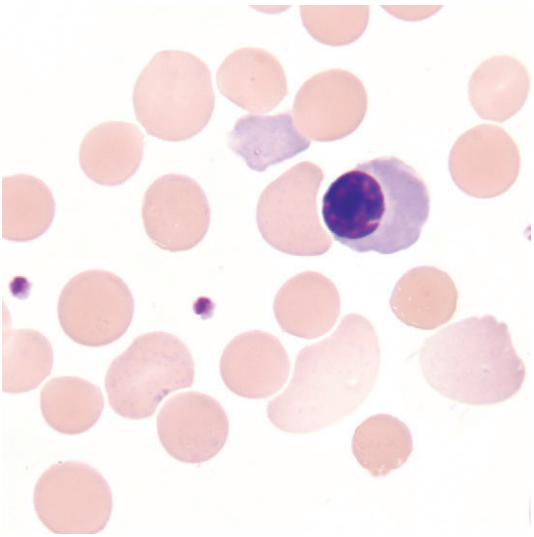
Peripheral Blood: Pancytopenia, normocytic, normochromic (occasional macrocytes)
Bone Marrow: Hypocellular; lymphocytes may predominate
Associated with: Bone marrow failure

IMMUNE HEMOLYTIC ANEMIA



A

FIGURE 13–8A Immune hemolytic anemia (PB ×500).

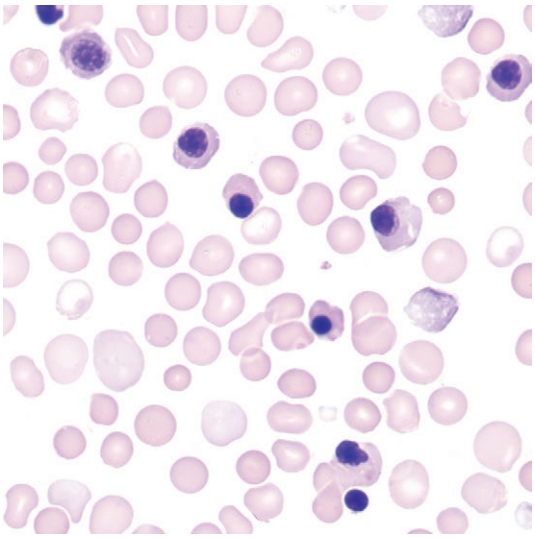


B

FIGURE 13–8B Immune hemolytic anemia (PB ×1000).

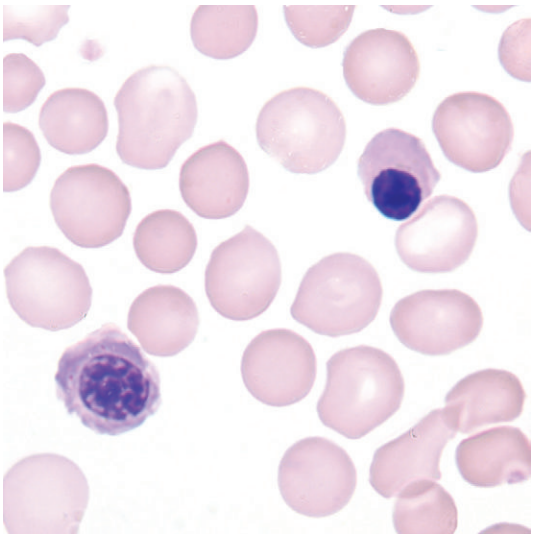
Peripheral Blood: Spherocytes, schistocytes, polychromasia, nucleated erythrocytes
Associated with: Autoimmune, alloimmune (see also hemolytic disease of the fetus and newborn, Figure 13-9), drug-induced hemolytic anemia
NOTE: Erythrocyte morphology varies with cause and severity of disease.

HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN



A

FIGURE 13–9A Hemolytic disease of the fetus and newborn (PB ×500).

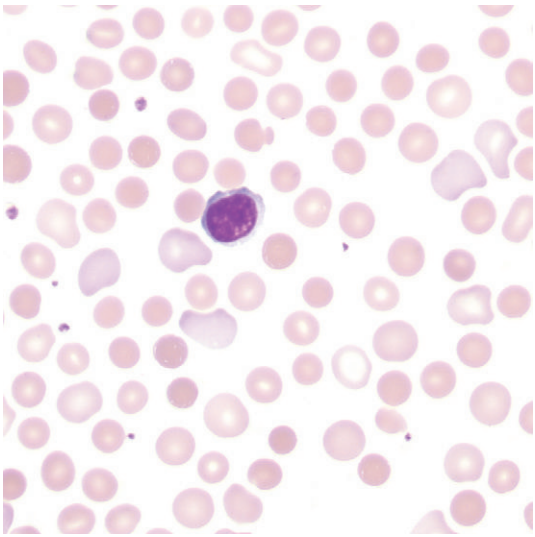


B

FIGURE 13–9B Hemolytic disease of the fetus and newborn (PB ×1000).

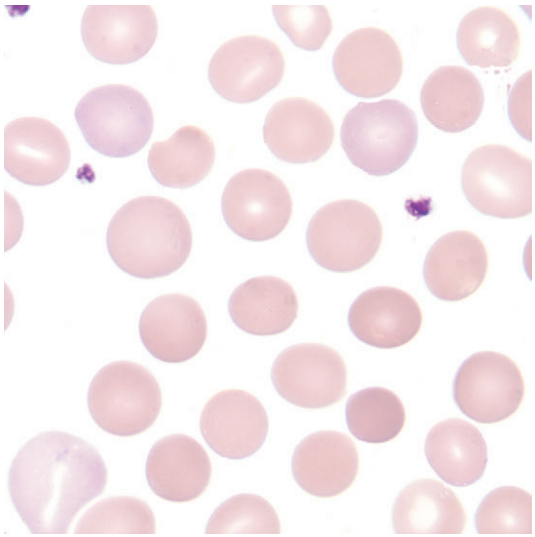
Peripheral Blood: Polychromasia, increased number of nucleated erythrocytes, macrocytic/normochromic, spherocytes—more common in ABO incompatibility
Associated with: Fetal-maternal Rh and/or ABO incompatibility
NOTE: Normal newborns have some nucleated erythrocytes (see Chapter 23).

HEREDITARY SPHEROCYTOSIS



A

FIGURE 13–10A Hereditary spherocytosis (PB ×500).



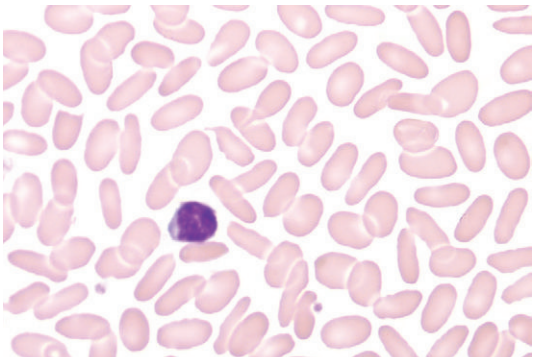
B

FIGURE 13–10B Hereditary spherocytosis (PB ×1000).

Peripheral Blood: Spherocytes (variable in number), polychromasia; nucleated erythrocytes possible

Associated with: Red cell membrane defects

HEREDITARY ELLIPTOCYTOSIS



A

FIGURE 13–11A Hereditary elliptocytosis (PB ×500).

Peripheral Blood: >25% elliptocytes, usually >60% elliptocytes; indices are normocytic, normochromic

Associated with: Red cell membrane defects

VARIANTS OF ELLIPTOCYTOSIS

Hemolytic

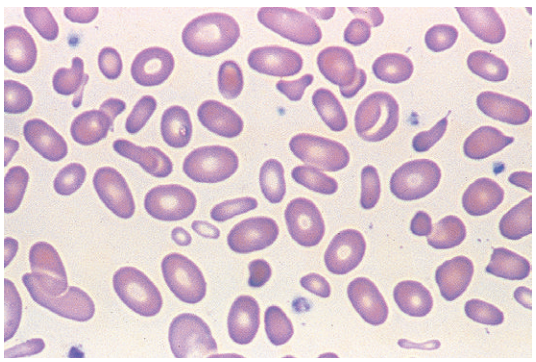
Peripheral Blood: Microelliptocytes, schistocytes, spherocytes

Associated with: Red cell membrane defects

Pyropoikilocytosis

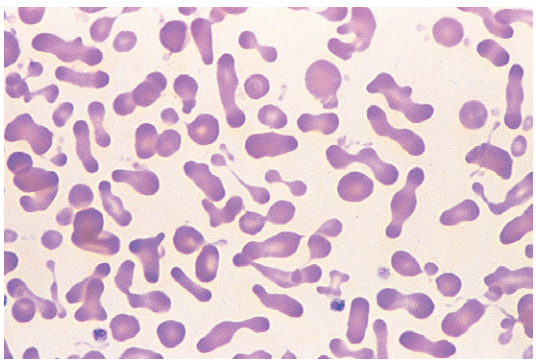
Peripheral Blood: Elliptocytes, schistocytes, microspherocytes (see Figure 11-4, *B*).

Associated with: Red cell membrane defects



B

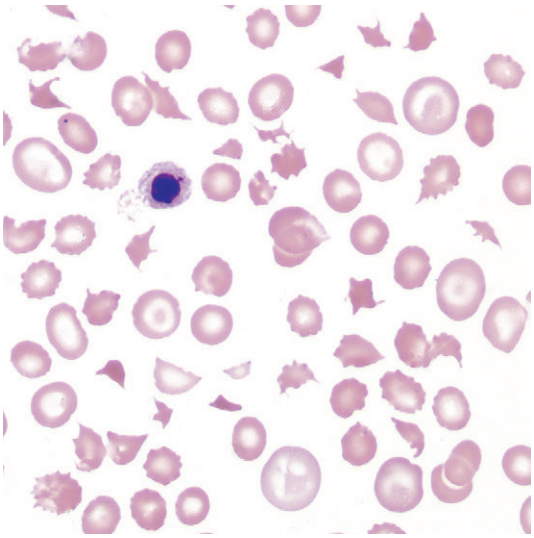
FIGURE 13–11B Hereditary pyropoikilocytosis—before incubation (PB ×500).



C

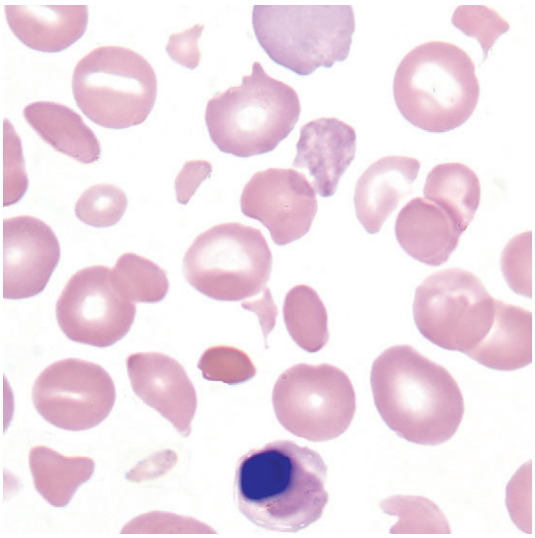
FIGURE 13–11C Hereditary pyropoikilocytosis—with incubation at 41° to 45° C for 1 hour (PB ×500).

MICROANGIOPATHIC HEMOLYTIC ANEMIA



A

FIGURE 13–12A Microangiopathic hemolytic anemia (PB ×500).



B

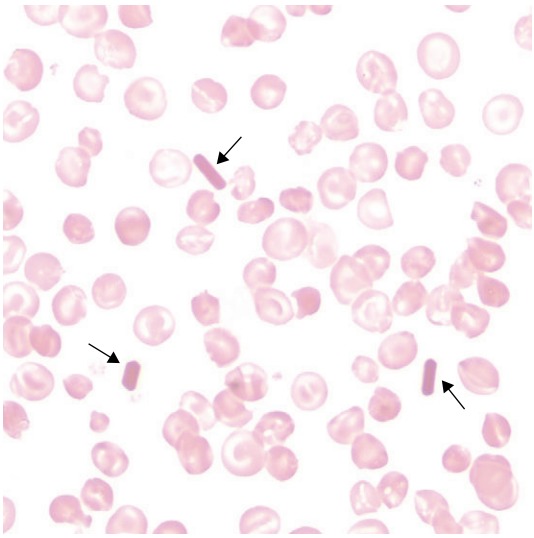
FIGURE 13–12B Microangiopathic hemolytic anemia (PB ×1000).

Peripheral Blood: Schistocytes, spherocytes, polychromasia, nucleated erythrocytes, decreased platelet count

Associated with: Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, HELLP syndrome (Hemolytic anemia, Elevated Liver enzymes and Low Platelet count), disseminated intravascular coagulation, hypertensive crises

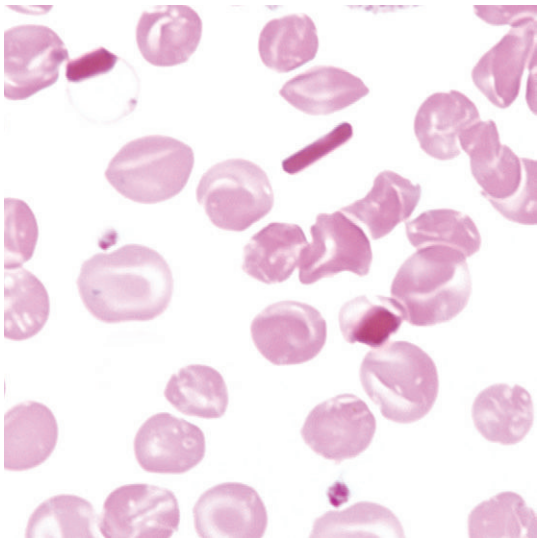
NOTE: The degree of morphological change correlates directly with severity of the disease.

HEMOGLOBIN CC DISEASE



A

FIGURE 13–13A Hemoglobin CC (PB ×500).



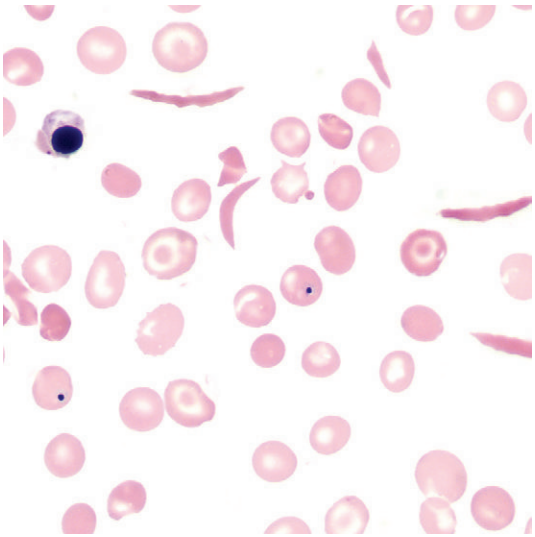
B

FIGURE 13–13B Hemoglobin CC (PB ×1000).

Peripheral Blood: Polychromasia, target cells, spherocytes, microcytes, intracellular and/or extracellular rod-shaped crystals possible

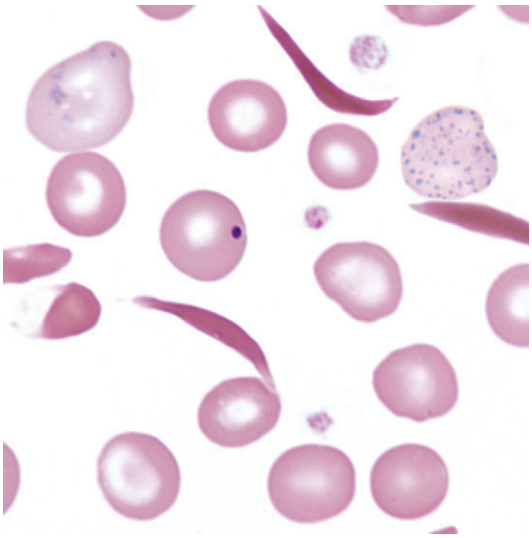
Associated with: Homozygous hemoglobin C (see Figure 11-7)

HEMOGLOBIN SS DISEASE



A

FIGURE 13–14A Hemoglobin SS (PB ×500).



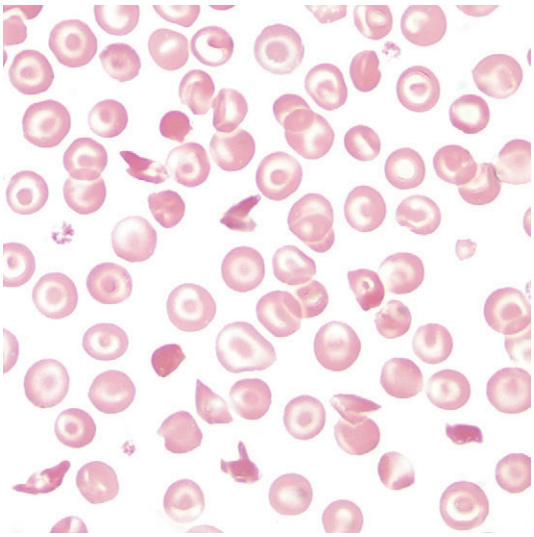
B

FIGURE 13–14B Hemoglobin SS (PB ×1000).

Peripheral Blood: Sickle cells (in crises), target cells, nucleated erythrocytes, schistocytes, Howell-Jolly bodies, basophilic stippling, Pappenheimer bodies, polychromasia, increased leukocyte count with neutrophilia, thrombocytosis

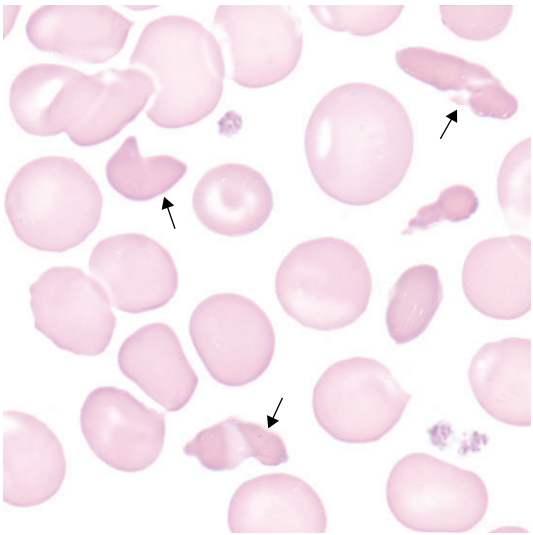
Associated with: Homozygous hemoglobin S (see Figure 11-6)

HEMOGLOBIN SC DISEASE



A

FIGURE 13–15A Hemoglobin SC (PB 500).



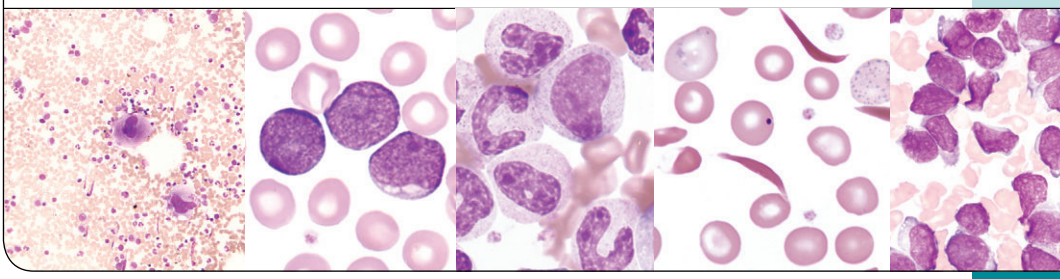
B

FIGURE 13–15B Hemoglobin SC (PB ×1000).

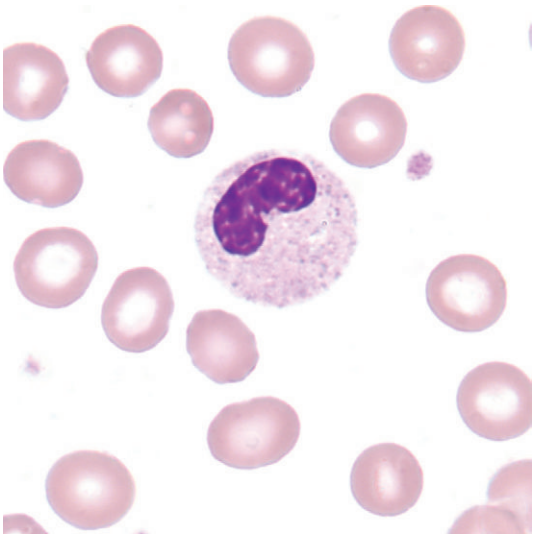
Peripheral Blood: Few sickle cells, target cells, intraerythrocytic crystals; crystalline aggregates of hemoglobin SC may protrude from the erythrocyte membrane.
Associated with: Hemoglobin SC (see Figure 11-8)

14

NUCLEAR AND CYTOPLASMIC CHANGES IN LEUKOCYTES

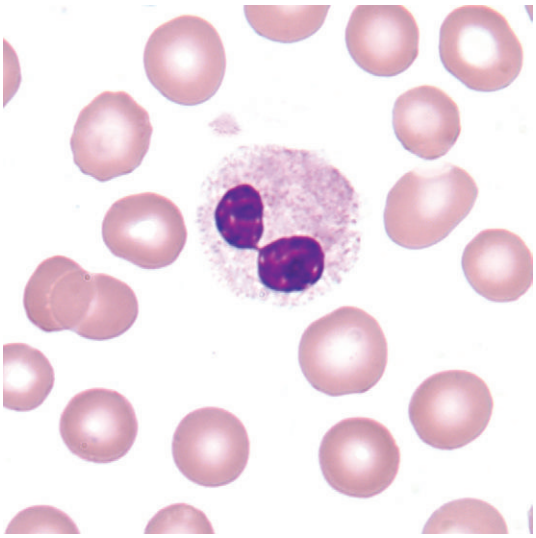


HYPOSEGMENTATION OF NEUTROPHILS



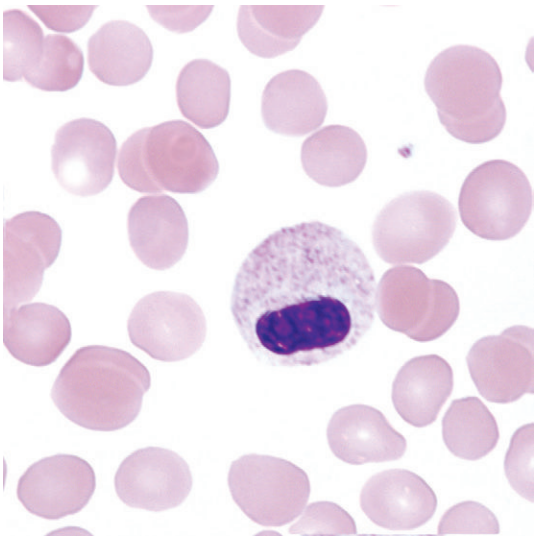
A

FIGURE 14–1A Hyposegmentation—peanut-shaped nucleus (PB ×1000).



B

FIGURE 14–1B Hyposegmentation—bilobed nucleus (PB ×1000).



C

FIGURE 14–1C Non-segmented nucleus (PB ×1000)

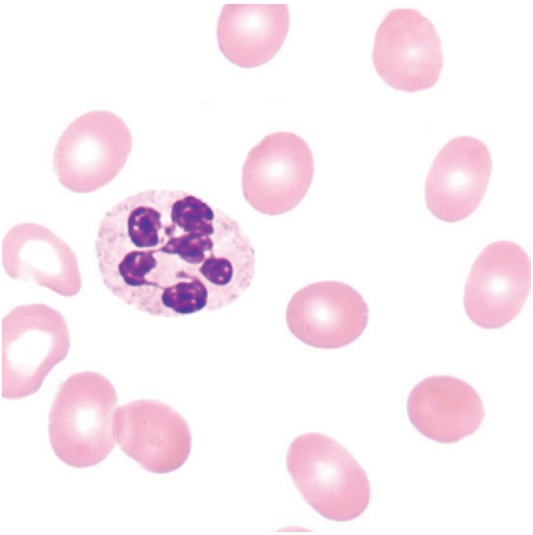
DESCRIPTION: Peanut-shaped, bilobed or non-segmented, granulocyte nucleus with the coarse chromatin of a mature cell.

Associated with: Pelger-Hüet anomaly, pseudo-Pelger-Hüet anomaly

NOTE: Pelger-Hüet anomaly is inherited and affects the majority of granulocytes. Pseudo-Pelger–Hüet is acquired, affects less than 50% of granulocytes and is usually accompanied by other morphologic indications of malignancy such as seen in myeloproliferative or myelodysplastic disorders (see Chapters 17 and 18).

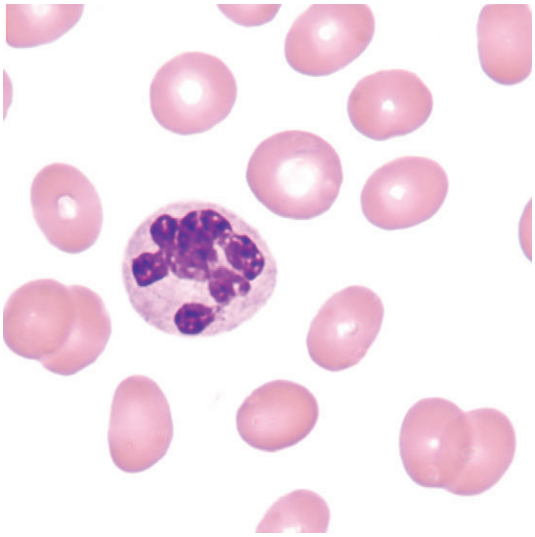
All photomicrographs are ×1000 with Wright-Giemsa stain unless stated otherwise.

HYPERSEGMENTATION OF NEUTROPHILS



A

FIGURE 14–2A Hypersegmented neutrophil (PB ×1000).



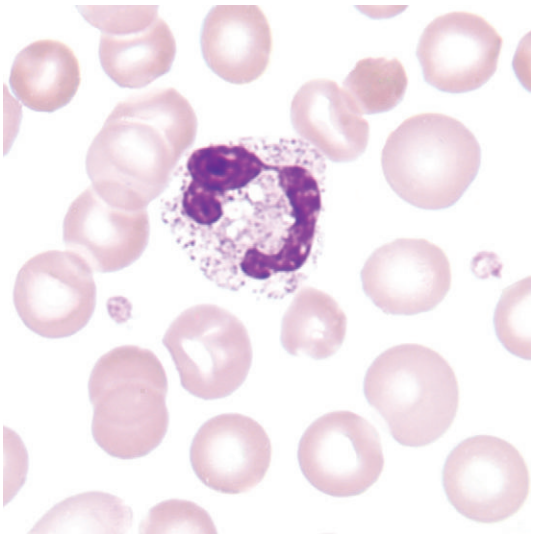
B

FIGURE 14–2B Hypersegmented neutrophil (PB ×1000).

DESCRIPTION: Six or more lobes in granulocyte nucleus

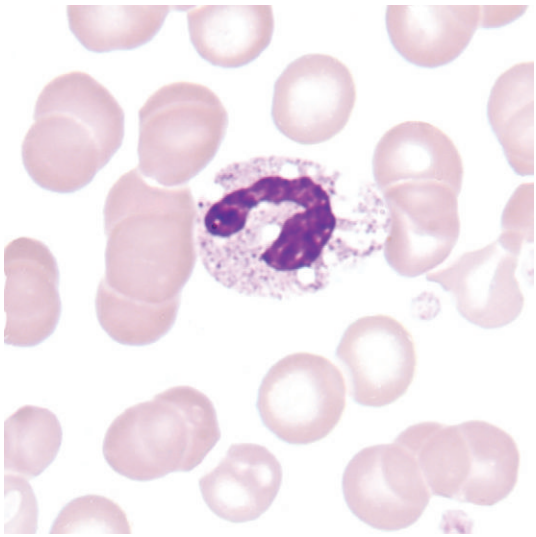
Associated with: Megaloblastic anemias; chronic infections; myelodysplastic syndrome; rarely inherited

VACUOLATION



A

FIGURE 14–3A Vacuoles.

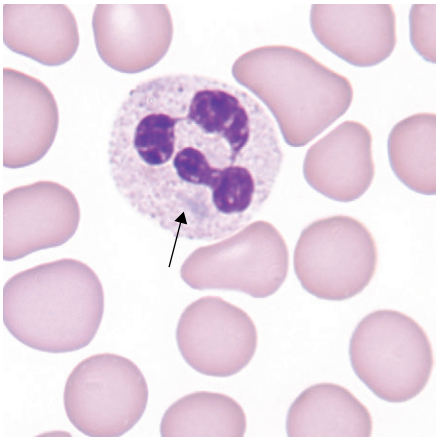


B

FIGURE 14–3B Vacuoles.

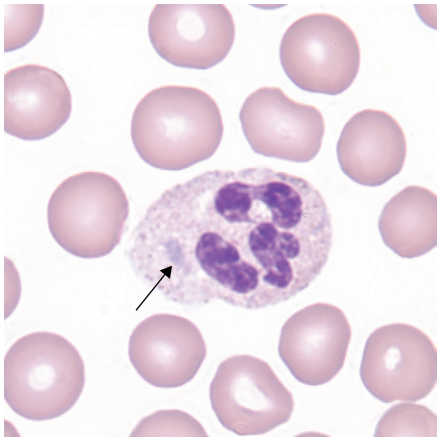
DESCRIPTION: Unstained circular area within the cytoplasm.
NUMBER: Few to many
Associated with: Bacterial or fungal infection, poisoning, burns, chemotherapy, artifact
NOTE: Rarely may contain micro-organisms or pigment.

DÖHLE BODY



A

FIGURE 14–4A Döhle body.

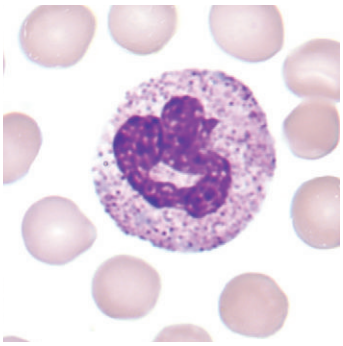


B

FIGURE 14–4B Döhle body

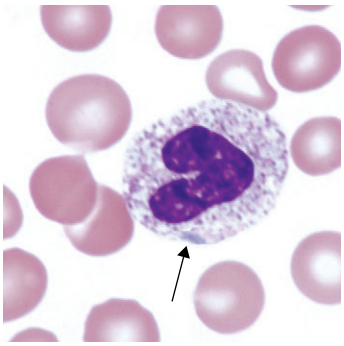
DESCRIPTION: Gray-blue, variably shaped
LOCATION: Cytoplasm
COMPOSITION: Ribosomal RNA
NUMBER: Single or multiple
Associated with: Wide range of conditions, including bacterial infection, sepsis and normal pregnancy
NOTE: May be seen in cells with toxic granulation or on same slide with toxic granulation. (see Figure 14-5, *B*)

TOXIC GRANULATION



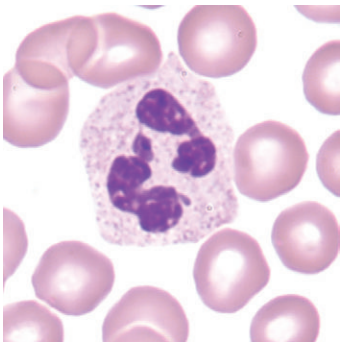
A

FIGURE 14–5A Toxic granulation.



B

FIGURE 14–5B Toxic granulation and Döhle body (*arrow*). Cytoplasm may retain blue color due to cell's early release from bone marrow.

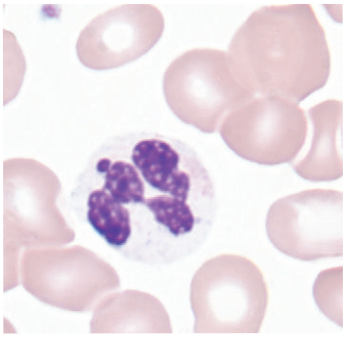


C

FIGURE 14–5C Normal segmented neutrophil for comparison.

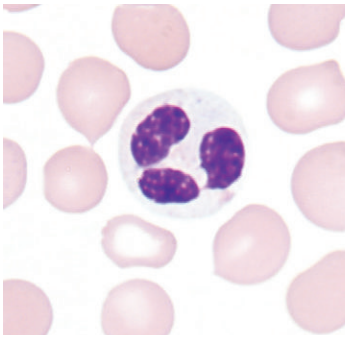
DESCRIPTION: Prominent dark purple-black granules
LOCATION: Cytoplasm of neutrophils, unevenly distributed
COMPOSITION: Primary granules
NUMBER: Few to many
Associated with: Wide range of conditions including bacterial infection, sepsis and following administration of granulocyte colony-stimulating factor.

HYPOGRANULATION/AGRANULATION



A

FIGURE 14–6A Hypogranulation.

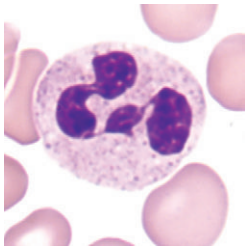


B

FIGURE 14–6B Agranulation.

DESCRIPTION: Decreased number or absence of specific granules giving the cytoplasm a colorless appearance

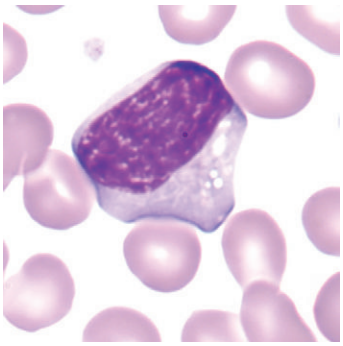
Associated with: Myelodysplastic syndrome, myeloproliferative neoplasms, infection



C

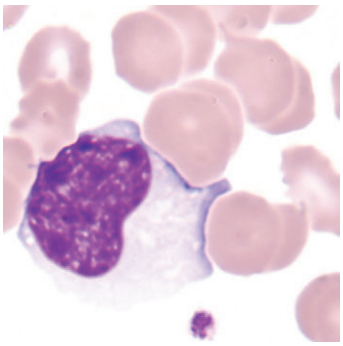
FIGURE 14–6C Normal segmented neutrophil for comparison.

REACTIVE LYMPHOCYTES



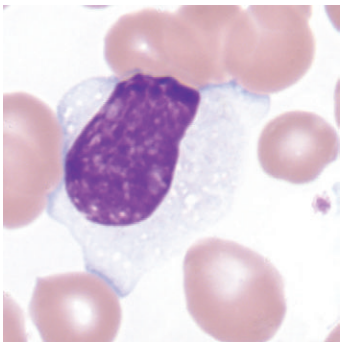
A

FIGURE 14–7A Reactive lymphocyte, vacuolated cytoplasm.



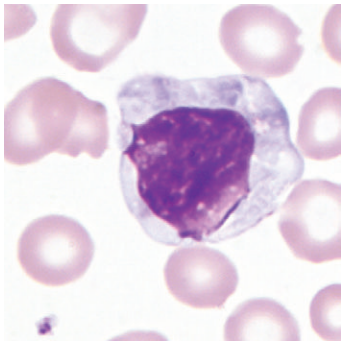
B

FIGURE 14–7B Reactive lymphocyte, peripheral basophilia.



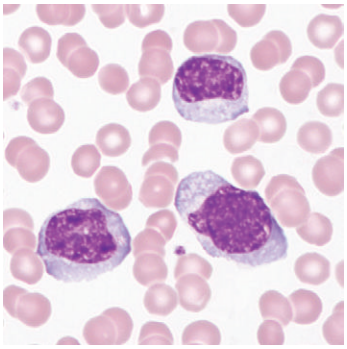
C

FIGURE 14–7C Reactive lymphocyte, cytoplasm indented by adjacent cells.



D

FIGURE 14–7D Reactive lymphocyte, radial basophilia.



E

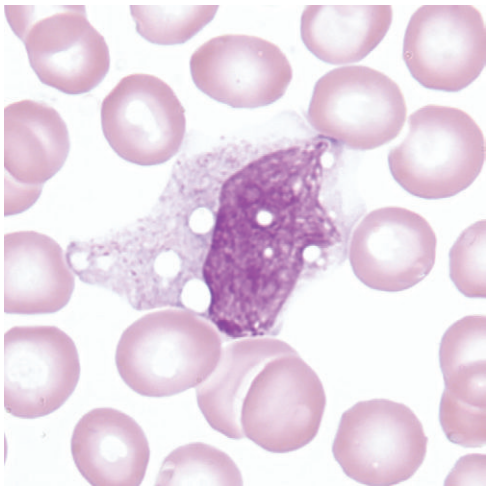
FIGURE 14–7E Reactive lymphocytes, characteristic of viral diseases, such as infectious mononucleosis (PB ×500).

SHAPE: Pleomorphic; easily indented by surrounding cells
SIZE: 10-30 μm
NUCLEUS: Irregular
Nucleoli: Occasionally present
Chromatin: When compared with that of a resting lymphocyte, chromatin coarse to fine and dispersed.
CYTOPLASM: Pale blue to deeply basophilic, may stain unevenly with peripheral or radial basophilia
Granules: May have increased numbers of azurophilic granules
Vacuoles: Occasional
Associated with: Viral infections and other antigenic stimulation, including organ transplantation

TABLE 14-1 Monocyte versus Reactive Lymphocyte

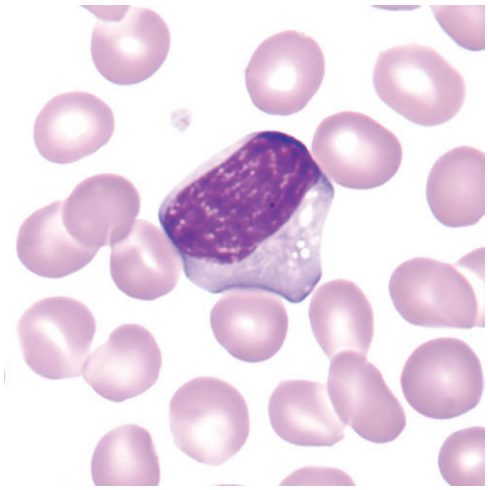
	Monocyte	Reactive Lymphocyte
Shape	Pleomorphic; may have pseudopodia, which tend to "push away" surrounding cells	Pleomorphic, easily indented by surrounding cells
Size	12-20 μm	10-30 μm
Nucleus	Round, oval, horseshoe, or kidney shaped, may have brainlike convolutions	Irregular, elongated, stretched, occasionally round
Nucleoli	Absent	Occasionally present
Chromatin	Loosely woven, lacy	Variable; coarse to fine and dispersed
Cytoplasm	Blue-gray	Pale blue to deeply basophilic, may stain unevenly
Granules	Many fine red—may give ground glass appearance	May be a few prominent azurophilic granules
Vacuoles	Absent to numerous	Occasional

Use as many criteria as possible to identify cells. It is often difficult to differentiate cells in isolation; multiple fields should be examined for nuclear and cytoplasmic characteristics. Consider "the company they keep."



A

FIGURE 14-8A Monocyte. Note the blue-gray cytoplasm with fine red granules. Nucleus has brainlike convolutions. Cell "pushes away" surrounding cells. Vacuoles are present in both of these figures.



B

FIGURE 14-8B Reactive lymphocyte. Note the blue cytoplasm with darker blue periphery. Cell is indented by surrounding cells. Nucleus is elongated. Vacuoles are present in both of these figures.

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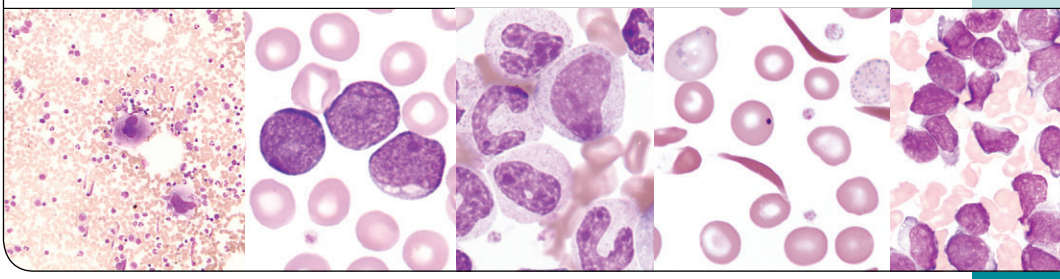
15

ACUTE MYELOID LEUKEMIA



APPROACH TO ACUTE MYELOID LEUKEMIA

The World Health Organization (WHO) *Classification of Tumours and Haematopoietic and Lymphoid Tissues* is based on morphology; immunophenotyping; genetic features, including karyotype and molecular testing; and clinical features. WHO lists the characteristic features of acute myeloid leukemia (AML) as increased bone marrow cellularity with ≥20% blasts, variable white blood cell count, anemia, and thrombocytopenia in the peripheral blood.

AML is separated into four categories:

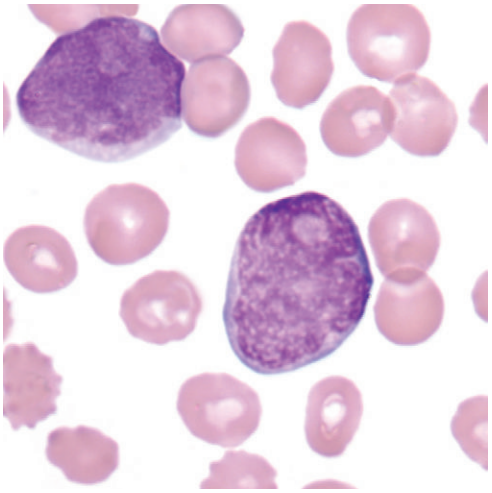
1. Acute myeloid leukemia with recurrent genetic abnormalities
2. Acute myeloid leukemia with myelodysplasia-related changes*
3. Therapy-related myeloid neoplasms*
4. Acute myeloid leukemia, not otherwise specified

This atlas presents characteristic peripheral blood and bone marrow morphology for each of the AMLs with recurrent genetic abnormalities and those not otherwise specified together with the associated cytochemical reactions, genetic abnormalities, and immunophenotypes.

*Diagnosis is, in part, based on patient presentation and as such will not be addressed in this atlas.

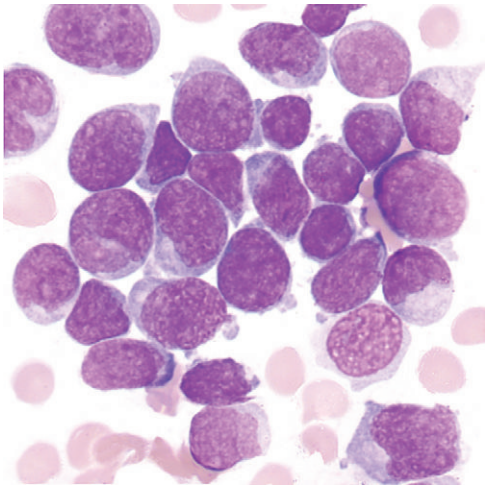
ACUTE MYELOID LEUKEMIA, MINIMALLY DIFFERENTIATED

FAB† M0



A

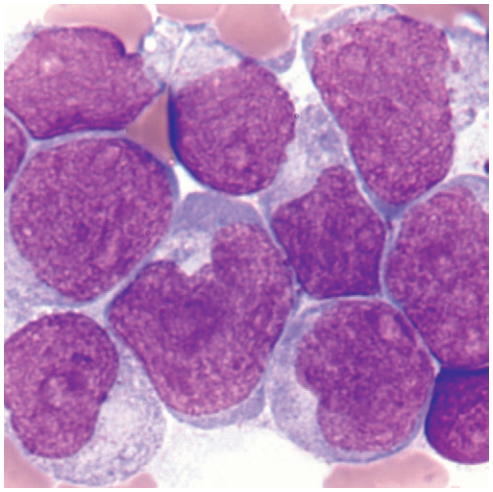
FIGURE 15–1A Peripheral blood (×1000).



B

FIGURE 15–1B Bone marrow (×500).

MORPHOLOGY
Peripheral Blood: Large agranular blasts
Bone Marrow: Large agranular blasts
CYTOCHEMISTRY
Myeloperoxidase: negative
Sudan Black B: negative
Nonspecific Esterase: negative
GENETICS
Recurrent genetic abnormalities: not defined
IMMUNOPHENOTYPE
$CD13^+$, $CD33^+$, $CD117^+$, HLA-$DR^{\pm}$, $CD34^{\pm}$, $CD38^+$



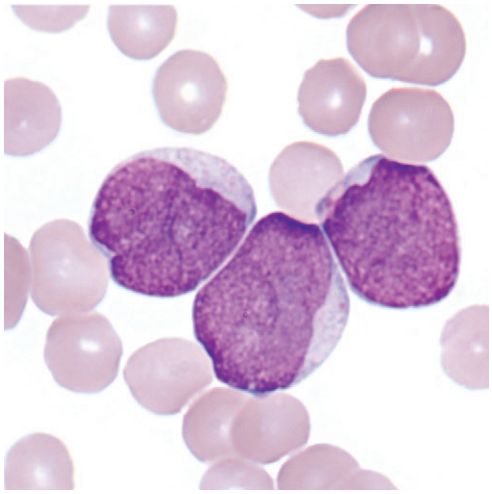
C

FIGURE 15–1C Bone marrow (×1000).

†French-American-British classification of acute leukemia.

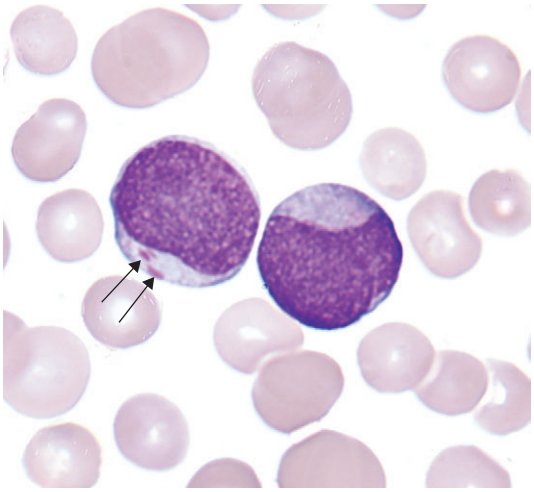
ACUTE MYELOID LEUKEMIA WITHOUT MATURATION

FAB M1



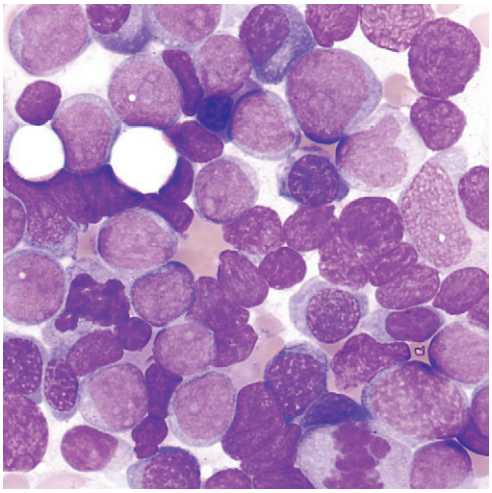
A

FIGURE 15–2A Peripheral blood (×1000).



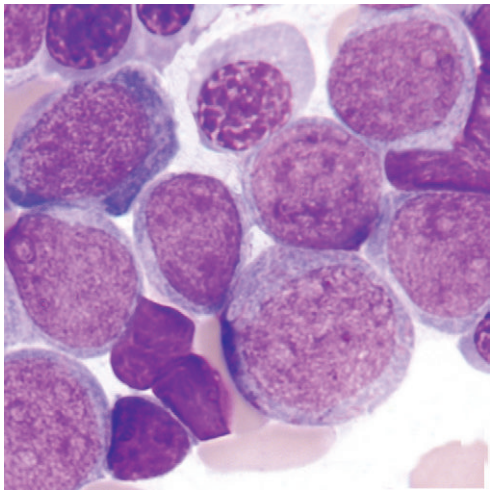
B

FIGURE 15–2B Peripheral blood: Auer rods in myeloblast (×1000). Auer rods are composed of fused primary granules usually rod shaped but may be round in appearance. Single or multiple Auer rods may be seen in malignant myeloblasts and malignant promyelocytes.



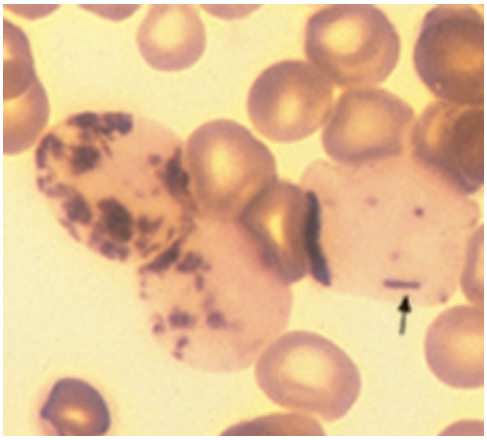
C

FIGURE 15–2C Bone marrow (×500).



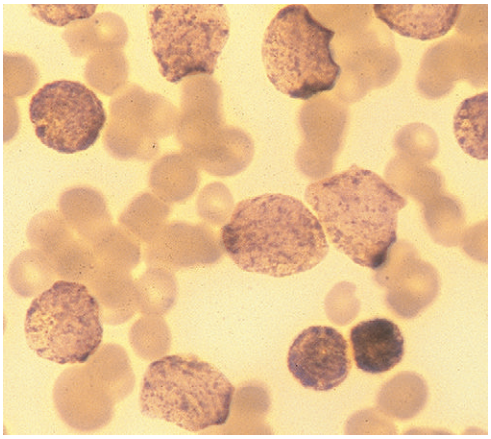
D

FIGURE 15–2D Bone marrow (×1000).



E

FIGURE 15–2E Positive myeloperoxidase stain.



F

FIGURE 15–2F Positive Sudan Black B stain.

MORPHOLOGY

Peripheral Blood: Blasts

± Auer rods (see Figure 15-2, *A* and *B*)

Bone Marrow: ≥90% of nonerythroid cells are blasts

CYTOCHEMISTRY

Myeloperoxidase: positive (see Figure 15-2, *E*)
Sudan Black B: positive (see Figure 15-2, *F*)
Nonspecific Esterase: negative

GENETICS

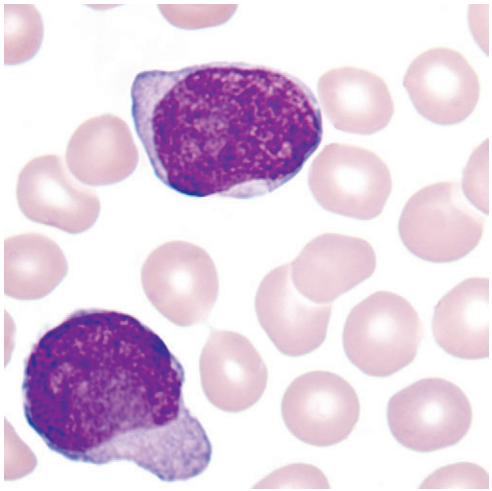
Recurrent genetic abnormalities: not defined

IMMUNOPHENOTYPE

$CD13^{+}$, $CD33^{+}$, $CD34^{\pm}$, $HLA\text{-}DR^{\pm}$, $CD117^{+}$

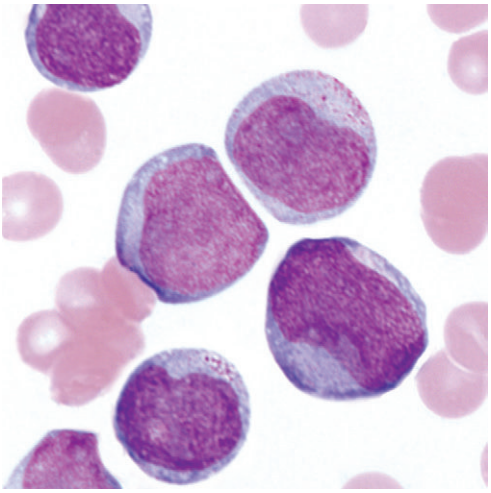
ACUTE MYELOID LEUKEMIA WITH MATURATION

FAB M2



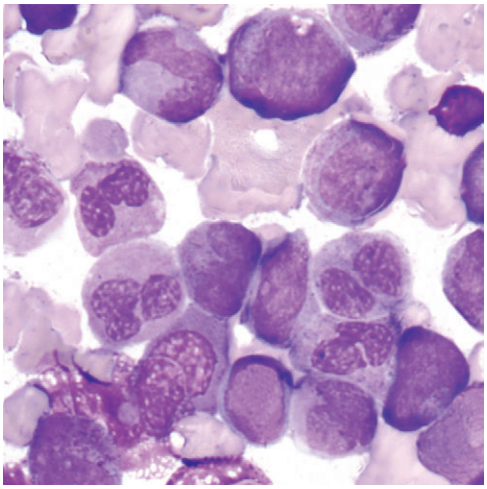
A

FIGURE 15–3A Peripheral blood Type I myeloblast (×1000).



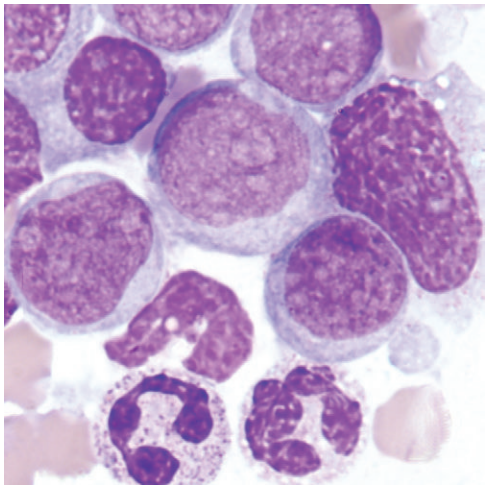
B

FIGURE 15–3B Peripheral blood Type II myeloblast (×1000).



C

FIGURE 15–3C Bone marrow (×500).



D

FIGURE 15–3D Bone Marrow (×1000).

MORPHOLOGY

Peripheral Blood: Blasts with some maturation

± Auer rods (see Figures 15-2, *A* and *B*)

Bone Marrow: Blasts, some with large azurophilic granules, perinuclear hof

± Auer rods
<90% of nonerythroid cells are blasts
≥10% neutrophilic component
<20% monocytic component

CYTOCHEMISTRY

Myeloperoxidase: positive (see Figure 15-2, *E*)
Sudan Black B: positive (see Figure 15-2, *F*)

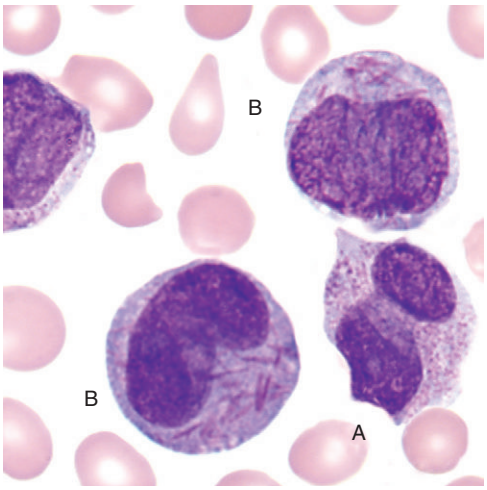
GENETICS

Subset with t(8;21) is designated as AML with recurrent genetic abnormalities. In this subset, blasts are large with abundant basophilic cytoplasm, azurophilic granules, and possible perinuclear hofs.

IMMUNOPHENOTYPE

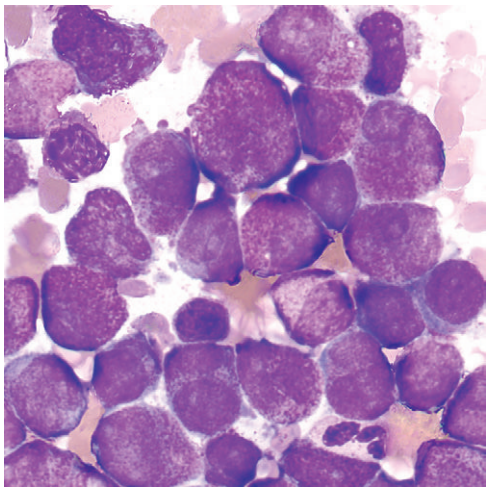
$CD13^+$, $CD33^+$, $CD65^+$, $CD11b^+$, CD15+, $HLA\text{-}DR^{\pm}$

ACUTE PROMYELOCYTIC LEUKEMIA



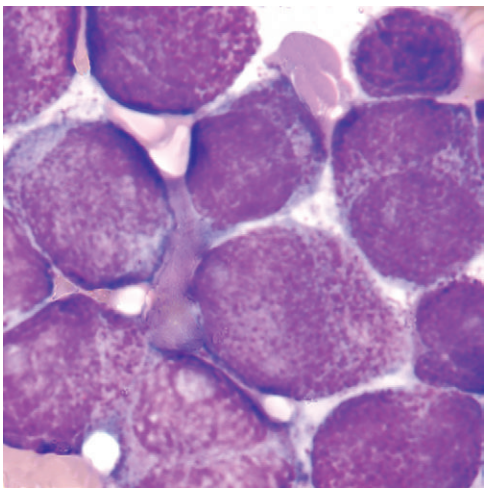
A

FIGURE 15–4A Peripheral blood. *A*, Hypergranular promyelocyte (×1000); *B*, Faggot cells.



B

FIGURE 15–4B Bone marrow (×500).



C

FIGURE 15–4C Bone marrow (×1000).

MORPHOLOGY

Peripheral Blood: White blood cell count may be low or only slightly elevated

Blasts, hypergranular promyelocytes, cytoplasm gray to blue, nucleus may be folded or bilobed

Multiple Auer rods possible, may be in bundles (Faggot cells), schistocytes

Bone Marrow: Blasts, hypergranular promyelocytes, nuclei often bilobed or kidney shaped

± Multiple Auer rods

CYTOCHEMISTRY

Myeloperoxidase: strongly positive (see Figure 15-2, *E*)
Sudan Black B: strongly positive (see Figure 15-2, *F*)

GENETICS

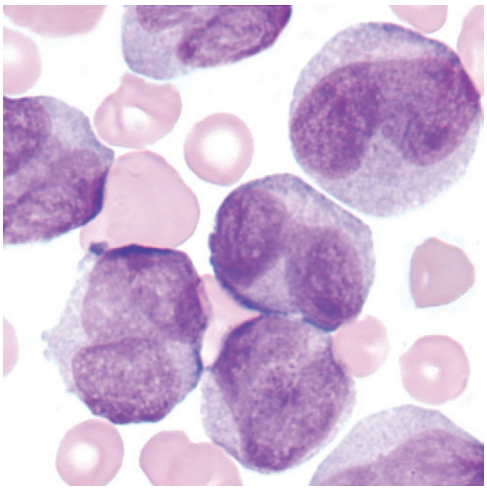
t(15;17) is sufficient for diagnosis as AML with recurrent genetic abnormalities regardless of blast/promyelocyte count.‡

IMMUNOPHENOTYPE

$CD13^{\pm}$, $CD33^{+}$, $CD34^{-}$, $HLA\text{-}DR^{-}$

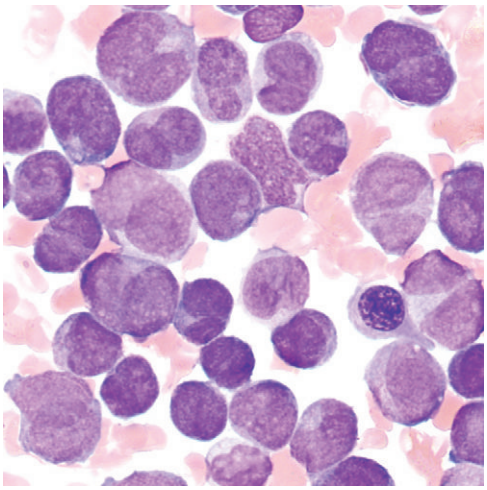
‡1. Abnormal promyelocytes are considered blast equivalents for the purpose of diagnosis.
2. May be associated with disseminated intravascular coagulopathy.

ACUTE PROMYELOCYTIC LEUKEMIA—MICROGRANULAR VARIANT



A

FIGURE 15–5A Peripheral blood (×1000).



B

FIGURE 15–5B Bone marrow (×500).

MORPHOLOGY

Peripheral Blood: White blood cell count markedly elevated, deeply notched nuclei

Cytoplasm may appear agranular because of small size of granules, which are evident with electron microscopy

Bone Marrow: Agranular promyelocytes, with deeply notched nuclei

CYTOCHEMISTRY

Myeloperoxidase: strongly positive (see Figure 15-2, *E*)

Sudan Black B: strongly positive (see Figure 15-2, *F*)

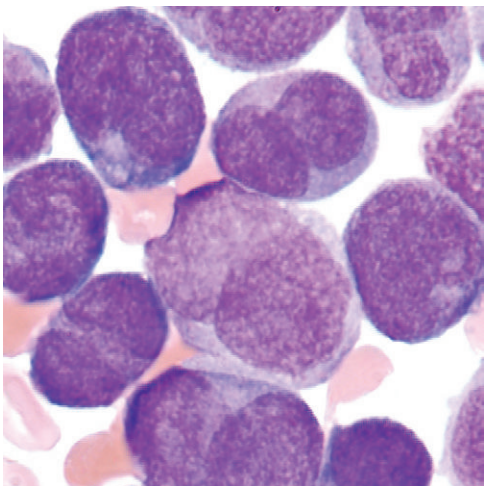
GENETICS

t(15;17) is sufficient for diagnosis as AML with recurrent genetic abnormalities regardless of blast/promyelocyte count.

IMMUNOPHENOTYPE

$CD13^{\pm}$, $CD33^{+}$, $CD34^{-}$, HLA-DR^{-}, $CD64^{+}$, $CD117^{\pm}$

NOTE: Microgranular promyelocytes can be confused morphologically with monocyte precursors.

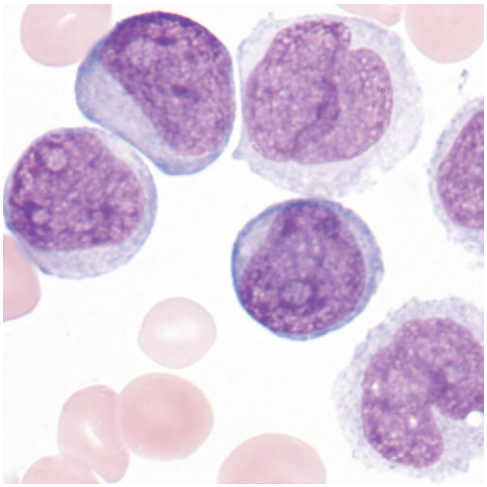


C

FIGURE 15–5C Bone marrow (×1000).

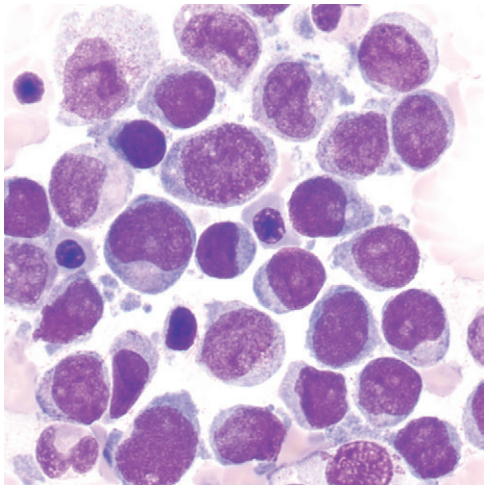
ACUTE MYELOMONOCYTIC LEUKEMIA

FAB M4



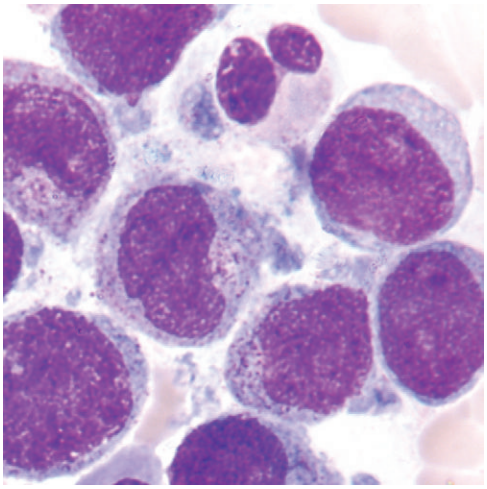
A

FIGURE 15–6A Peripheral blood (×1000).



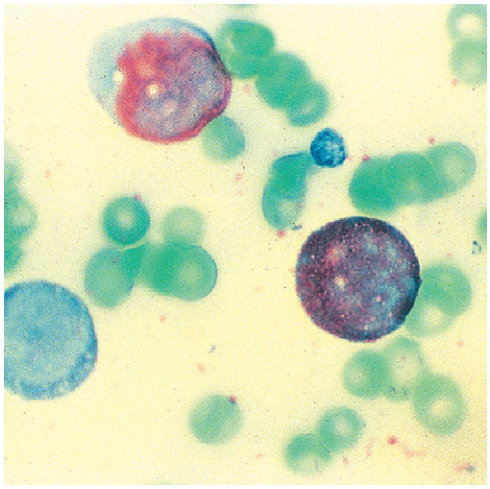
B

FIGURE 15–6B Bone marrow (×500).



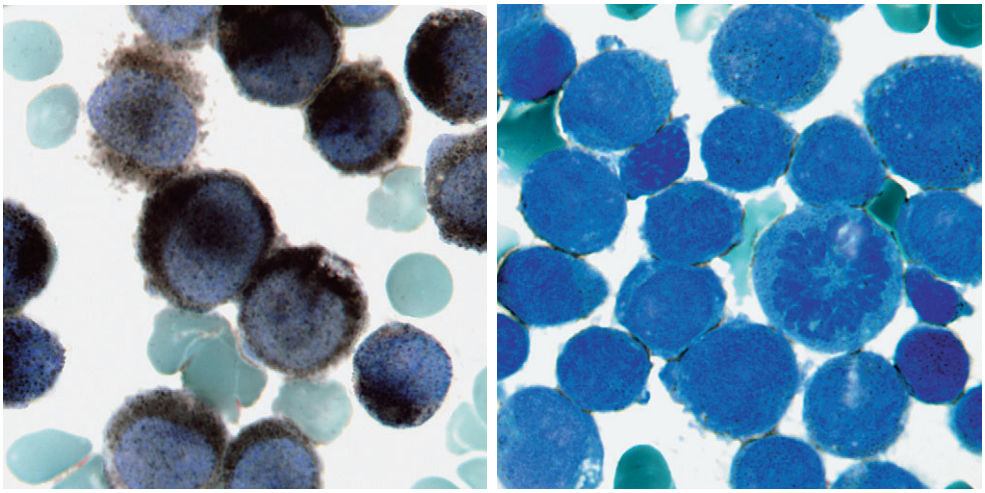
C

FIGURE 15–6C Bone marrow (×1000).



D

FIGURE 15–6D Positive naphthol-AS-D chloroacetate esterase (specific) stain.



E

FIGURE 15–6E Positive α-naphthyl esterase (nonspecific) esterase stain in monocytes (*left*). Naphthyl esterase stain inhibited by NaFl (*right*).

MORPHOLOGY

Peripheral Blood: Myeloblasts, promyelocytes, and other immature myeloid precursors

Monoblasts and promonocytes and monocytes—frequently more mature then those seen in bone marrow
± Auer rods (see Figure 15-2, *B*)

Bone Marrow: Monoblasts large with abundant, moderate basophilic cytoplasm; some with folded nuclei, one or more prominent nucleoli

Promonocytes, irregular, convoluted nucleus, cytoplasm slightly basophilic; granules; occasional vacuoles
± Auer rods
Granulocytes and their precursor and monocytes and their precursors each comprise ≥20%

CYTOCHEMISTRY

Myeloperoxidase: positive (see Figure 15-2, *E*)
Specific Esterase: naphthol-AS-D chloroacetate esterase is positive in granulocytic cells and weak in monocytic cells (see Figure 15-6, *D*)
Nonspecific Esterase:

- α-Naphthyl acetate esterase: positive in monocytic cells; inhibited by NaFl (Figure 15-6, *E*)
- α-Naphthyl butyrate esterase: positive in monocytic cells (see Figure 15-8, *D*)

GENETICS

Recurrent genetic abnormalities: not defined
NOTE: inv(16) or t(16;16) and abnormal eosinophils are excluded from this category.

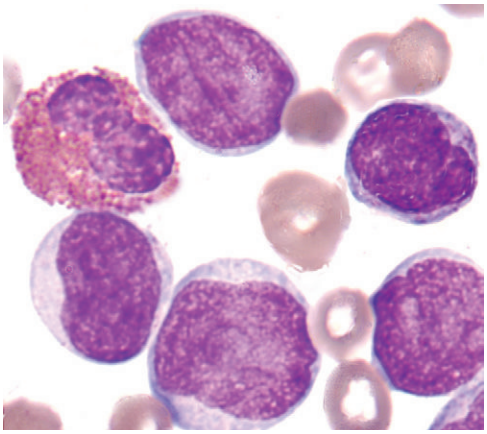
IMMUNOPHENOTYPE

$CD13^+$, $CD33^+$, $CD14^+$ $CD4^+$, $CD11b^+$, $CD64^+$, $CD15^+$, $CD36^+$

ACUTE MYELOID LEUKEMIA WITH inv(16) (p13.1q22) OR t(16;16)(p13.1;q22); CBFB-MYH11

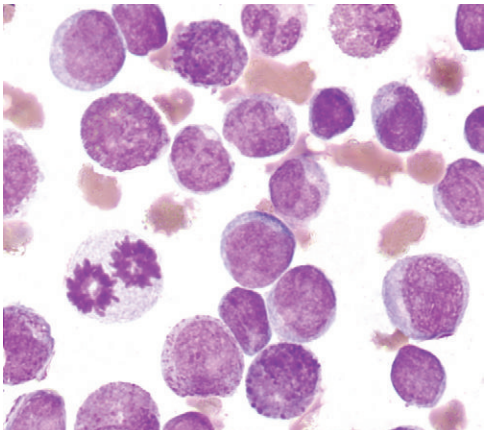
Acute myeloid leukemia with abnormal marrow eosinophils

FAB M4EO



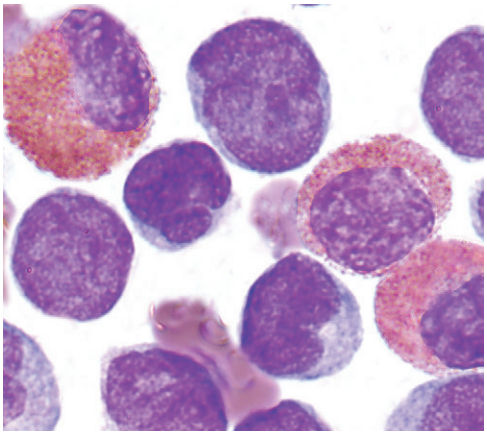
A

FIGURE 15–7A Peripheral blood (×1000).



B

FIGURE 15–7B Bone marrow (×500).



C

FIGURE 15–7C Bone marrow (×1000).

MORPHOLOGY

Peripheral Blood: Myeloblasts, promyelocytes, and other immature myeloid precursors

Monoblasts and promonocyte and monocytes
± Auer rods (see Figure 15-2, *B*)

Bone Marrow: Monoblasts large with abundant, moderate basophilic cytoplasm; some with folded nuclei, one or more prominent nucleoli

Promonocytes, irregular, convoluted nucleus, cytoplasm slightly basophilic; granules; occasional vacuoles
Eosinophils increased and dysplastic with many large granules, some basophilic
± Auer rods

CYTOCHEMISTRY

Myeloperoxidase: positive (see Figure 15-2, *E*)
Nonspecific Esterase: positive (see Figure 15-6, *E*)
Specific Esterase: weakly positive in abnormal eosinophils

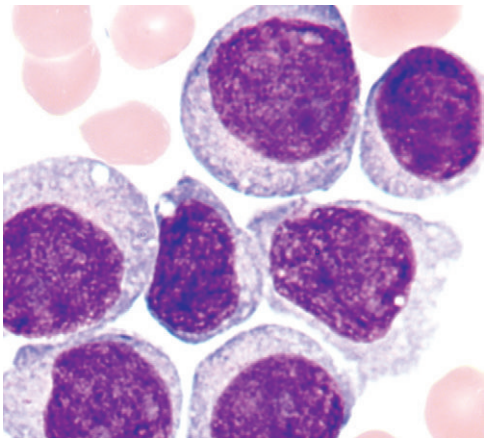
GENETICS

Recurrent genetic abnormality: inv(16) (p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
NOTE: These genetic abnormalities are diagnostic of AML and do not require a ≥20% blast count.

IMMUNOPHENOTYPE

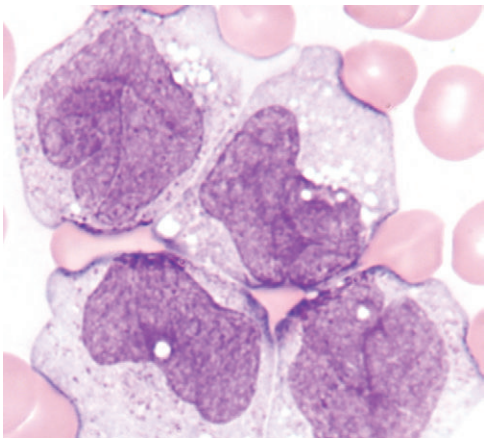
$CD34^+$, $CD117^+$, $CD13^+$, $CD33^+$, $CD15^+$, $CD4^+$, $CD11b^+$, $CD11c^+$, $CD14^+$, $CD64^+$, $CD36^+$, $CD65^+$

ACUTE MONOBLASTIC AND MONOCYTIC LEUKEMIA



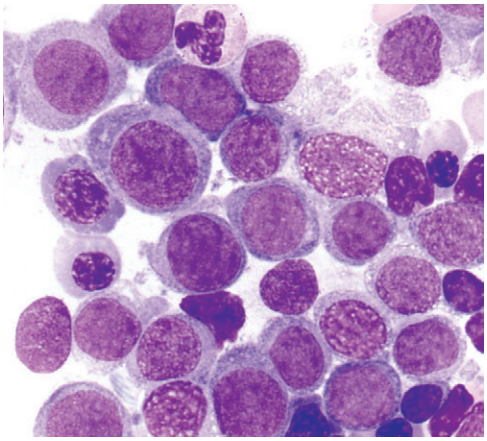
A

FIGURE 15–8A Peripheral blood—monoblasts predominate (×1000).



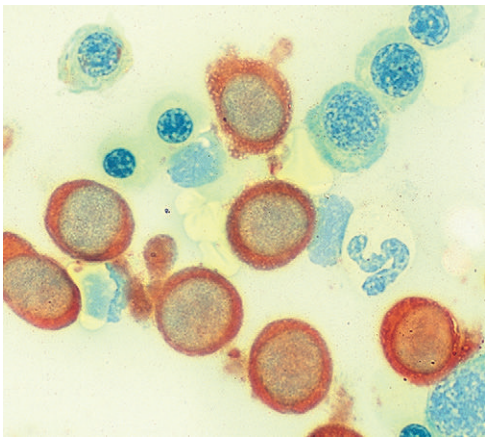
B

FIGURE 15–8B Peripheral blood—promonocytes predominate (×1000).



C

FIGURE 15–8C Bone marrow showing monoblast predominance (×500).



D

FIGURE 15–8D Positive α-naphthyl butyrate esterase (nonspecific) esterase stain.

MORPHOLOGY

Peripheral Blood: Monoblasts, promonocytes

Bone Marrow: Monoblasts large with abundant, moderate basophilic cytoplasm, some with folded nuclei, one or more prominent nucleoli

Promonocytes, irregular, convoluted nucleus, cytoplasm slightly basophilic; granules; occasional vacuoles

≥80% have monocytic morphology

Granulocytic component <20%

NOTE: Monoblastic leukemia is diagnosed when ≥80% leukemic cells are monoblasts. Monocytic leukemia predominant cell type is promonocytes.

CYTOCHEMISTRY

Myeloperoxidase: negative

Nonspecific Esterase: positive (see Figures 15-6, *E*, and 15-8, *D*)

GENETICS

Subset with t(9;11)(p22;q23);MLLT3-MLL is diagnosed as AML with recurrent genetic abnormalities.

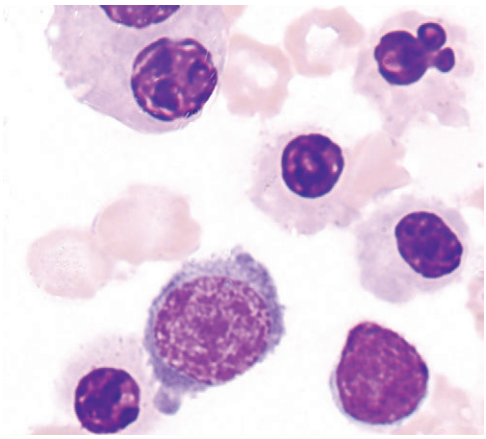
IMMUNOPHENOTYPE

$CD33^+$, $CD13^+$, $CD4^+$, $CD14^+$, $CD11b^+$, $CD64^+$, $CD15^+$, $CD65^+$, $CD11c^+$, $CD36^+$, $CD68^+$, $HLA\text{-}DR^+$

ACUTE ERYTHROID LEUKEMIA

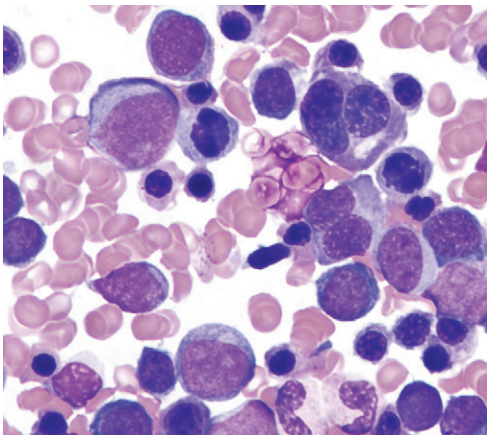
FAB M6a

(Erythroid/myeloid leukemia)



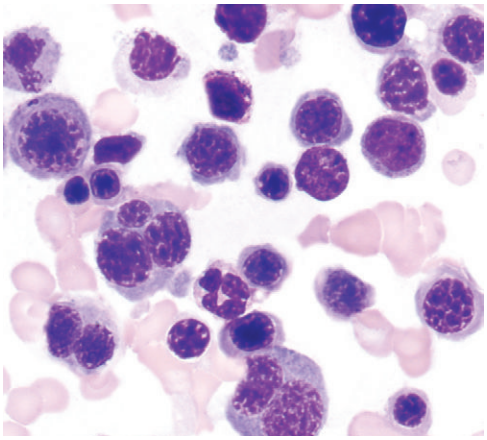
A

FIGURE 15–9A Peripheral blood (×1000).



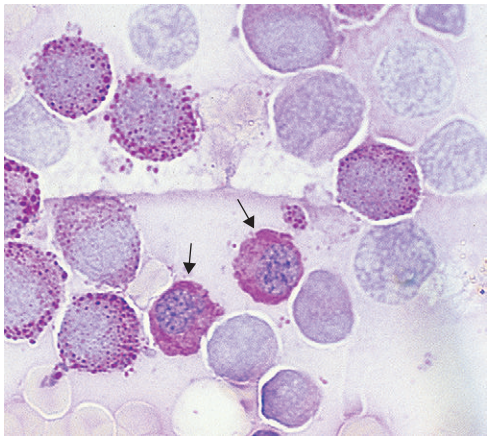
B

FIGURE 15–9B Bone marrow erythroleukemia (×500).



C

FIGURE 15–9C Bone marrow—pure erythroid leukemia (×500).



D

FIGURE 15–9D Positive periodic acid–Schiff stain.

MORPHOLOGY

Peripheral Blood: Myeloblasts, ± Auer rods

Oval macrocytes, microcytes, dimorphic red blood cell population, ± basophilic stippling, dysplastic nucleated red blood cells, multiple nuclei ± abnormal nuclear shapes (see Figure 18-1 A-D)

Bone Marrow: ≥20% of nonerythroid cells are myeloblasts, ± Auer rods

≥50% of all nucleated cells are erythroid precursors
Dysplastic erythroid precursors with megaloblastoid nuclei, round nuclei, fine chromatin, multiple nucleoli, nuclear bridging
Cytoplasm deeply basophilic, often contain vacuoles that may fuse together (see dyserythropoiesis, Figure 18-1 E-H)

Neutrophils: ± dysplastic changes
Megakaryocytes: ± dysplastic changes

CYTOCHEMISTRY

Myeloperoxidase: positive (see Figure 15-2, *E*)
Sudan Black B: positive (see Figure 15-2, *F*)
Periodic Acid–Schiff: block positivity in erythroblasts (see Figure 15-9, *D*)
Iron Stain: ± Ringed sideroblasts (see Figure 18-1 *I*)

GENETICS

Recurrent genetic abnormalities: not defined

IMMUNOPHENOTYPE

Hemoglobin +, glycophorin +, $CD13^{+}$, $CD33^{+}$, $CD117^{\pm}$

FAB M6b
Pure Erythroid Leukemia

MORPHOLOGY

Peripheral Blood: Dysplastic nucleated red blood cells, multiple nuclei ± abnormal nuclear shapes

Oval macrocytes, microcytes, dimorphic red blood cell population, ± basophilic stippling

Bone Marrow: ≥80% erythroid precursors without evidence of a myeloid component

Immature erythroid cells with deeply basophilic cytoplasm, round nuclei, one or more nucleoli, vacuoles (some coalesced)

CYTOCHEMISTRY

Myeloperoxidase: negative
Sudan Black B: negative
Nonspecific Esterase: positive or negative
Periodic Acid–Schiff: block positivity in erythroblasts (see Figure 15-9, *D*)
Iron Stain: ± Ringed sideroblasts (see Figure 18-1 *I*)

GENETICS

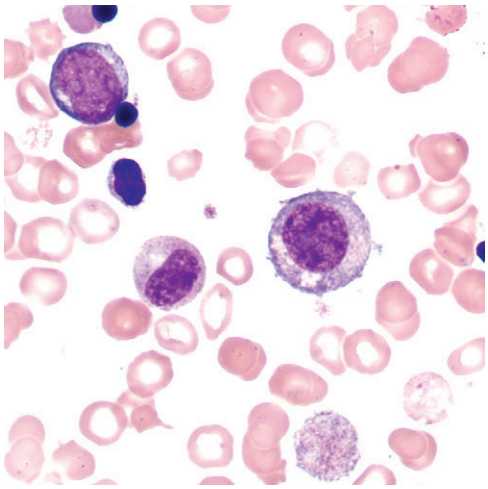
Recurrent genetic abnormalities: not defined

IMMUNOPHENOTYPE

$CD71^{+}$, glycophorin +, hemoglobin +, $CD13^{-}$, $CD\ 33^{-}$, $CD117^{\pm}$

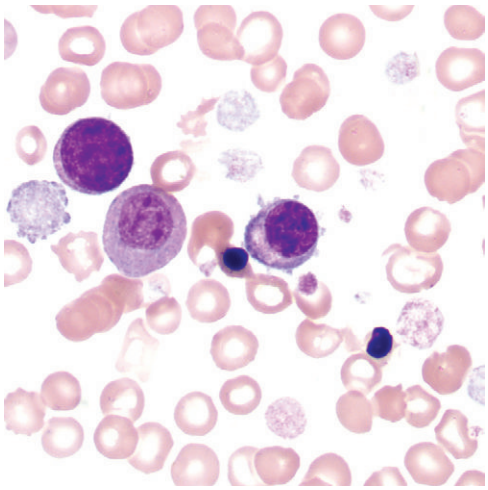
ACUTE MEGAKARYOCYTIC LEUKEMIA

FAB M7



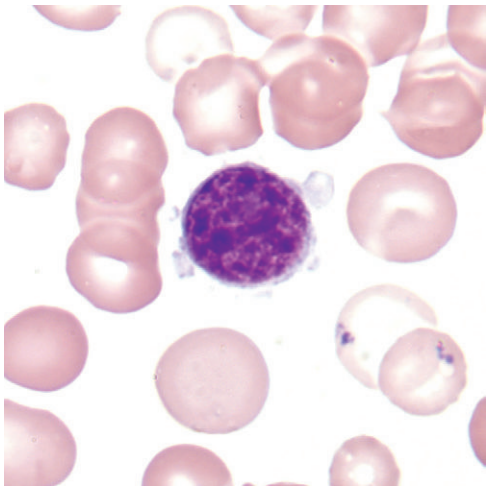
A

FIGURE 15–10A Peripheral blood (×500).



B

FIGURE 15–10B Peripheral blood (×500).



C

FIGURE 15–10C Micromegakaryocyte. Peripheral blood (×1000).

MORPHOLOGY

Peripheral Blood: Blasts with abundant, budding cytoplasm

Micromegakaryocytes ± (see Figures 15-10, *C* and 18-3, *E*)

Large atypical platelets with irregular borders
Hypogranular neutrophils

Bone Marrow: Usually results in dry tap

≥20% blasts
≥50% of blasts are megakaryoblasts
Two types of blasts may be present:

Small blasts: resembling lymphoblasts, round nucleus, dense chromatin, scanty cytoplasm
Large blasts: fine nuclear chromatin, nucleoli, cytoplasm abundant, basophilic, agranular, ± pseudopods

CYTOCHEMISTRY

Myeloperoxidase: negative
Sudan Black B: negative
Specific Esterase: negative
Periodic Acid–Schiff: positive or negative
Nonspecific Esterase: focal positivity

GENETICS

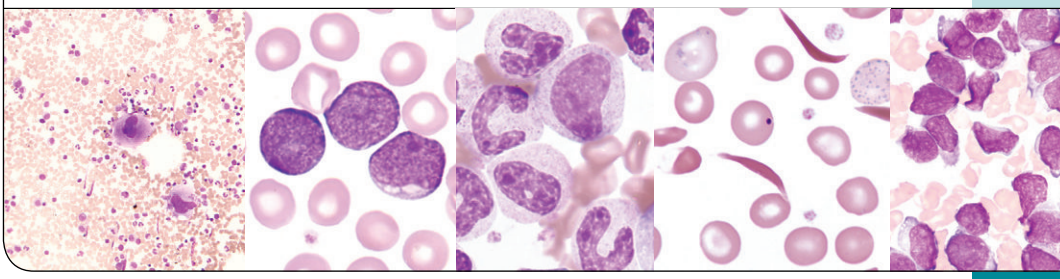
Recurrent genetic abnormalities: not defined
NOTE: In infants may be associated with t(1:22)(p13;q13).

IMMUNOPHENOTYPE

$CD41^+$, $CD61^+$, $CD36^+$

16

PRECURSOR LYMPHOID NEOPLASMS



The World Health Organization classifies precursor lymphoid neoplasms into two major groups: B lymphoblastic leukemia/lymphoma and T lymphoblastic leukemia/lymphoma. Leukemia is primarily a disease of peripheral blood and bone marrow, whereas the primary site of involvement for lymphoma is the lymph system. Because this is an atlas of blood cells, only the leukemia morphology will be presented. Acute lymphoblastic leukemia (ALL) is not classified morphologically or by cytochemistry, but by a combination of cytogenetic profiles, genotype, and immunophenotype. B lymphoblastic leukemia is subdivided into seven subtypes that are associated with recurrent genetic abnormalities (Box 16-1). Those cases of B-ALL that do not fall within one of these groups are classified as B lymphoblastic leukemia, not otherwise specified. Although 50% to 70% of patients with T-ALL do have abnormal karyotypes, none of the abnormalities is clearly associated with distinctive biologic features, and thus T-ALL is not further subdivided.

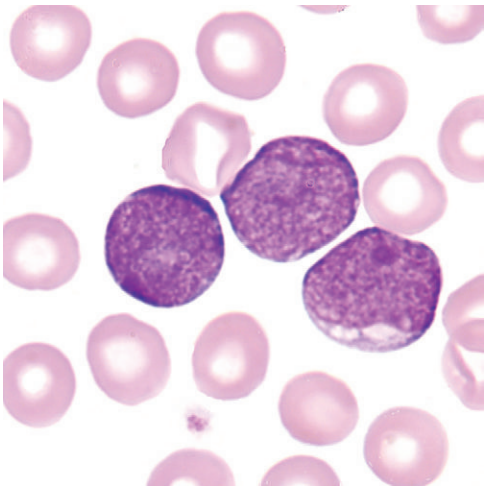
Lymphoblasts may be either small and homogeneous or large and heterogeneous. Further testing is needed to determine the phenotype and genotype.

BOX 16-1 B Lymphoblastic Leukemia/Lymphoma with Recurrent Genetic Abnormalities (2008 World Health Organization Classification)

B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); *TEL-AML1*(*ETV6-RUNX1*)
B lymphoblastic leukemia/lymphoma with hyperdiploidy
B lymphoblastic leukemia/lymphoma with hypodiploidy
B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *E2A-PBX1 (TCF3-PBX1)*

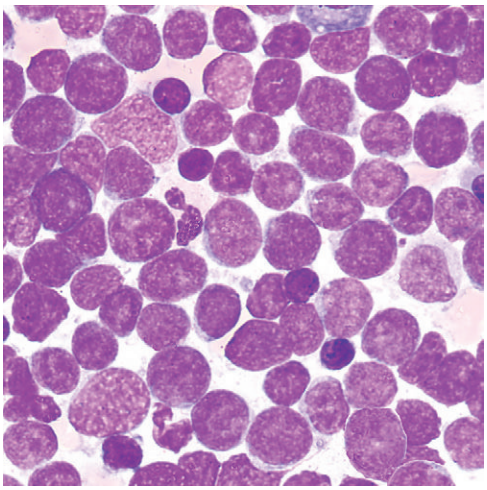
From Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO classification of tumours of haematopoietic and lymphoid tissues*, ed 4, Lyon, France, 2008, IARC Press.

ACUTE LYMPHOBLASTIC LEUKEMIA, SMALL BLASTS



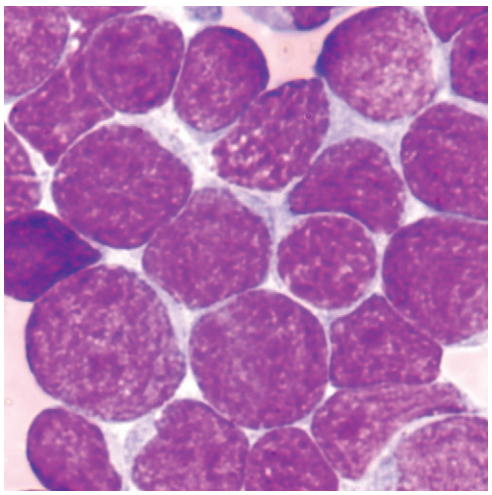
A

FIGURE 16–1A Peripheral blood (×1000).



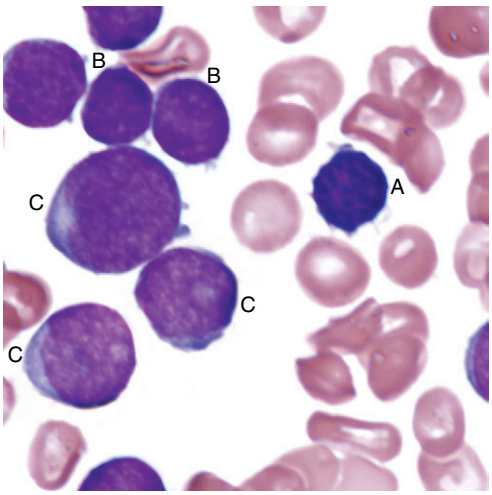
B

FIGURE 16–1B Bone marrow (×500).



C

FIGURE 16–1C Bone marrow demonstrating homogeneous blasts in acute lymphoblastic leukemia (×1000).



D

FIGURE 16–1D Bone marrow demonstrating the comparison between hemotogones and lymphoblasts. *A,* Normal lymphocyte; *B,* hematogones; and *C,* lymphoblasts (×1000).

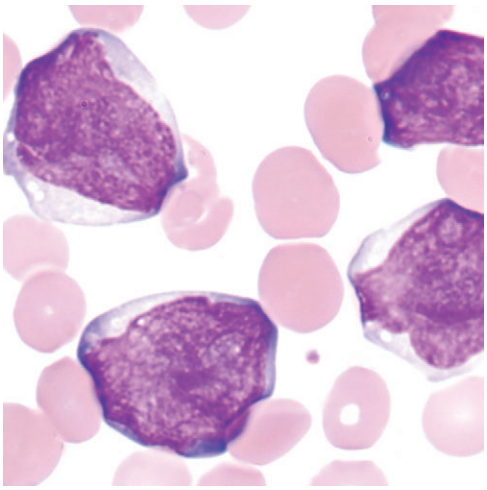
MORPHOLOGY

Peripheral Blood: ± Blasts, small blasts (about one to two-and-a-half times the size of a resting lymphocyte) with scant blue cytoplasm, condensed chromatin and indistinct nucleoli, thrombocytopenia

Bone Marrow: ≥20% of all nucleated cells make up a homogeneous population of blasts

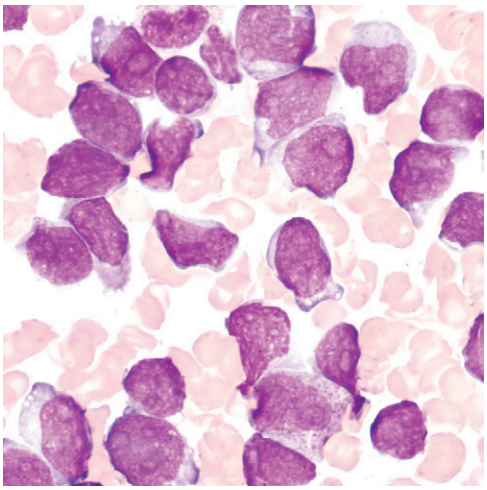
NOTE: Hematogones (immature B cells) may be seen in bone marrow and peripheral blood of newborns, or in patients during bone marrow recovery. Care must be taken not to confuse hematogones with small lymphoblasts (see figures 16-1D and 23-4).

ACUTE LYMPHOBLASTIC LEUKEMIA, LARGE BLASTS



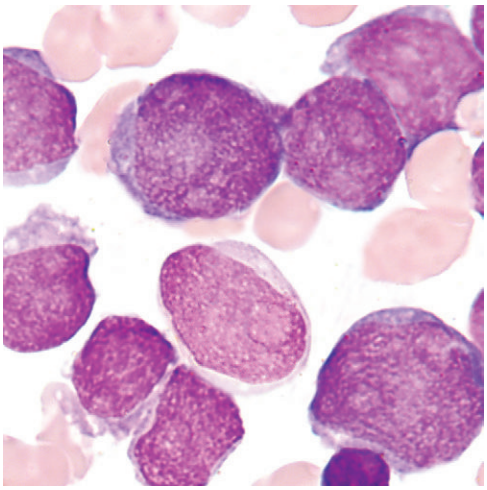
A

FIGURE 16–2A Peripheral blood (×1000).



B

FIGURE 16–2B Bone marrow (×500).



C

FIGURE 16–2C Bone marrow (×1000).

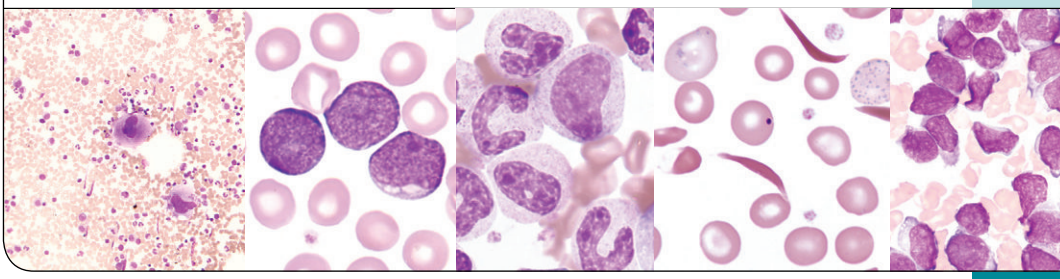
MORPHOLOGY

Peripheral Blood: Blasts—two to three times the size of a resting lymphocyte, moderate cytoplasm, irregular nuclear membrane, prominent nucleoli, thrombocytopenia, morphologically difficult to distinguish from acute myeloid leukemia

Bone Marrow: ≥20% of all nucleated cells comprise a heterogeneous population of blasts

17

MYELOPROLIFERATIVE NEOPLASMS



Myeloproliferative neoplasms are clonal hematopoietic stem cell diseases with expansion, excessive production, and overaccumulation of erythrocytes, granulocytes, and platelets individually or in some combination.

The World Health Organization classification of tumors of the hematopoietic and lymphoid tissues has divided these disorders into four major categories:

1. Chronic myelogenous leukemia, BCR-ABL1^{+} (CML)
2. Polycythemia vera (PV)
3. Essential thrombocythemia (ET)
4. Primary myelofibrosis (PMF)

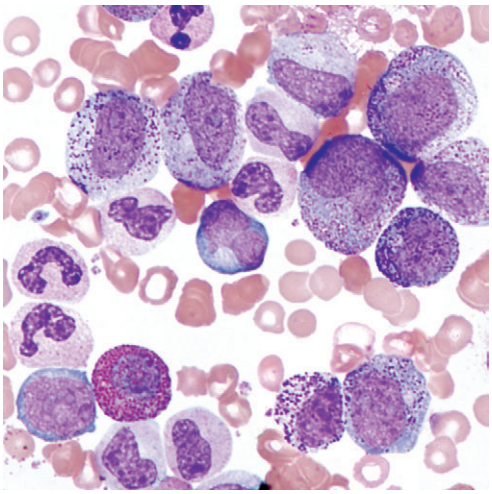
These neoplasms have common clinical features, laboratory findings, and pathogenetic similarities (Table 17-1).

TABLE 17-1 Laboratory Features of Myeloproliferative Neoplasms

Parameter	CML	PV	ET	PMF
WBC	Increased	Normal or increased	Normal or slightly increased	Normal, increased, or decreased
RBC	Normal or decreased	Increased	Normal or slightly decreased	Normal or decreased
Platelets	Normal or increased	Normal or increased	Increased	Normal, increased, or decreased
Molecular abnormalities	BCR-ABL1	JAK2 V617F or other JAK2 mutation	± JAK2	± JAK2

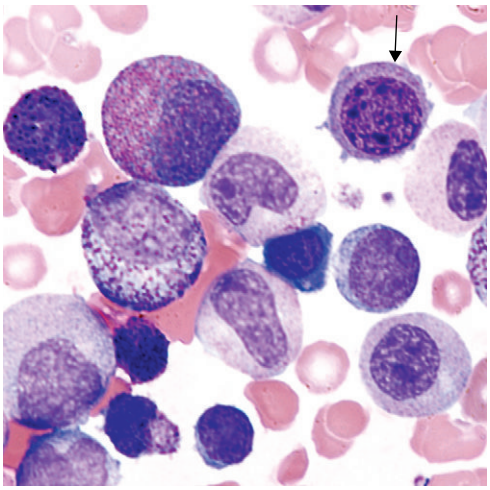
CML, Chronic myelogenous leukemia; *ET*, essential thrombocythemia; *PMF*, primary myelofibrosis; *PV*, polycythemia vera; *RBC*, red blood cells; *WBC*, white blood cells.

CHRONIC MYELOGENOUS LEUKEMIA, BCR-ABL1 POSITIVE



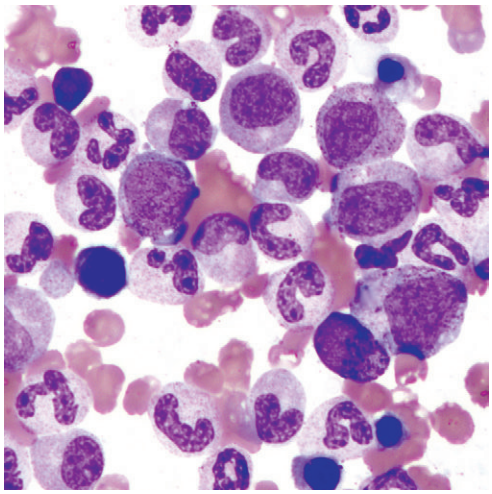
A

FIGURE 17–1A Peripheral blood. Note immature basophils and eosinophil (Original size ×500).



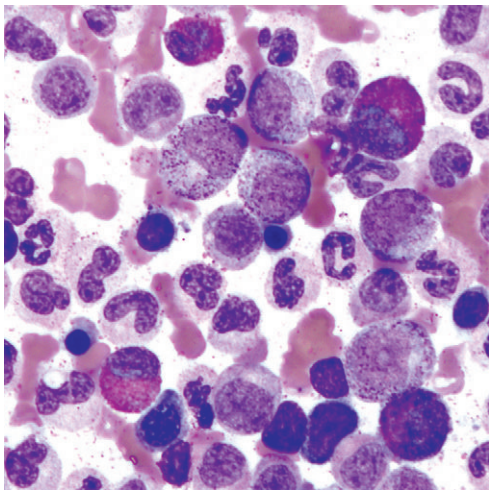
B

FIGURE 17–1B Peripheral blood. Arrow shows a micromegakaryocyte.



C

FIGURE 17–1C BM ×500. A spectrum of granulocytes, including multiple myelocytes, bands, and an immature basophil.



D

FIGURE 17–1D BM ×500. Multiple eosinophils, some of them immature.

NOTE: BCR-ABL positivity must be present for diagnosis.

MORPHOLOGY

Peripheral Blood: Chronic phase*

LEUKOCYTES

Marked leukocytosis (12-1000 × 10^9/L)

- Spectrum of myeloid cells with a predominance of myelocytes and segmented neutrophils
- Myeloblasts <5%
- ± Pseudo-Pelger-Huët cells
- Basophilia
- Eosinophilia
- ± Monocytosis
- Leukocyte alkaline phosphatase (LAP) markedly decreased (Figure 17-2)

ERYTHROCYTES

Normal or decreased in number

PLATELETS

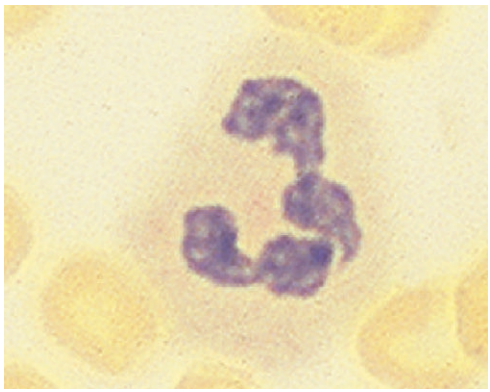
- Normal or increased
- ± Circulating micromegakaryocytes

Bone Marrow:

- Hypercellular with expansion of granulocyte pool
- Myeloid:Erythroid (M:E) ratio increased
- Myeloblasts <5%
- Megakaryocytes normal to increased; may be immature and/or atypical
- ± Pseudo-Gaucher cells (see Figure 22-1A)
- ± Sea blue histiocytes (see Figure 22-5A)

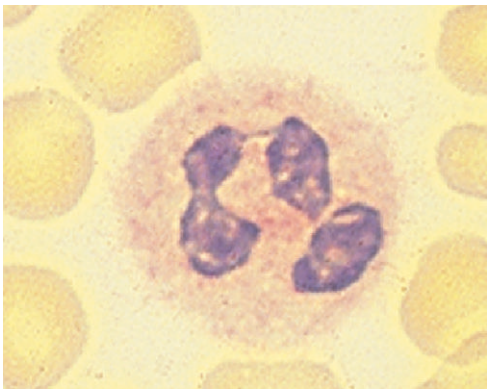
*Before development of tyrosine kinase inhibitors for treatment, CML would progress through phases, from chronic to accelerated to blast phase, with increasing numbers of blasts, basophils, micromegakaryocytes, and dysplasia. (See a hematology textbook for complete discussion of progression of CML.)

LEUKOCYTE ALKALINE PHOSPHATASE



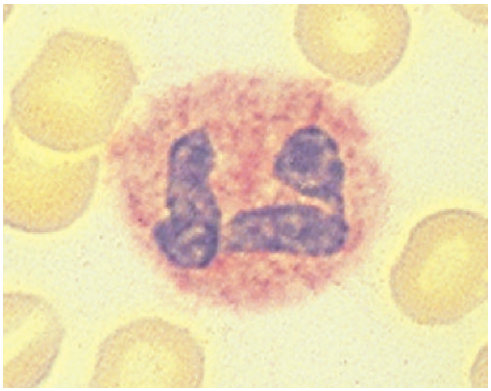
A

FIGURE 17–2A Leukocyte alkaline phosphatase–negative reaction (0) (PB ×1000).



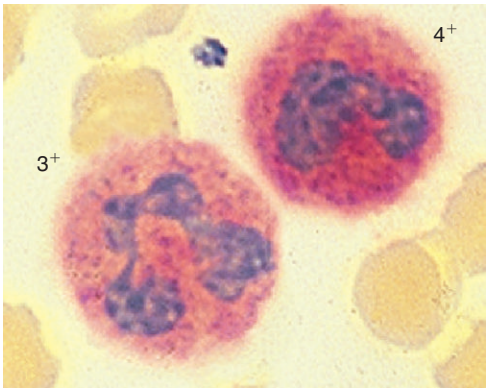
B

FIGURE 17–2B Leukocyte alkaline phosphatase stain (1+) (PB × 1000).



C

FIGURE 17–2C LAP (2+) (PB ×1000).



D

FIGURE 17–2D LAP (3+, 4+) (PB × 1000).

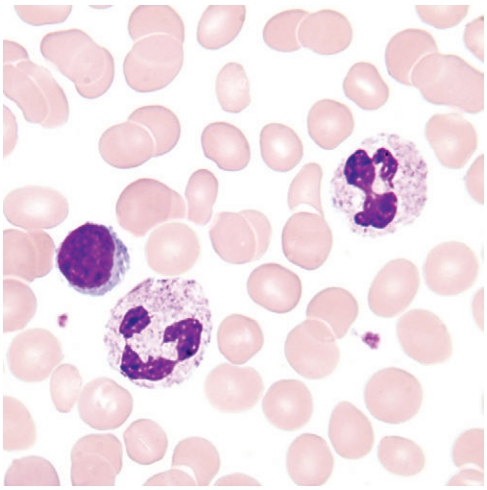
LAP is an enzyme found in secondary granules of neutrophils. LAP activity is scored from 0 to 4+ in the mature segmented neutrophils and bands. One hundred cells are scored and results are added together for the LAP score. A normal score is approximately 20 to 100. Low (<20) scores may be found in untreated CML, paroxysmal nocturnal hemoglobinuria, sideroblastic anemia, and myelodysplastic syndromes. Higher scores may be found in leukemoid reactions (Table 17-2).

TABLE 17-2 Comparison of Chronic Myelogenous Leukemia and Leukemoid Reaction in Peripheral Blood

	CML	Leukemoid Reaction
Neutrophils	Increased with immature cells; peaks at myelocyte and segmented neutrophil stages	Increased with immature forms; orderly progression of maturation stages with no peaks
Eosinophils Basophils	Increased with immature forms	Normal
Platelets	Abnormal number Abnormal morphology	Normal
Dyspoiesis	Present	Absent, but may be reactive changes
Leukocyte alkaline phosphatase	Markedly decreased	Increased

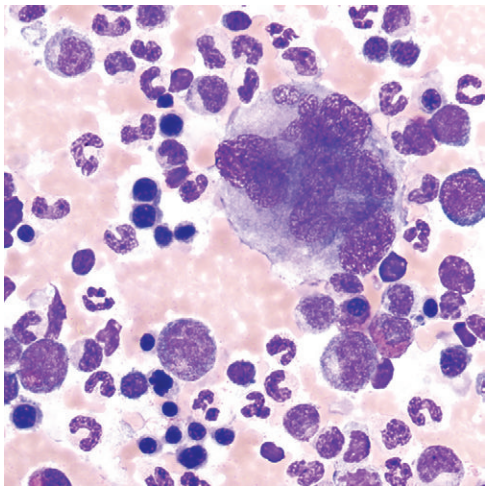
CML, Chronic myelogenous leukemia.

POLYCYTHEMIA VERA



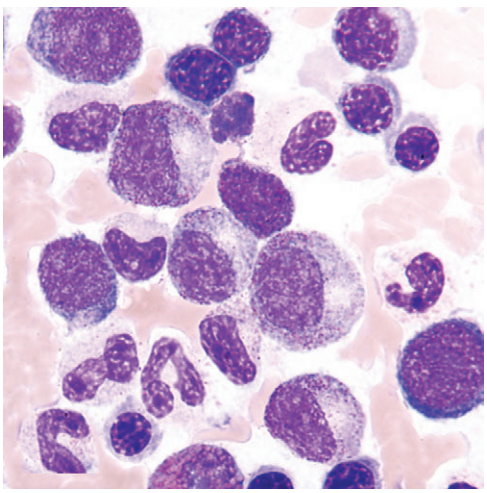
A

FIGURE 17–3A Peripheral blood (original magnification ×1000).



B

FIGURE 17–3B Bone marrow (original magnification ×500).



C

FIGURE 17–3C Bone marrow (original magnification ×1000).

MORPHOLOGY

Peripheral Blood:

LEUKOCYTES

Normal or increased

- Neutrophilia with few metamyelocytes, rare myelocytes
- Promyelocytes and myeloblasts extremely rare
- ± Eosinophilia and/or basophilia

ERYTHROCYTES

Absolute erythrocytosis

- Hemoglobin > 18.5 g/dL in male individuals
- Hemoglobin > 16.5 g/dL in female individuals

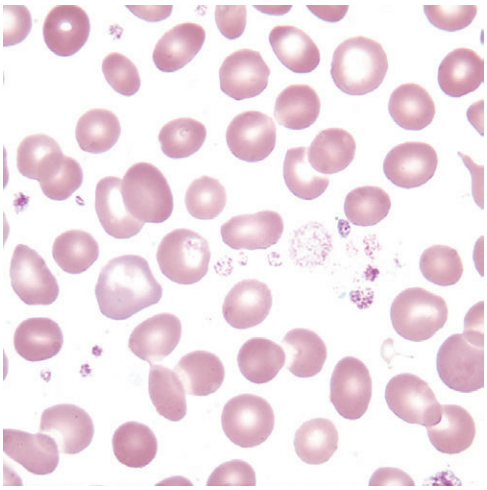
PLATELETS

Normal or increased

Bone Marrow:

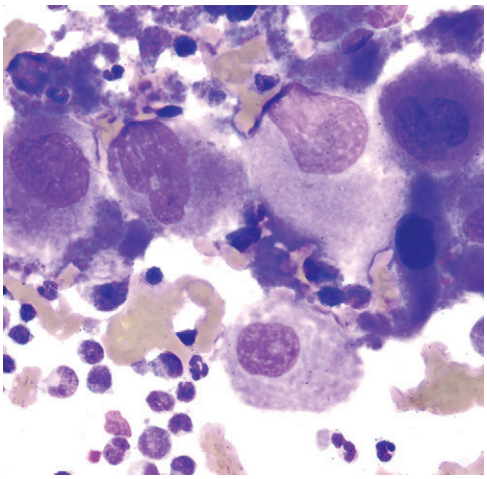
- Hypercellular with panmyelosis
- M:E ratio usually normal
- Megakaryocytes may be abnormal in size and morphology

ESSENTIAL THROMBOCYTHEMIA



A

FIGURE 17–4A Peripheral blood (original magnification ×1000).



B

FIGURE 17–4B Bone marrow (original magnification ×500).

MORPHOLOGY

Peripheral Blood:

LEUKOCYTES

Normal or slightly increased
Normal maturation and distribution

ERYTHROCYTES

Normal or slightly decreased

PLATELETS

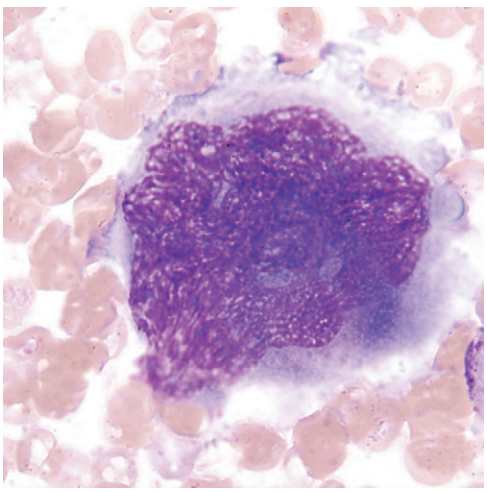
Marked sustained thrombocytosis
Variation in size from tiny to giant

Bone Marrow:

Hypercellular with expansion of the megakaryocyte pool

- Large megakaryocytes with abundant cytoplasm
- May exhibit hyperlobulation

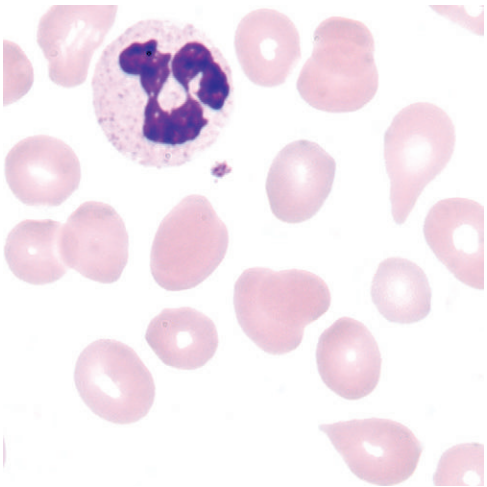
Mild granulocytic hyperplasia
Mild erythrocytic hyperplasia



C

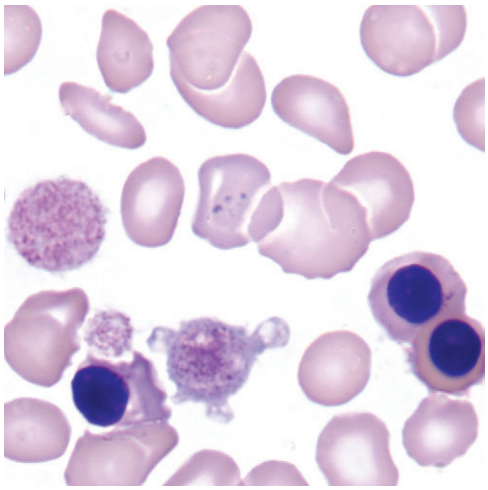
FIGURE 17–4C Bone marrow (original magnification ×1000).

PRIMARY MYELOFIBROSIS



A

FIGURE 17–5A Peripheral blood (×1000; subtle changes).



B

FIGURE 17–5B Peripheral blood (×1000; more advanced case).

MORPHOLOGY

Peripheral Blood:

LEUKOCYTES

Normal, increased, or decreased

- Immature granulocytes
- <5% blasts

ERYTHROCYTES

Normal or decreased

- Tear drop cells common, nucleated erythrocytes, polychromasia

PLATELETS

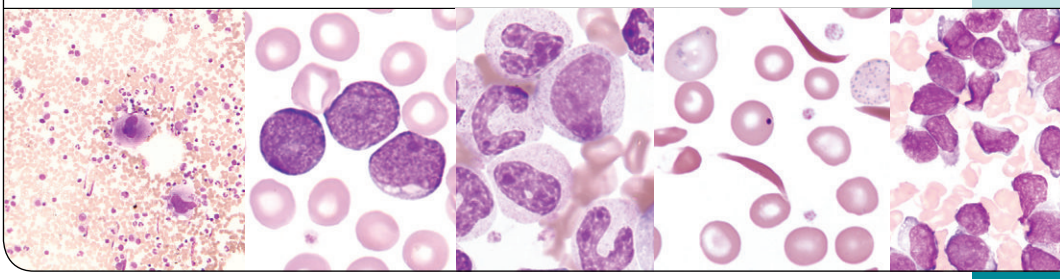
Low, normal, or increased

- May be giant with atypical shapes
- Abnormal granulation
- ± Circulating micromegakaryocytes

Bone Marrow: Aspiration attempts often result in a dry tap; biopsy results exhibit marked fibrosis with islands of hematopoietic activity and pockets of clumped megakaryocytes.

18

MYELODYSPLASTIC SYNDROMES



Myelodysplastic syndromes (MDSs) are acquired clonal hematological disorders characterized by normocellular/hypercellular marrow, ineffective hematopoiesis that leads to progressive cytopenia, and dysplasia in peripheral blood, reflecting maturation defects in erythrocytes, leukocytes, and/or platelets.

MDSs are heterogeneous and have a multitude of expressions; however, two morphologic findings are common to all types of MDS: the presence of progressive cytopenias in spite of a cellular bone marrow and dyspoiesis in one or more cell lines. Subtypes of the 2008 World Health Organization classification of MDSs are listed in Box 18-1.

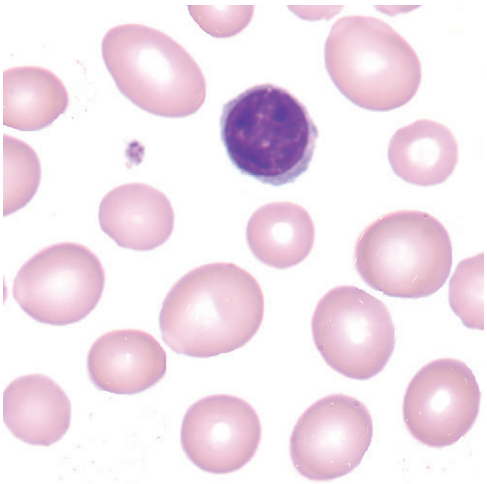
BOX 18-1 World Health Organization Classification of Myelodysplastic Syndromes (2008)

Refractory cytopenia with unilineage dysplasia
Refractory anemia with ringed sideroblasts
Refractory cytopenia with multilineage dysplasia
Refractory anemia with excess blasts
Myelodysplastic syndrome with isolated del(5q)
Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome (provisional)

From Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO classification of tumours of haematopoietic and lymphoid tissues*, ed 4, Lyon, France, 2008, IARC Press.

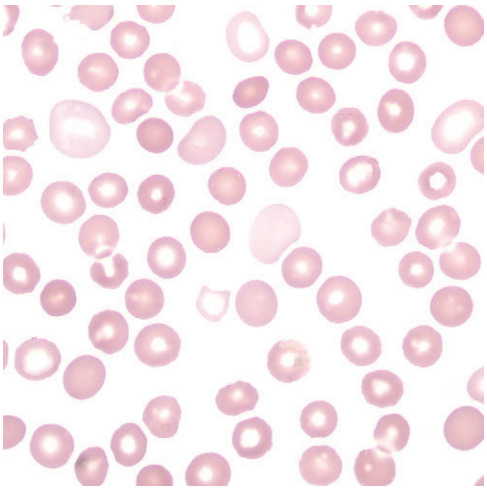
All photomicrographs are ×1000 original magnification with Wright-Giemsa staining unless stated otherwise.

DYSERYTHROPOIESIS



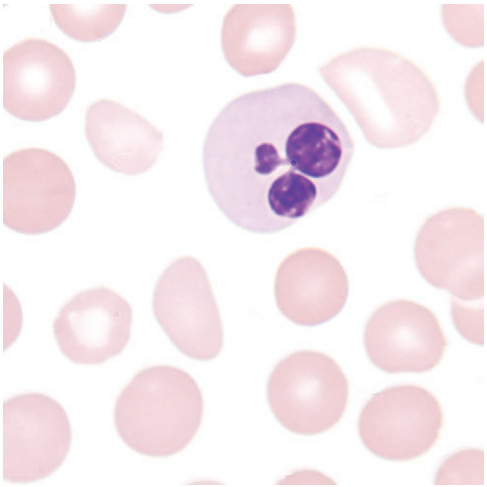
A

FIGURE 18–1A Oval macrocytes (PB ×1000).



B

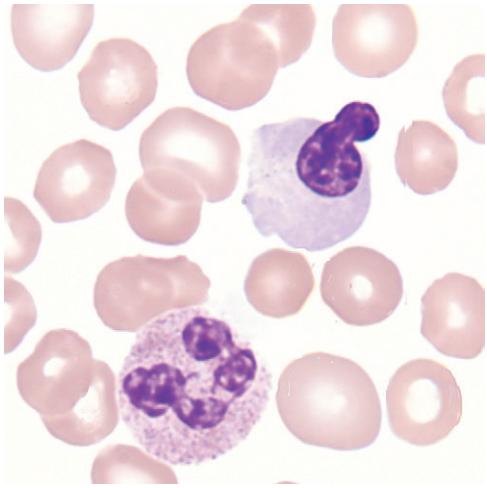
FIGURE 18–1B Dimorphic erythrocyte population (PB ×500).



C

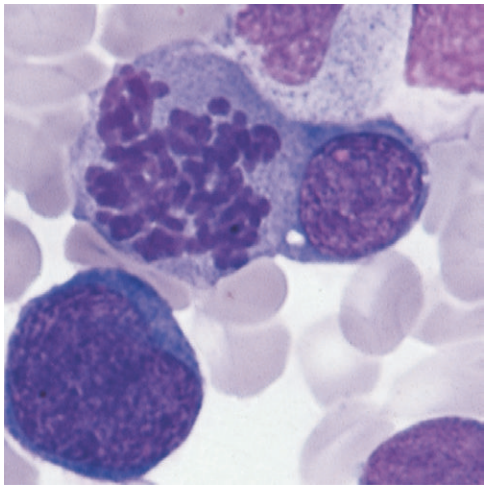
FIGURE 18–1C Nucleated erythrocyte with abnormal nuclear shape (PB ×1000).

Evidence of dyserythropoiesis (Figure 18-1, *A-I*) may include any or all of the following: oval macrocytes, hypochromic microcytes, dimorphic erythrocyte population, erythrocyte precursors with more than one nucleus, abnormal nuclear shapes, nuclear bridging, uneven cytoplasmic staining, and/or ringed sideroblasts.



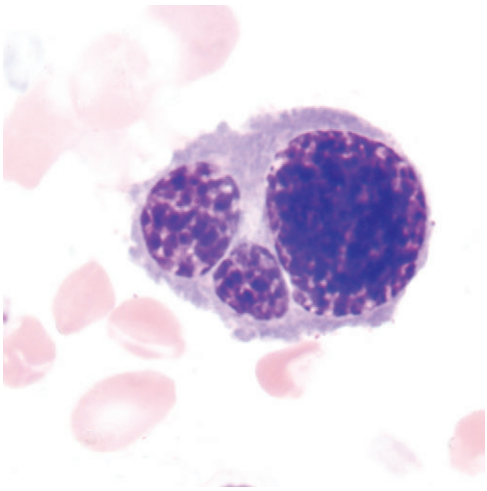
D

FIGURE 18–1D Erythrocyte precursor with partial loss of nucleus (PB ×1000).



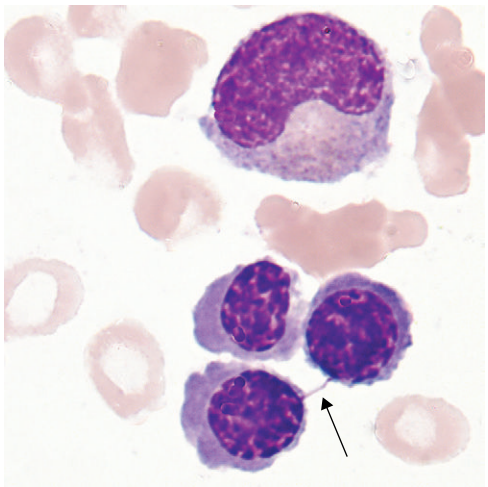
E

FIGURE 18–1E Erythrocyte precursor with abnormal nuclear shape (bilobed, with one nucleus in mitosis, demonstrating asynchrony; BM ×1000).



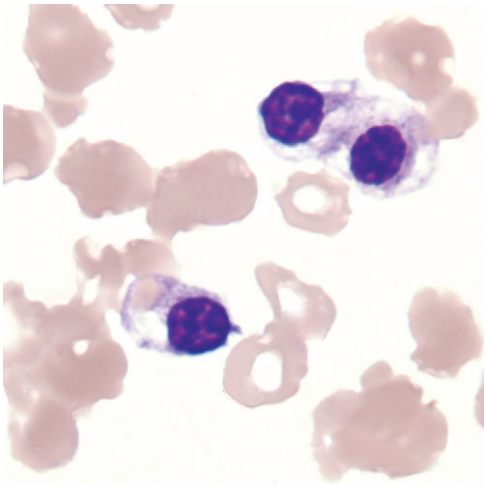
F

FIGURE 18–1F Erythrocyte precursor with three uneven nuclei (BM ×1000).



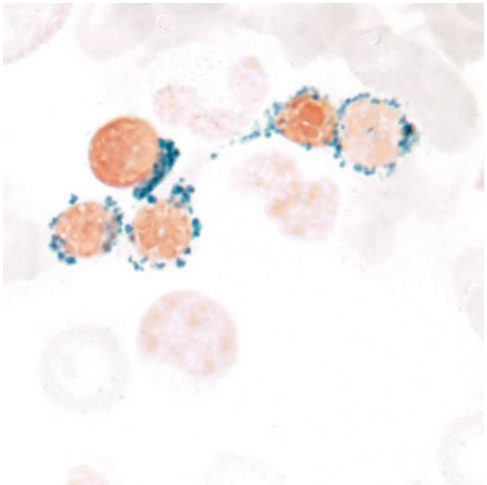
G

FIGURE 18–1G Erythrocyte precursor with nuclear bridging (BM ×1000).



H

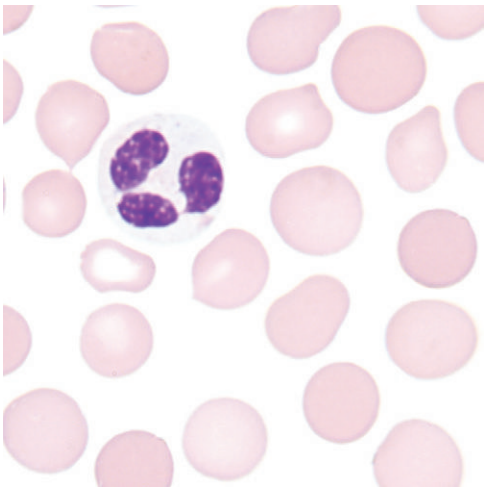
FIGURE 18–1H Erythrocyte precursors with uneven cytoplasmic staining (BM ×1000).



I

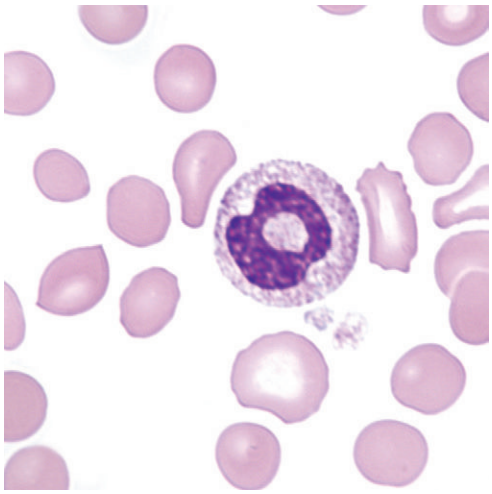
FIGURE 18–1I Ringed sideroblasts (iron stain, BM ×1000). Ringed sideroblasts are precursor RBCs containing at least five iron granules that circle at least one third of the nucleus.

DYSMYELOPOIESIS



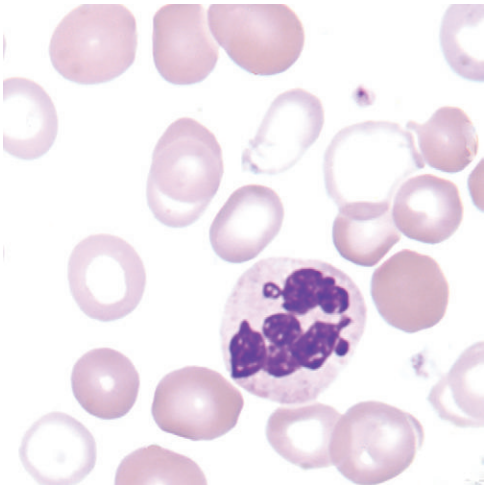
A

FIGURE 18–2A Abnormal granulation, agranular segmented neutrophil.



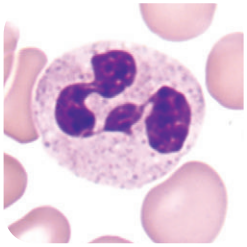
B

FIGURE 18–2B Abnormal nuclear shapes, neutrophil with circular (donut) nucleus.



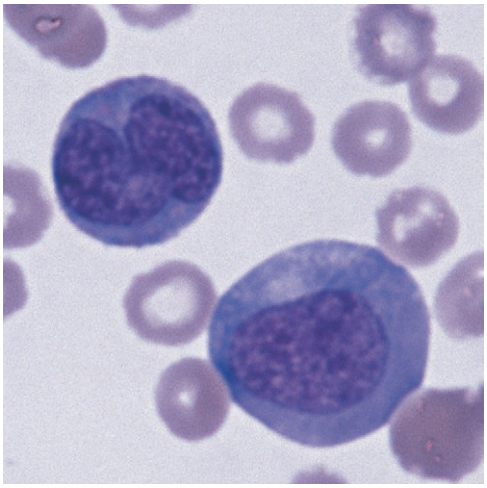
C

FIGURE 18–2C Abnormal nuclear shapes, neutrophil with hypersegmented nucleus; also exhibits hypogranulation.



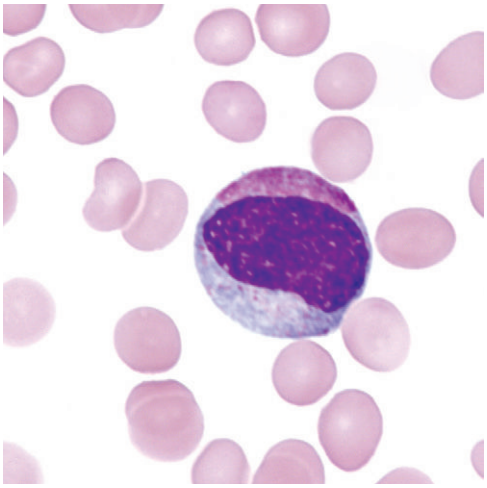
Normal neutrophil for comparison.

Evidence of dysmyelopoiesis (Figure 18-2, *A-E*) may include any or all of the following: abnormal granulation, abnormal nuclear shapes, persistent basophilic cytoplasm, and/or uneven cytoplasmic staining and pseudo-Pelger-Hüet cells (see Figure 14-1).



D

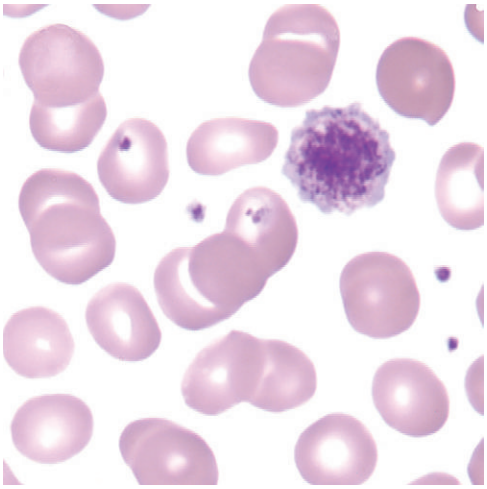
FIGURE 18–2D Persistent basophilic cytoplasm.



E

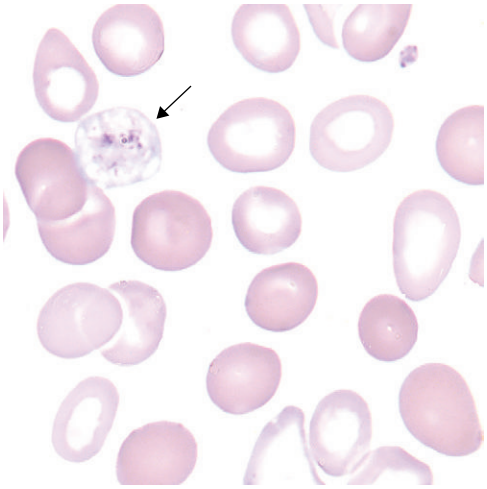
FIGURE 18–2E Uneven cytoplasmic staining with uneven granulation. This characteristic does not necessarily imply malignancy, but is often found in MDS.

DYSMEGAKARYOPOIESIS



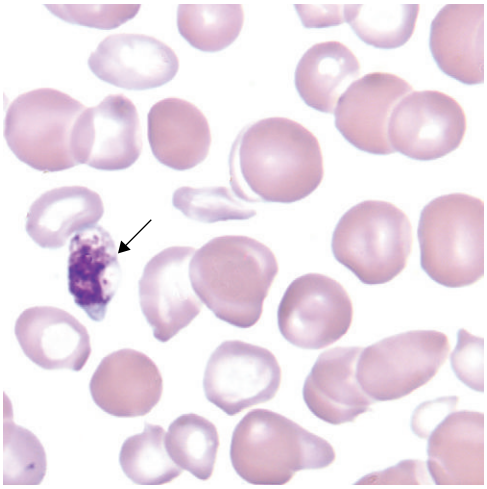
A

FIGURE 18–3A Giant platelet.



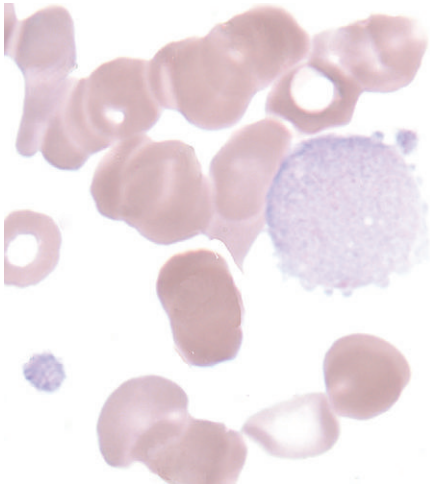
B

FIGURE 18–3B Platelet with hypogranulation.



C

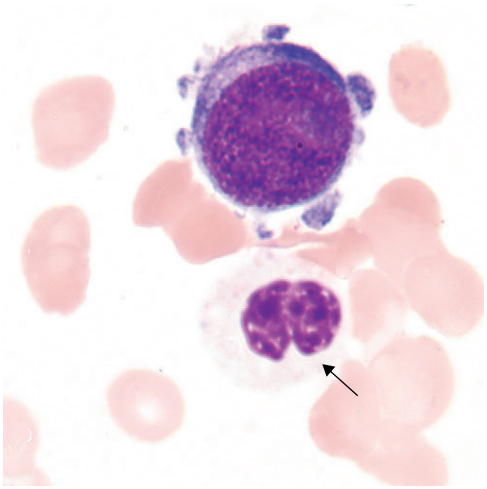
FIGURE 18–3C Platelet with hypergranulation.



D

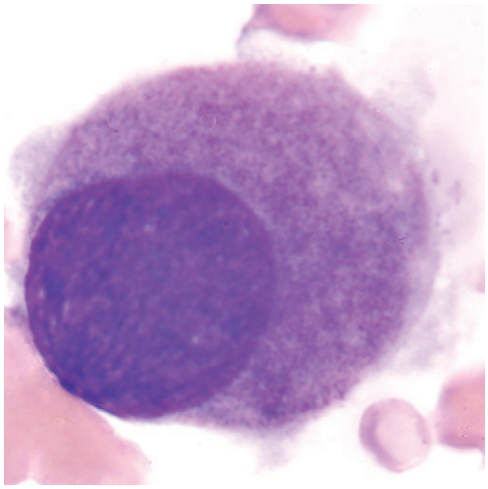
FIGURE 18–3D Giant platelet.

Evidence of dysmegakaryopoiesis (Figure 18-3, *A-H*) may include any or all of the following: giant platelets, platelets with abnormal granulation, circulating micromegakaryocytes, large mononuclear megakaryocytes, and abnormal nuclear shapes.



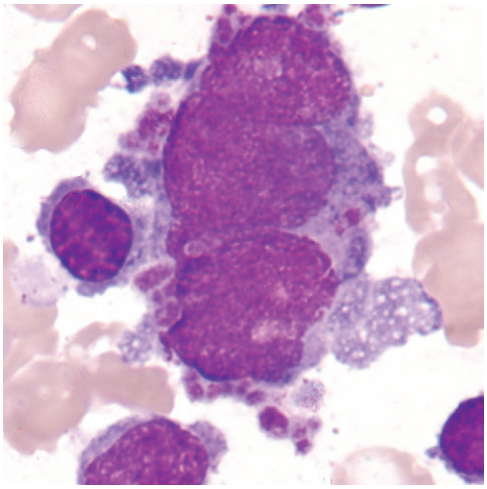
E

FIGURE 18–3E Circulating micromegakaryocyte. Hypogranular pseudo-Pelger-Hüet cell at *arrow*.



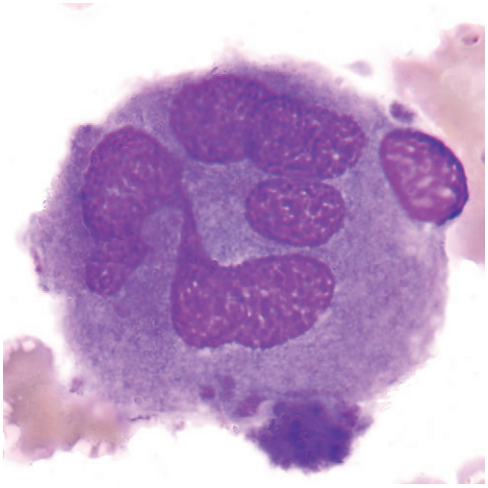
F

FIGURE 18–3F Large mononuclear megakaryocyte (BM ×1000).



G

FIGURE 18–3G Abnormal nuclear shape, uneven number of nuclei (BM ×1000).



H

FIGURE 18–3H Abnormal nuclear shapes, separate nuclei (BM, original magnification ×1000).

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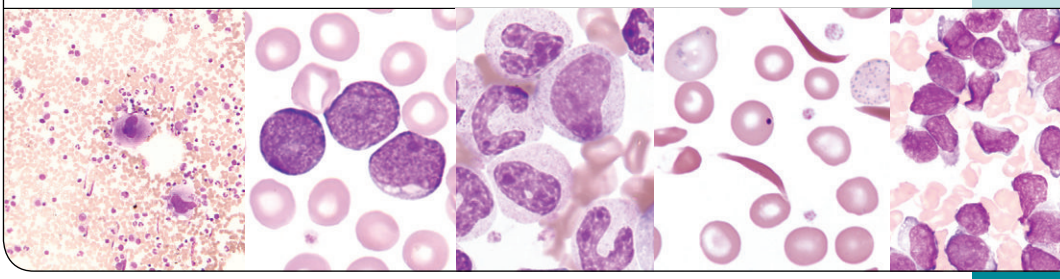
19

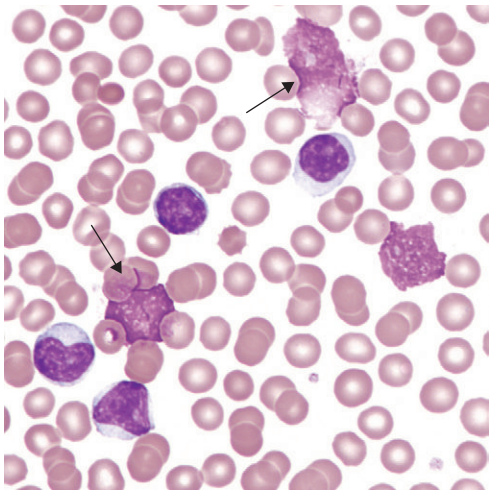
MATURE LYMPHOPROLIFERATIVE DISORDERS



Mature lymphoproliferative disorders frequently are derived from a single clone of cells. Although this group of diseases involves lymphocytes, the morphological presentation is variable. The integration of clinical and morphological disease features with immunophenotyping is necessary for appropriate recognition and classification. Only representative samples are included in this atlas.

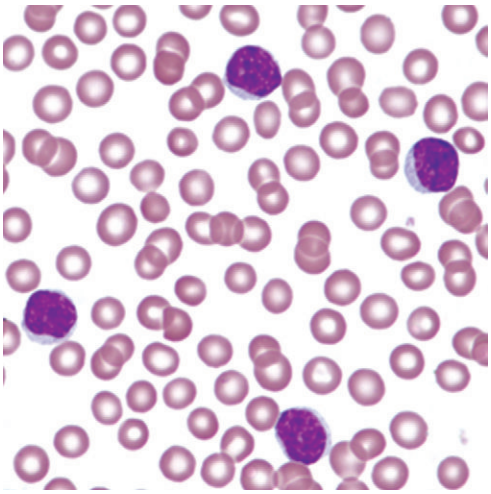
NOTE: Sustained absolute lymphocytosis in an adult should be investigated to differentiate reactive from malignant processes. Characteristics of reactive lymphocytes are listed in Table 14-1.

CHRONIC LYMPHOCYTIC LEUKEMIA



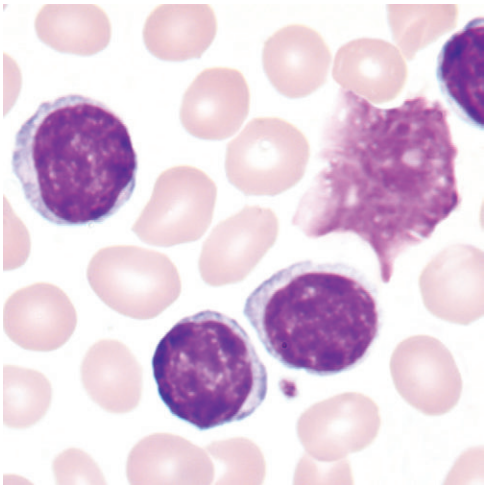
A

FIGURE 19–1A Small lymphocytes with smudge cells at *arrows* (PB ×500).



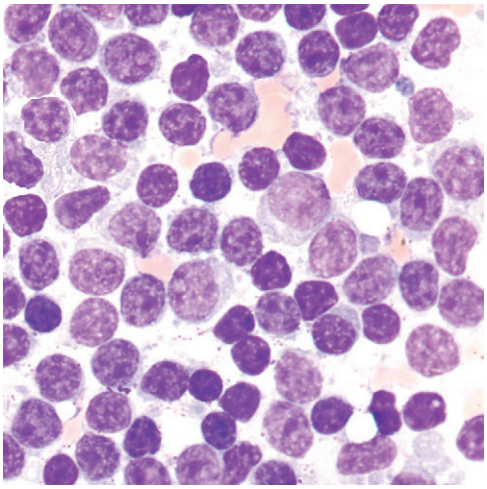
B

FIGURE 19–1B Albumin smear-same patient as presented in figure 19-1A (PB ×500).



C

FIGURE 19–1C Small lymphocytes with smudge cell (PB ×1000).



D

FIGURE 19–1D (BM ×500).

MORPHOLOGY

Peripheral Blood: Mature-appearing lymphocytes with round nuclei and block-type chromatin; inconspicuous nucleoli, scant cytoplasm; homogeneous appearance within a given patient; lymphocytes more fragile than normal, leading to "smudge" cells

Absolute sustained lymphocytosis
± Normocytic normochromic anemia (approximately 10% of patients develop an autoimmune hemolytic anemia)
± Thrombocytopenia

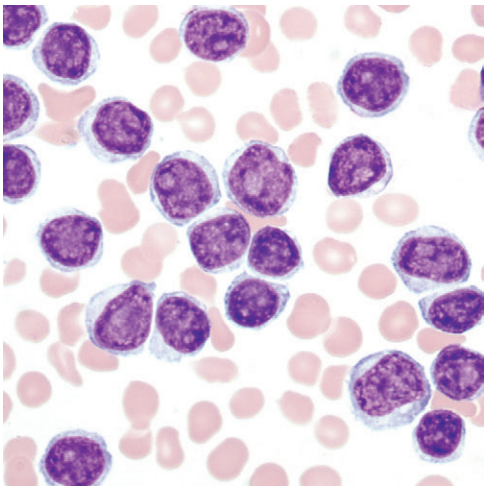
Bone Marrow: ≥30% lymphocytes

IMMUNOPHENOTYPE

$CD20^+$, $CD19^+$, $CD5^+$, $CD23^+$

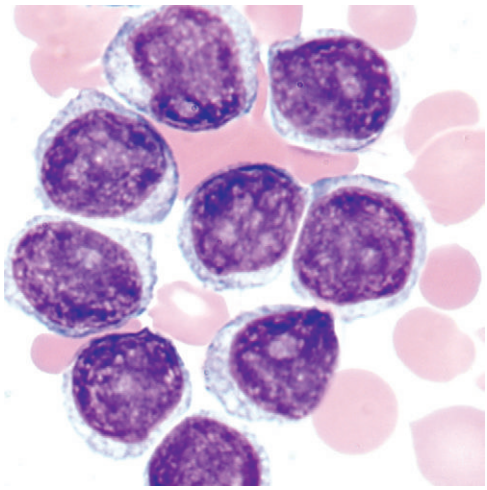
NOTE: Addition of albumin to blood prior to smear preparation stabilizes CLL cells, decreasing the formation of smudge cells and allows for accurate cell classification.

B CELL PROLYMPHOCYTIC LEUKEMIA



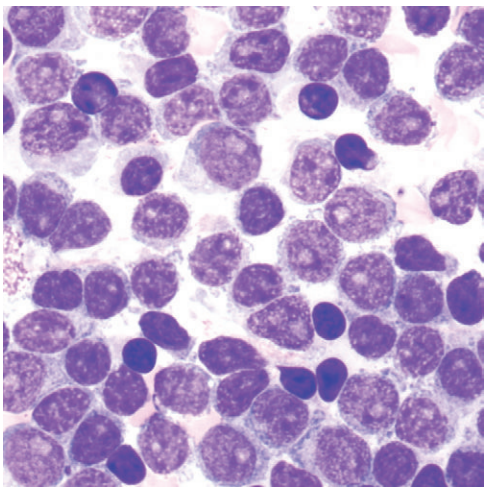
A

FIGURE 19–2A (PB ×500).



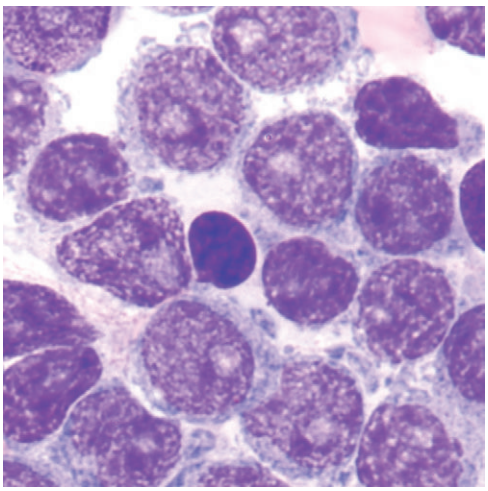
B

FIGURE 19–2B (PB ×1000).



C

FIGURE 19–2C (BM ×500).



D

FIGURE 19–2D (BM ×1000).

MORPHOLOGY

Peripheral Blood: Medium-sized cells (approximately twice the size of small lymphocyte), one prominent nucleolus, moderately condensed chromatin; small-to-moderate slightly basophilic cytoplasm

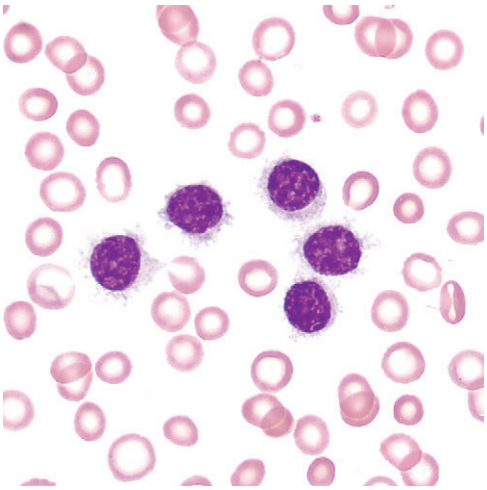
Absolute lymphocytosis, usually $>100 \times 10^9$/L
Anemia
Thrombocytopenia

Bone Marrow: Predominantly prolymphocytes with few residual hematopoietic cells

IMMUNOPHENOTYPE

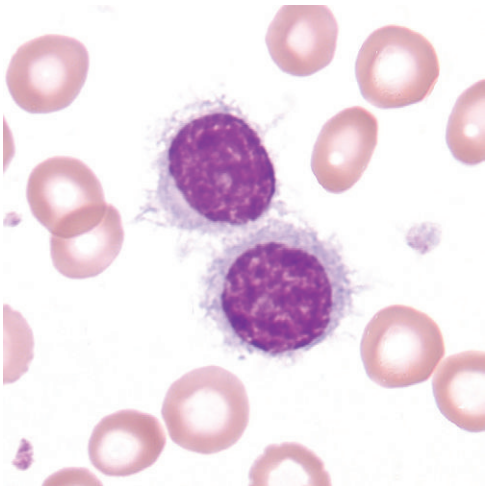
$CD20^+$, $CD19^+$, $FMC7^+$

HAIRY CELL LEUKEMIA



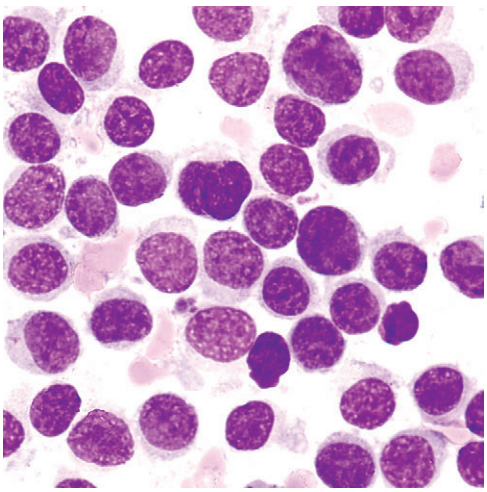
A

FIGURE 19–3A (PB ×500).



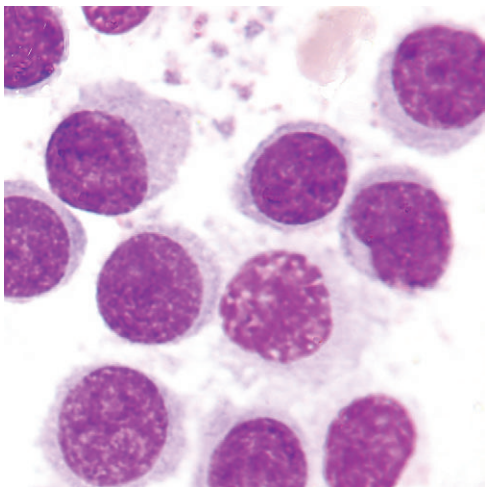
B

FIGURE 19–3B (PB ×1000).



C

FIGURE 19–3C (BM ×500).



D

FIGURE 19–3D (BM ×1000).

MORPHOLOGY

Peripheral Blood: Small- to medium-sized lymphocytes, reniform to oval nucleus with diffuse homogeneous chromatin, ± nucleolus, cytoplasm irregular, and gray-blue hairlike projections

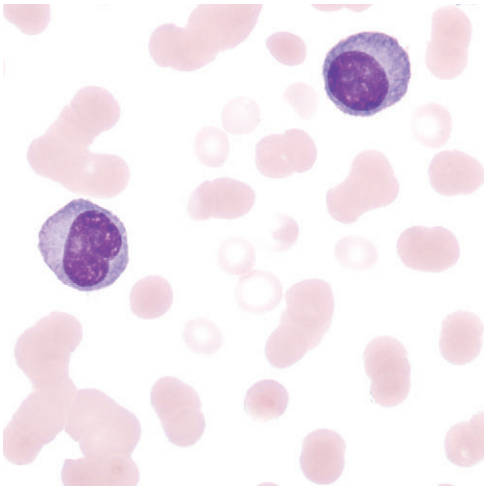
Pancytopenia

Bone Marrow: Aspirate difficult to obtain because of marrow fibrosis (dry tap), cells more easily distinguished by phase or electron microscopy

IMMUNOPHENOTYPE

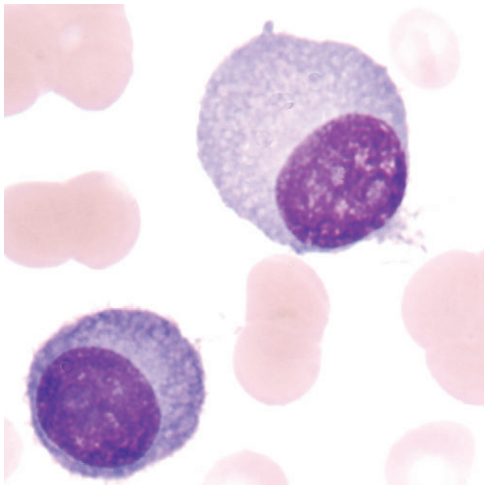
$CD19^+$, $CD20^+$, $CD22^+$, $CD11c^+$, Annexin $A1^+$

PLASMA CELL MYELOMA



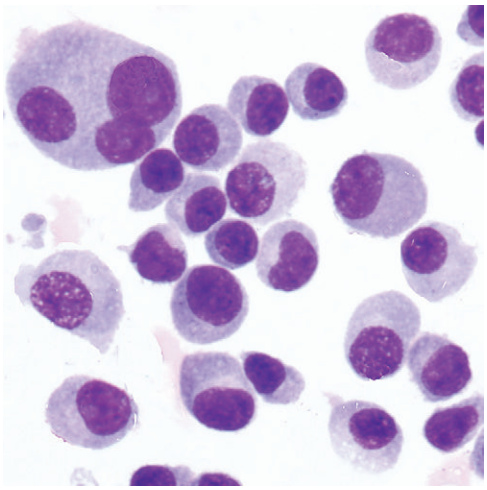
A

FIGURE 19–4A Plasma cells. Note rouleaux (PB ×500).



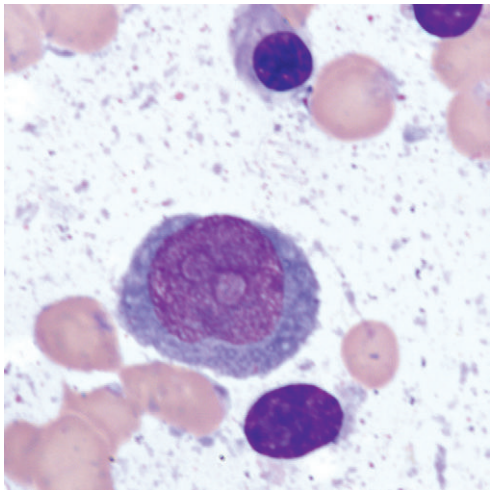
B

FIGURE 19–4B Plasma cells (PB ×1000).



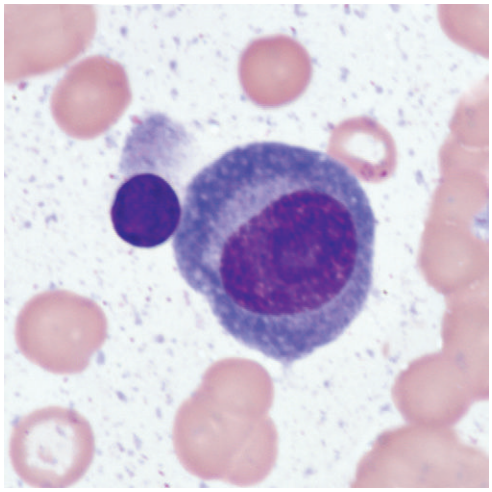
C

FIGURE 19–4C Plasma cells, one multi-nucleated (BM ×500).



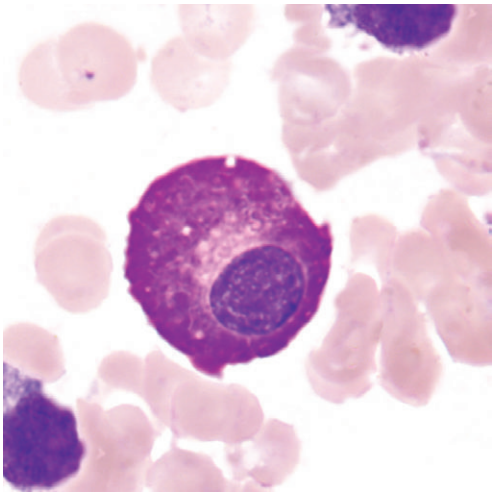
D

FIGURE 19–4D Plasmablast (BM ×1000). Note the lighter blue cytoplasm with the indistinct hof and the slightly eccentric nucleus with 2 distinct nucleoli.



E

FIGURE 19–4E Proplasmacyte (PB ×1000). The cytoplasm is darker blue and the perinuclear hof is distinct. The nucleus is eccentric and the nucleolus is partially masked by the clumped chromatin.



F

FIGURE 19–4F Flame cell (BM ×1000). Associated with plasma cells that are producing IgA.

MORPHOLOGY

Peripheral Blood: Rouleaux, rare circulating plasma cell; ± neutropenia

NOTE: >2 × 10^9/L circulating plasma cells suggest plasma cell leukemia.

± Normocytic, normochromic anemia
± Thrombocytopenia

NOTE: The background of Wright-stained blood smears may be blue because of abnormal amounts of immunoglobulin.

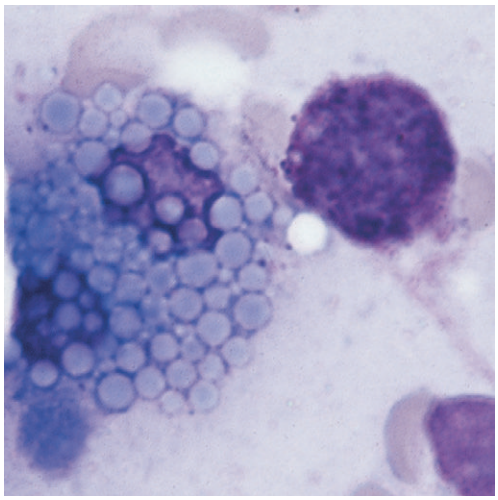
Bone Marrow: >10% plasma cells, often >30%

Immature ± larger than normal plasma cell with increased N/C ratio; abnormal nuclear chromatin; ± nucleoli, ± multinucleated
Cytoplasm pale blue or dark; cytoplasm may contain immunoglobulin inclusions

IMMUNOPHENOTYPE

$CD19^-$, $CD38^+$, $CD138^+$

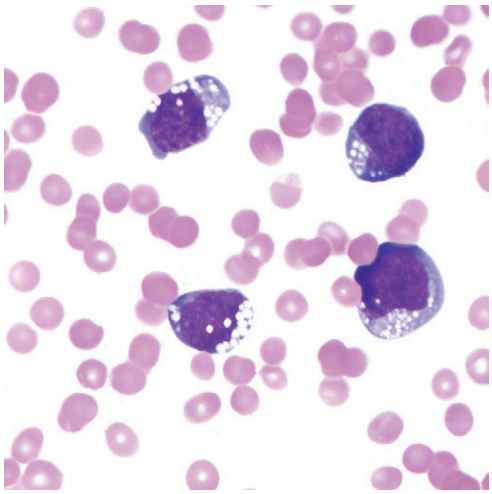
NOTE: This disease may be distinguished from Waldenström macroglobulinemia and heavy chain disease by immunoelectrophoresis.



G

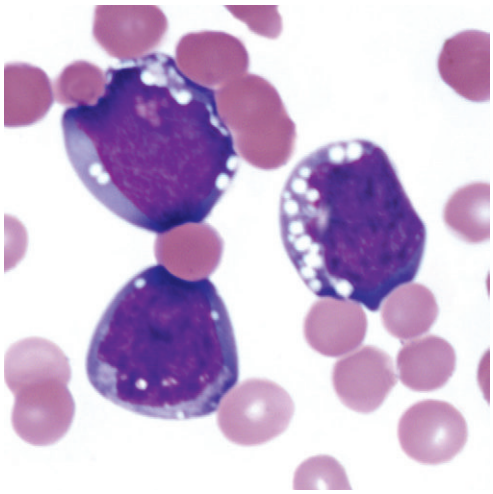
FIGURE 19–4G Mott cell (Morula cell, grape cell) (BM ×1000). Plasma cell containing multiple round globules of immunoglobulin, which stain pink, colorless, or blue.

BURKITT LEUKEMIA/LYMPHOMA



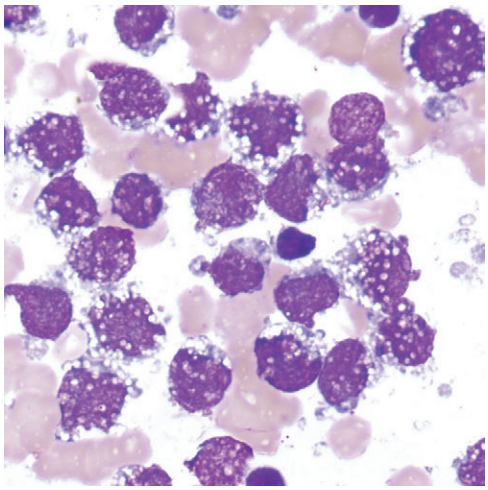
A

FIGURE 19–5A (PB ×500).



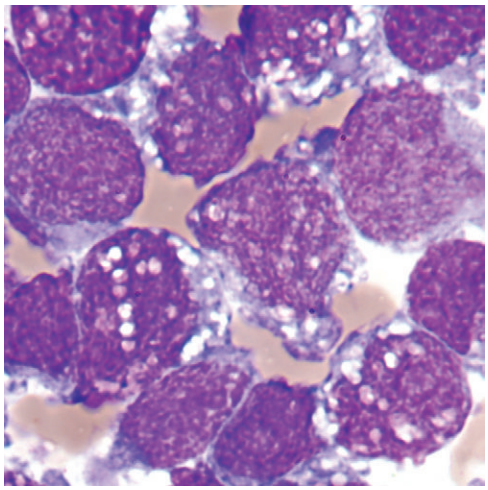
B

FIGURE 19–5B (PB ×1000).



C

FIGURE 19–5C (BM ×500).



D

FIGURE 19–5D (BM ×1000).

MORPHOLOGY

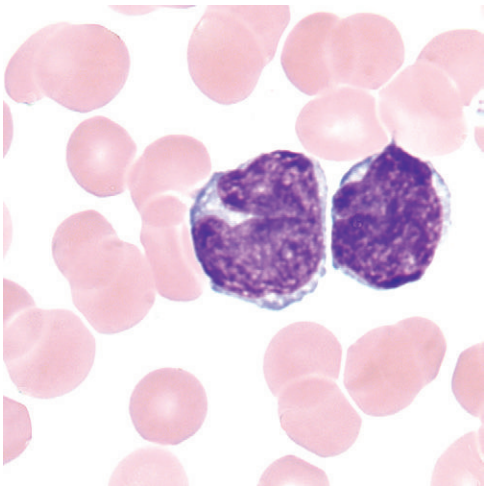
Peripheral Blood: Medium- to large-sized cells with dark blue vacuolated cytoplasm, inconspicuous nucleoli

Bone Marrow: Monotonous pattern of deeply basophilic cells with vacuolated cytoplasm

IMMUNOPHENOTYPE

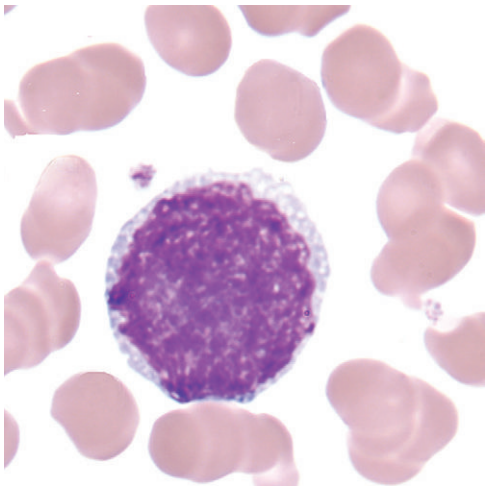
$CD5^-$, $CD20^+$, $CD19^+$, $CD10^+$

LYMPHOMA



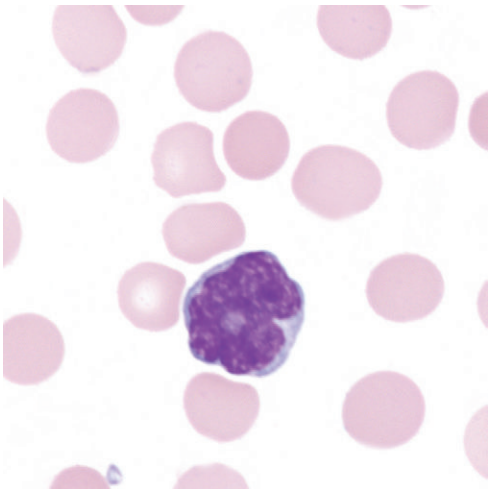
A

FIGURE 19–6A Cleaved lymphoma cells (PB ×1000).



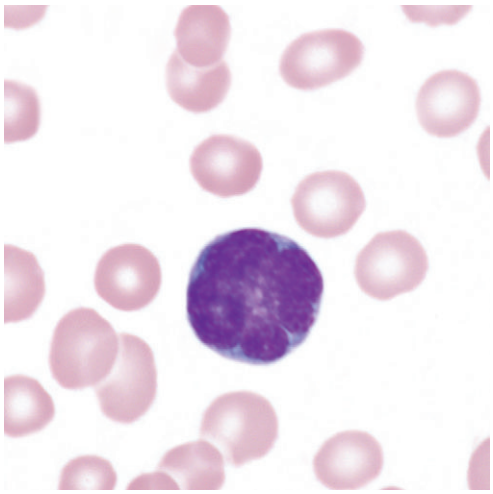
B

FIGURE 19–6B Large lymphoma cell (PB ×1000).



C

FIGURE 19–6C Lymphoma cell with prominent nucleoli (PB ×1000).



D

FIGURE 19–6D "Flower" nucleus suggestive of T-cell lymphoma (PB ×1000).

MORPHOLOGY

Peripheral Blood: Representative examples of lymphoma cells occasionally observed in peripheral blood.

Bone Marrow: NA

NOTE: The diagnosis of lymphoma is determined by lymph node biopsy, immunophenotyping, and molecular genetics.

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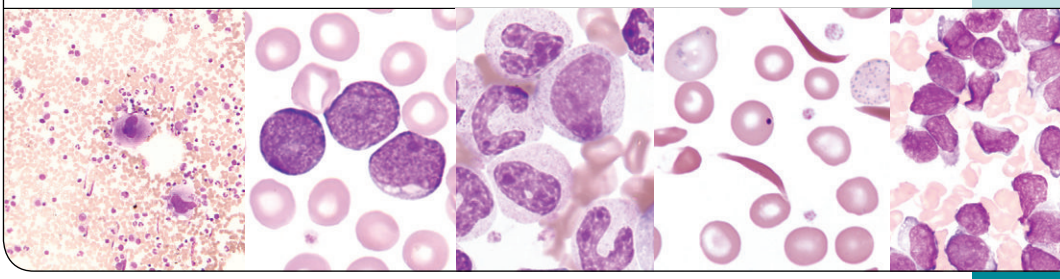
20

MORPHOLOGIC CHANGES AFTER MYELOID HEMATOPOIETIC GROWTH FACTORS



Cytokine therapies such as erythropoietin, thrombopoietin, and myeloid growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage-colony stimulating factor are becoming common. There are characteristic changes in the peripheral blood smear caused by these treatments. Although erythropoietin and thrombopoietin rarely create diagnostic challenges, the morphologic changes in the myeloid cell line may mimic severe infection, acute myeloid leukemia, or myelodysplastic or myeloproliferative neoplasm. Specific changes include transient leukocytosis with immature granulocytic cells, vacuolated and giant neutrophils, toxic granulation, Döhle bodies, hypogranulation, nucleated red blood cells, and as many as 5% blasts in the peripheral blood.*

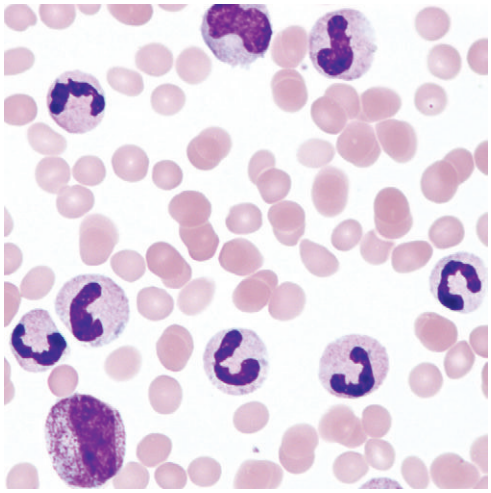


FIGURE 20-1 Leukocytosis in response to G-CSF (×500).

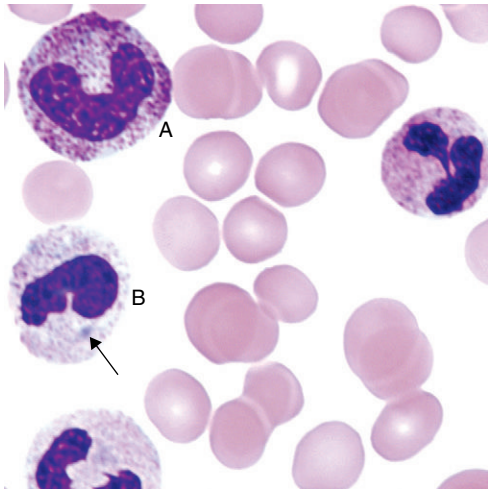


FIGURE 20-2 Neutrophils in peripheral blood exhibiting toxic granulation *(A)*, and hypogranulation *(B)*. A Döhle body is present at the *arrow*.

*Arber DA. Acute myeloid leukemia. In: Hsi ED, editor. *Hematopathology.* In: Goldblum JR, series editor. *Foundations in Diagnostic Pathology.* Philadelphia: Churchill Livingstone; 2007, p. 397–429.

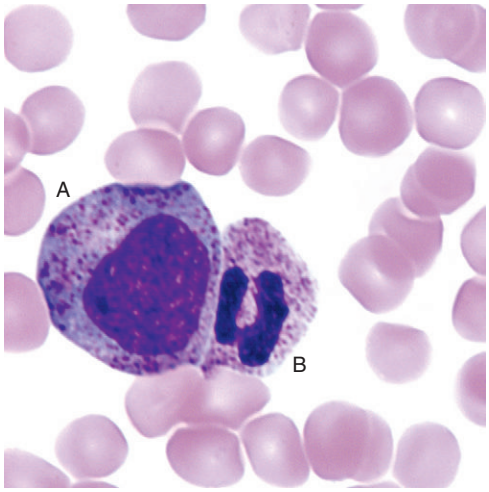


FIGURE 20-3 Immature asynchronous granulocyte *(A)*, and a mature neutrophil with toxic granulation *(B)*.

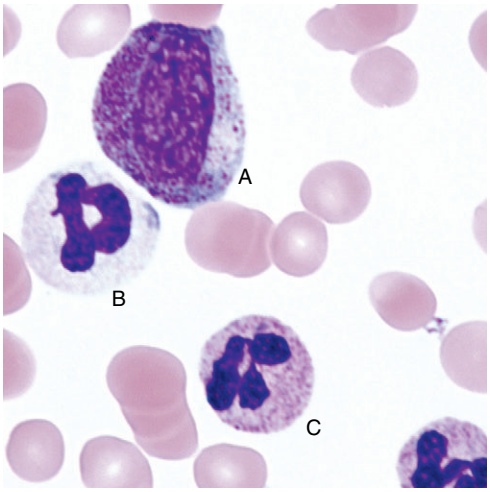


FIGURE 20-4 Giant asynchronous immature granulocyte *(A)*, a hypogranular neutrophil with a Döhle body *(B)*, and a granulocyte with normal granulation *(C)*.

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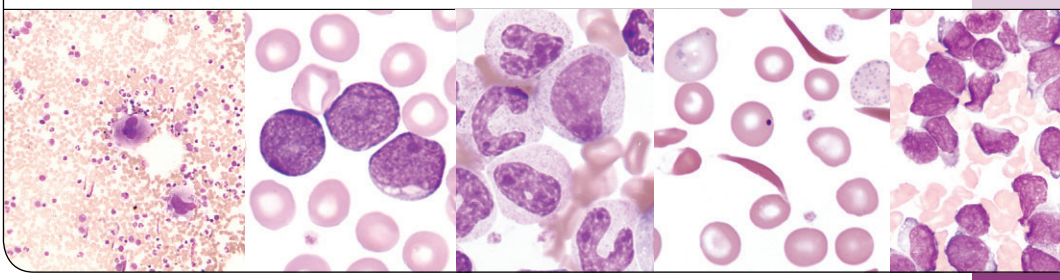
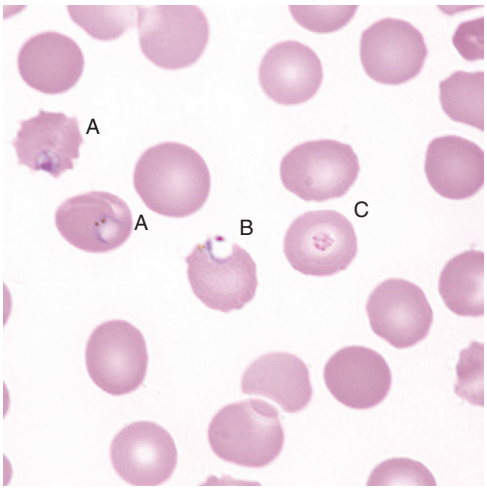
21

MICROORGANISMS



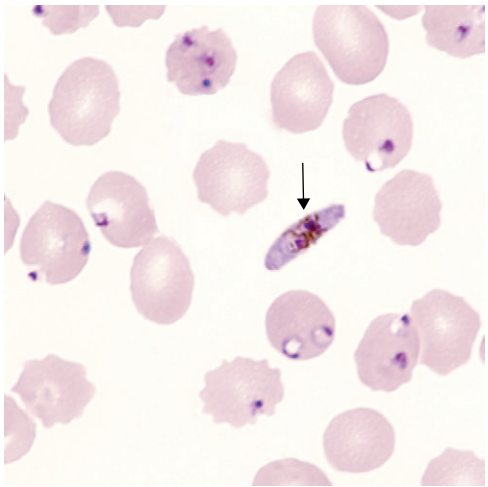
PLASMODIUM SPECIES

The following examples are representative of the developmental stages of malaria that can be seen in the peripheral blood. Detailed criteria for identification of species may be found in a parasitology text.



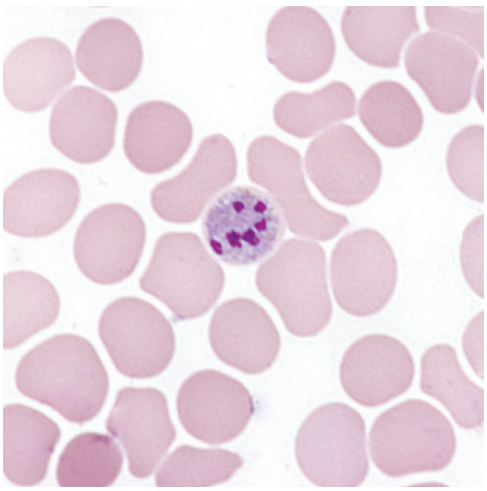
A

FIGURE 21–1A *Plasmodium falciparum* rings (*A*), including applique form (*B*), and platelet on RBC (*C*) (PB ×1000). (Courtesy Indiana Pathology Images).



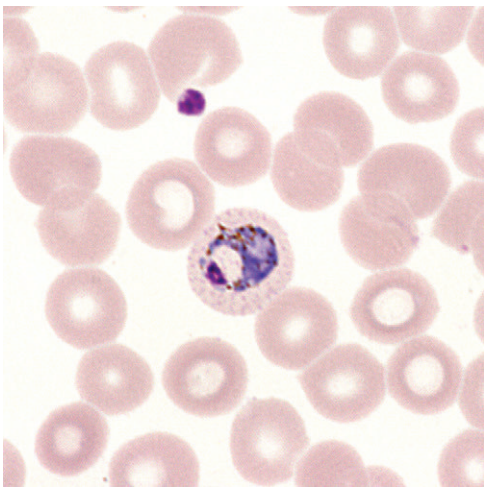
B

FIGURE 21–1B *Plasmodium falciparum* rings and crescent (banana-shaped) gametocyte (*arrow*) (PB ×1000). (Courtesy Indiana Pathology Images).



C

FIGURE 21–1C *Plasmodium malariae* schizont (with 6 merozoites) (PB ×1000). (Courtesy Indiana Pathology Images).



D

FIGURE 21–1D *Plasmodium vivax;* growing trophozoite with stippling (PB ×1000). (Courtesy Indiana Pathology Images.)

BABESIA SPECIES

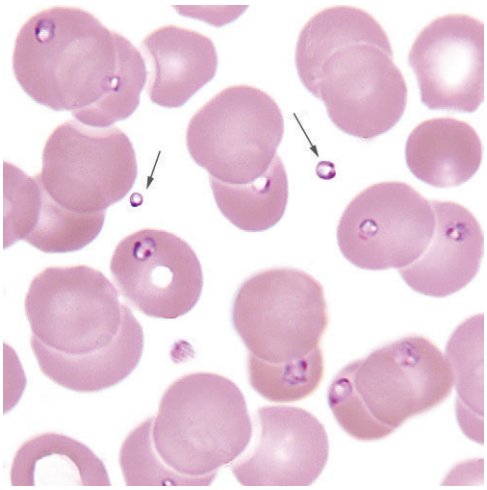


FIGURE 21–2 *Babesia microti* (PB ×1000).

Babesia species may be confused morphologically with *Plasmodium falciparum*, but lack of pigment and absence of life cycle stages help differentiate *Babesia* spp. from *P. falciparum*. Another differentiating factor is the presence of extracellular organisms (Figure 21-2, *arrows*) that may be seen in *Babesia* spp. but not in *P. falciparum*.

LOA LOA

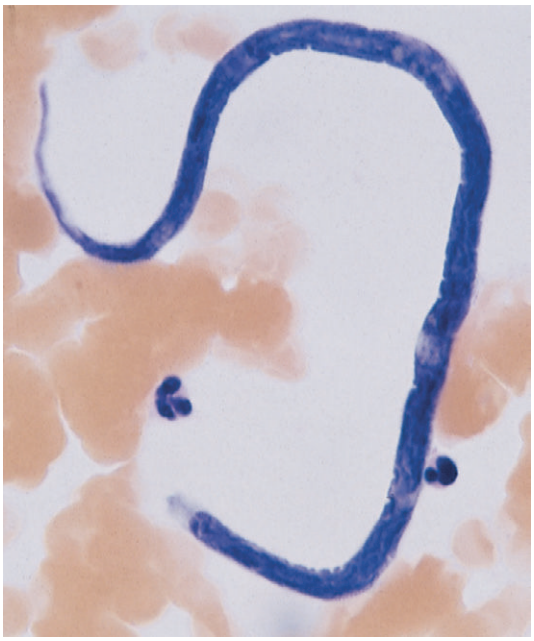
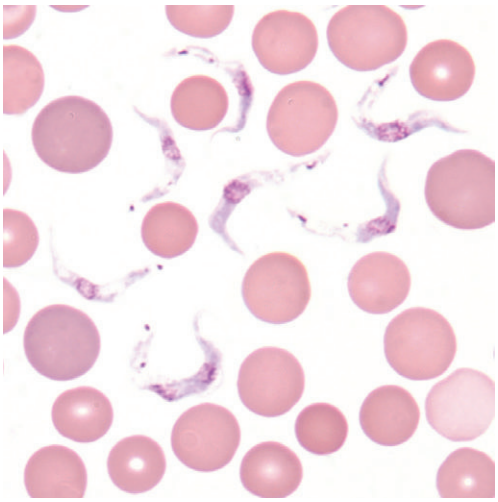


FIGURE 21–3 *Loa loa*, a microfilaria (PB original magnification ×1000).

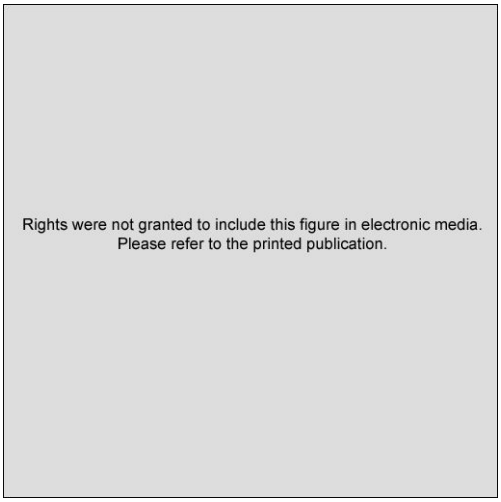
Loa loa is a microfilaria (Figure 21-3). Other microfilariae rarely may be seen in the peripheral blood.

TRYPANOSOMES



A

FIGURE 21–4A *Trypanosoma gambiense* (Giemsa stain, PB ×1000).

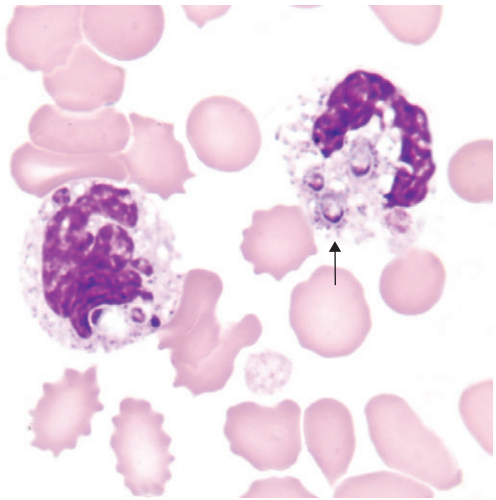


B

FIGURE 21–4B *Trypanosoma cruzi* (Giemsa stain, PB ×1000). (From Marler LM, Siders JA, Simpson A et al: *Parasitology image atlas CD-ROM*, Indianapolis, IN, 2003, Indiana Pathology Images.)

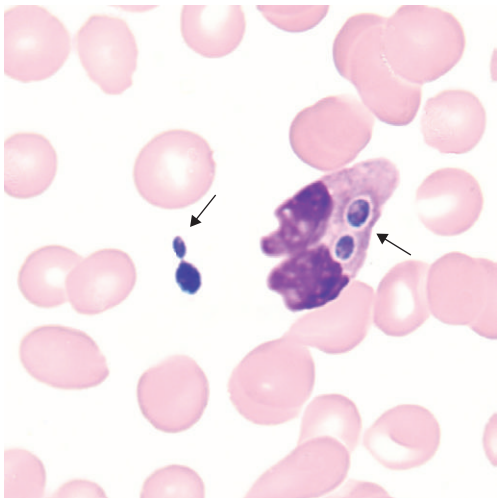
Trypanosomes are examples of hemoflagellates that may occasionally be encountered in the peripheral blood (Figure 21-4, *A* and *B*). Differentiating features may be found in a parasitology text.

FUNGI



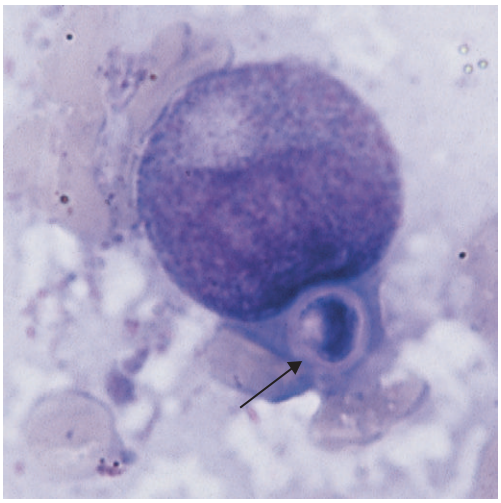
A

FIGURE 21–5A *Histoplasma capsulatum* in neutrophil (PB ×1000).



B

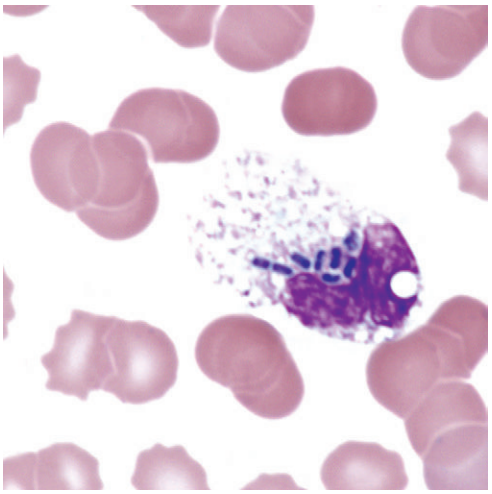
FIGURE 21–5B Intracellular and extracellular yeast in peripheral blood of an immunocompromised patient (PB ×1000).



C

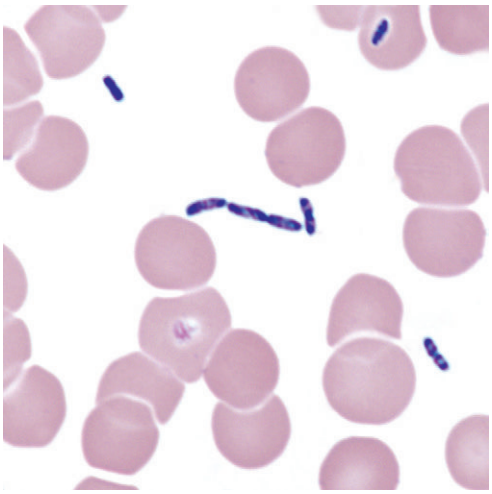
FIGURE 21–5C *Cryptococcus neoformans* (BM × 1000) (see also Figure 24-14).

BACTERIA



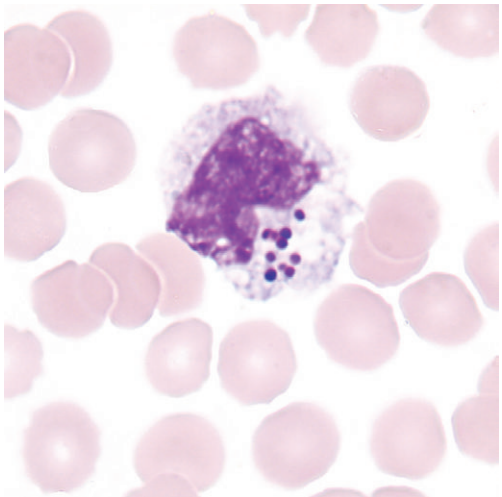
A

FIGURE 21–6A Bacilli engulfed by a leukocyte. Note vacuoles (PB ×1000).



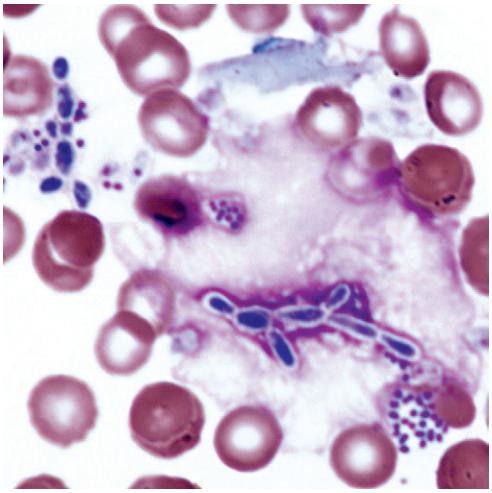
B

FIGURE 21–6B Extracellular bacteria from same specimen as Figure 21-6A. Extracellular bacteria alone may indicate contamination. Presence of intracellular bacteria may rule out contamination (PB ×1000).



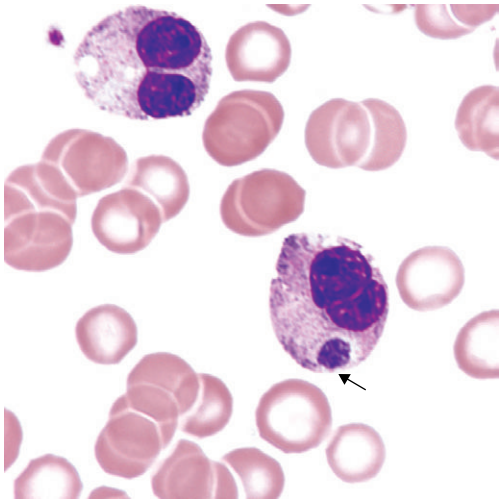
C

FIGURE 21–6C Cocci engulfed by a monocyte (PB ×1000).



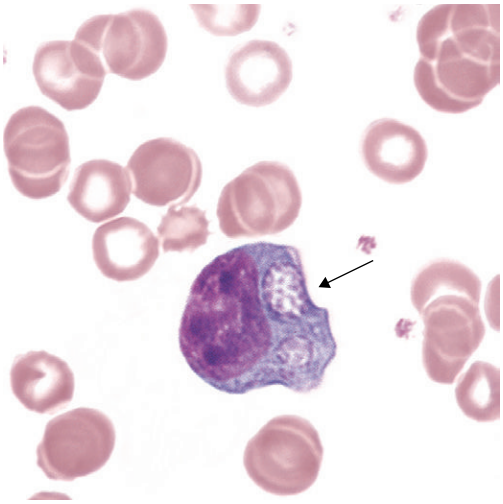
D

FIGURE 21–6D Multiple organisms, including yeast and cocci, most likely contamination from an intravenous line (PB ×1000).



E

FIGURE 21–6E *Anaplasma phagocytophilum* in a neutrophil (PB ×1000).

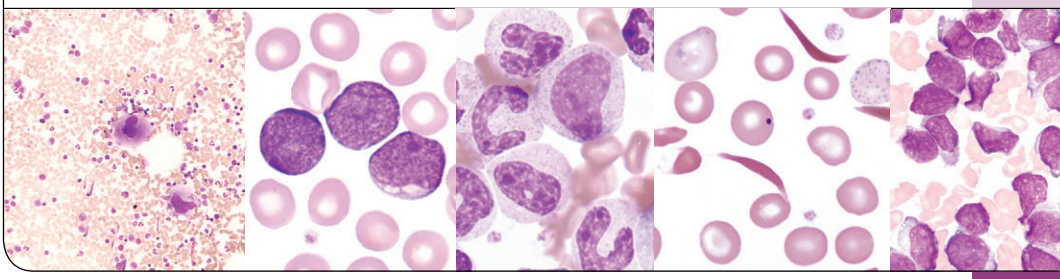


F

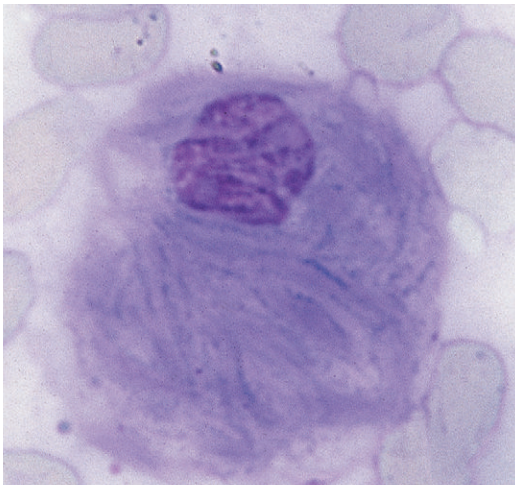
FIGURE 21–6F *Ehrlichia chaffeensis* in a monocyte (PBx1000). (Courtesy J. Stephen Dumler, MD, Division of Medical Microbiology, The Johns Hopkins Medical Institutions, Baltimore, MD.)

22

MISCELLANEOUS CELLS



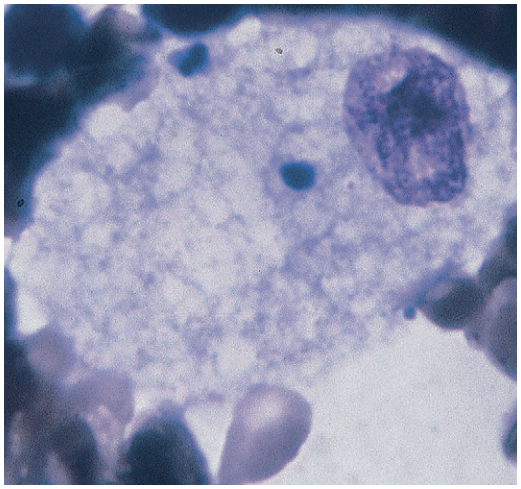
HEMATOLOGIC MANIFESTATIONS OF SYSTEMIC DISORDERS



A

FIGURE 22-1A Gaucher cell (BM ×1000).

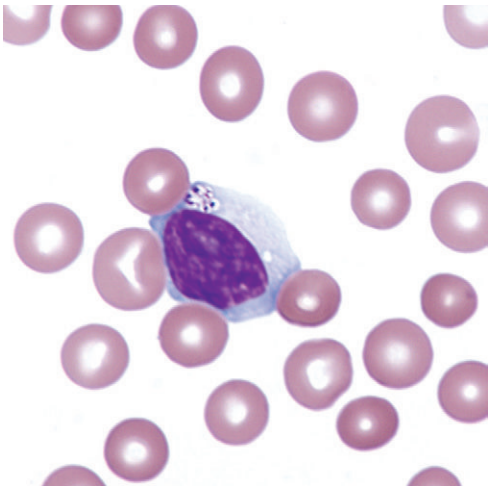
DESCRIPTION: The Gaucher cell is a macrophage 20 to 80 μm in diameter, with one or more small, round to oval eccentric nuclei; cytoplasm has crumpled tissue paper appearance; found in bone marrow, spleen, liver, and other affected tissue.



B

FIGURE 22-1B Niemann-Pick disease (BM ×1000).

DESCRIPTION: The Niemann-Pick cell is a macrophage, 20 to 90 μm in diameter, with a small eccentric nucleus and foamy cytoplasm. It is found in bone marrow and lymphoid tissue. The peripheral blood of patients with Niemann-Pick disease may exhibit vacuolated lymphocytes.

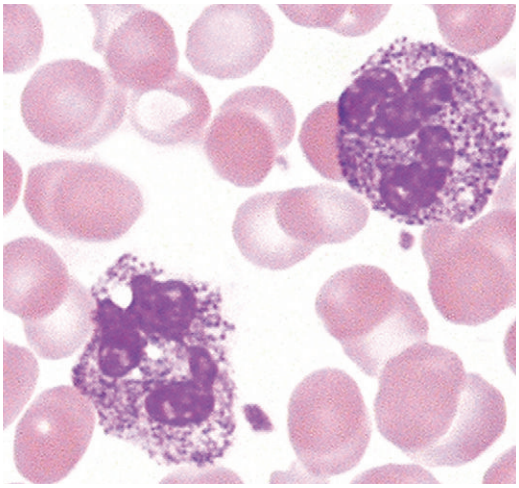


C

FIGURE 22-1C Lymphocyte from Sanfilippo Syndrome (PB ×1000).

DESCRIPTION: Peripheral blood lymphocytes containing azurophilic granules occasionally surrounded by halos

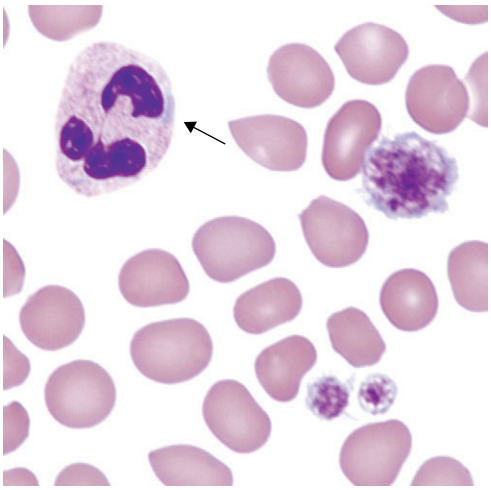
Associated with: Mucopolysaccharide storage disorders



D

FIGURE 22-1D Alder-Reilly anomaly (PB ×1000). (Courtesy Dennis P. O'Malley, MD, Clarient, Inc., Aliso Viejo, CA.)

DESCRIPTION: Deep purple to lilac granules difficult to distinguish from toxic granulation; occur in neutrophils and occasionally eosinophils and basophils

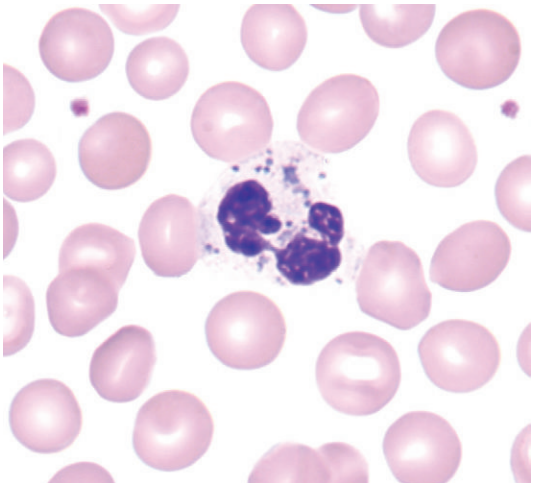


E

FIGURE 22–1E May-Hegglin Anomaly. Note Döhle-like body at *arrow* (PB ×1000).

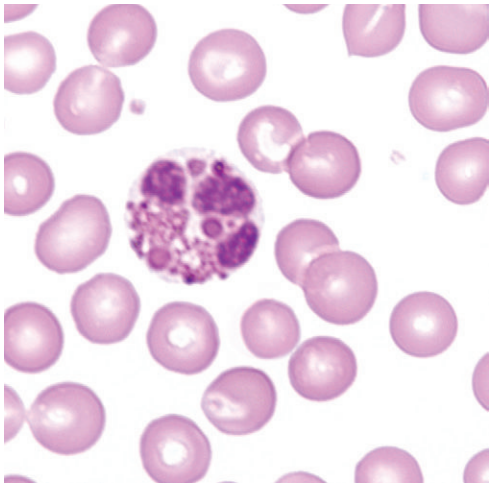
DESCRIPTION: Characterized by thrombocytopenia with large platelets and large basophilic inclusions resembling Döhle bodies in granulocytes and monocytes with the absence of toxic granulation

NOTE: These inclusions are sporadically visible by light microscopy but always detectable by electron microscopy. The ultrastructure varies from that of Döhle bodies.



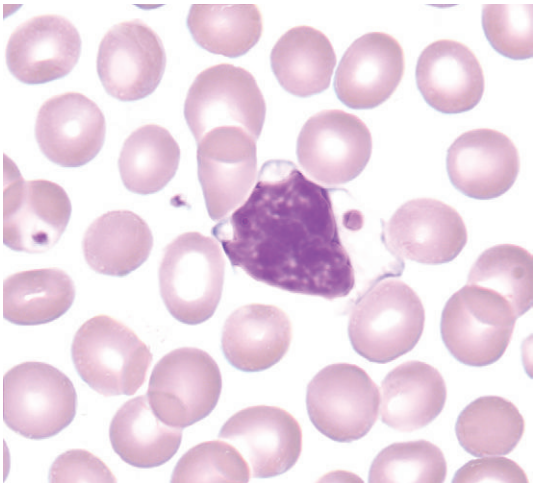
F

FIGURE 22–1F Chédiak-Higashi anomaly neutrophil with granules (PB ×1000).



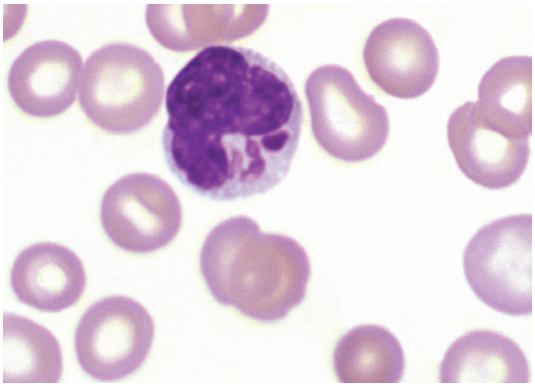
G

FIGURE 22–1G Chédiak-Higashi anomaly eosinophil with granules (PB ×1000).



H

FIGURE 22–1H Chédiak-Higashi anomaly lymphocyte with granule (PB ×1000).

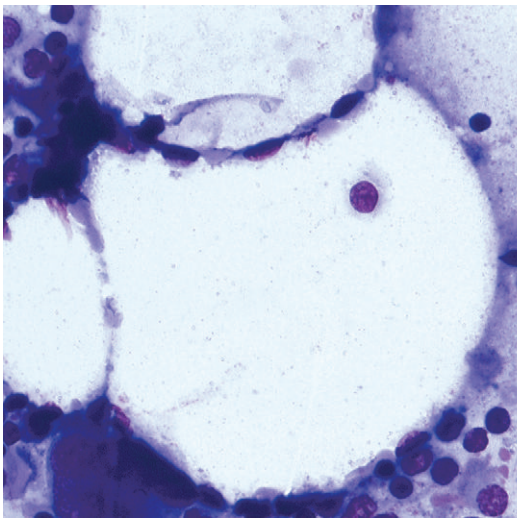


I

FIGURE 22–1I Chédiak-Higashi anomaly monocyte with granules (PB ×1000).

DESCRIPTION: Large gray-blue granules in the cytoplasm of many granulocytes. Monocytes, lymphocytes, and eosinophils may contain large red-purple granules.

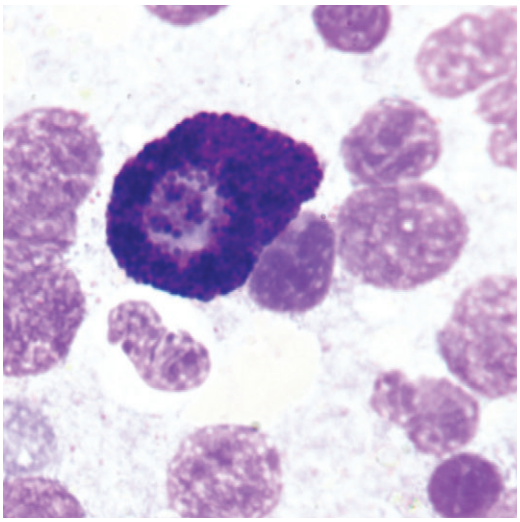
CELLS OCCASIONALLY SEEN IN BONE MARROW



A

FIGURE 22–2A Fat/adipose cell (BM ×500).

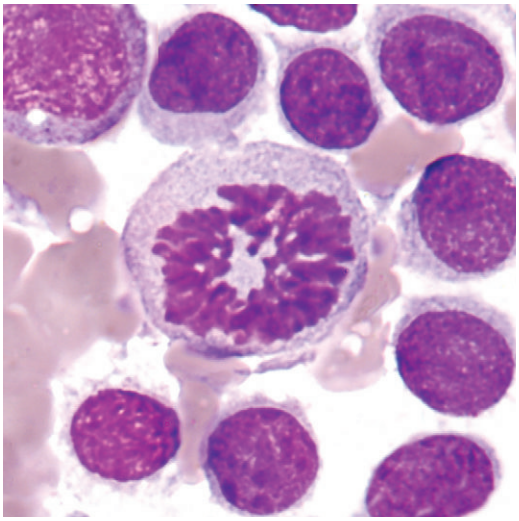
DESCRIPTION: Large, round cell, 50 to 80 μm; cytoplasm filled with one or several large fat vacuoles, colorless to pale blue; nucleus small, round to oval, and eccentric; chromatin coarse; nucleoli seldom seen



B

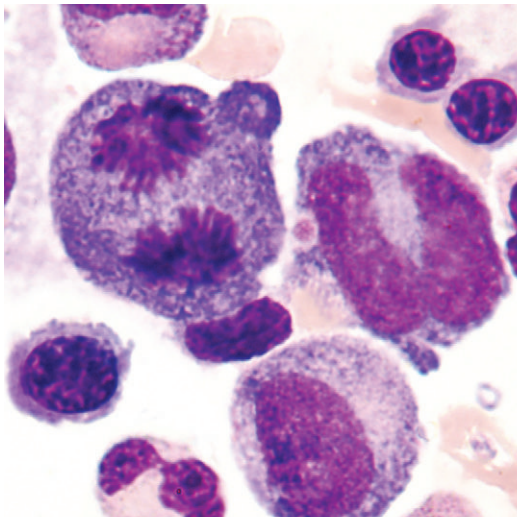
FIGURE 22–2B Mast cell (BM ×1000).

DESCRIPTION: Large cell (12-25 μm) with round to oval nucleus; cytoplasm is colorless to lavender with many dark blue to black granules that may partially obscure the nucleus. Constitute <1% of bone marrow cells. Increased numbers may be seen in allergic inflammation and anaphylaxis.



C

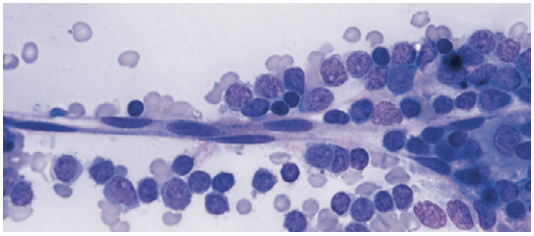
FIGURE 22–2C Mitosis (BM ×1000).



D

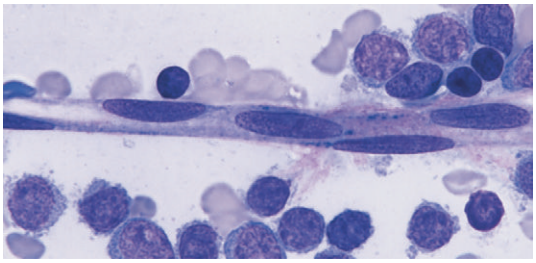
FIGURE 22–2D Mitosis (BM ×1000).

Mitotic figure—a cell that is dividing. Increased numbers may be seen in neoplastic disorders.



A

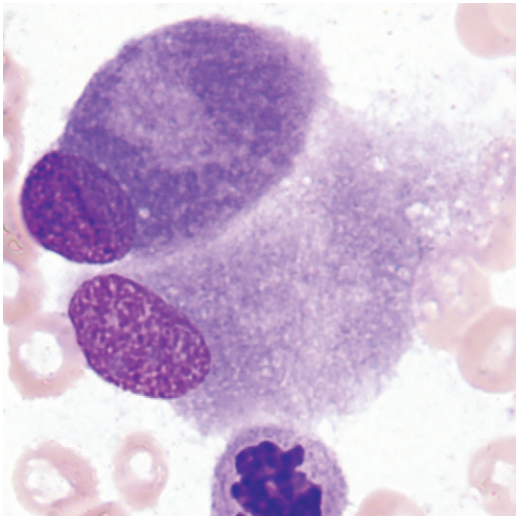
FIGURE 22–3A Endothelial cells lining a blood vessel (BM original magnification ×500).



B

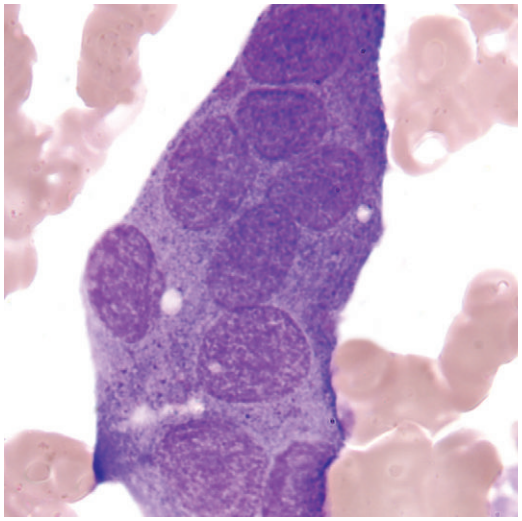
FIGURE 22–3B Endothelial cells (BM original magnification ×1000).

DESCRIPTION: Large, elongated cells, 20 to 30 μm; one oval nucleus with dense chromatin; nucleoli not visible; function is to line blood vessels; may also be seen in peripheral blood



C

FIGURE 22–3C Osteoblasts (BM original magnification ×1000).

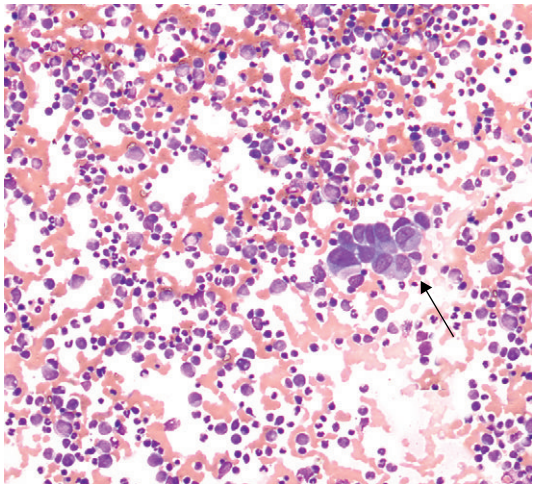


D

FIGURE 22–3D Osteoclast (BM original magnification ×1000).

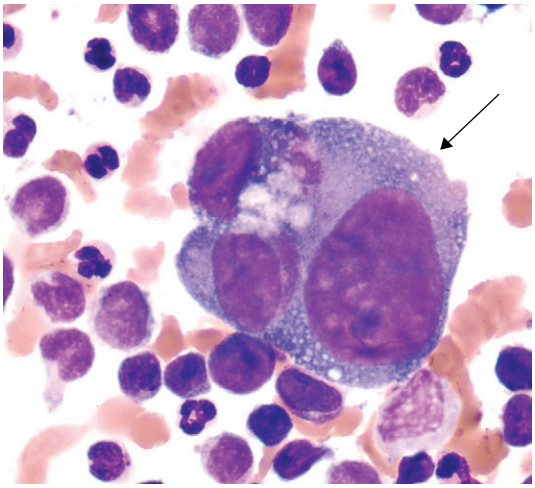
OSTEOBLAST
SIZE: 30 μm
DESCRIPTION: Comet or tadpole shaped; single, round, eccentrically placed nucleus, may be partially extruded; abundant cytoplasm with chromophobic area located away from nucleus; often appear in groups; function in synthesis of bone
NOTE: May be confused with plasma cells.

OSTEOCLAST
SIZE: Very large, >100 μm
DESCRIPTION: Multinucleated with irregularly shaped ruffled border; nuclei are round to oval, separate and distinct with little variation in nuclear size; cytoplasm may vary from slightly basophilic to very acidophilic; coarse granules may be present; osteoclasts function in the resorption of bone
NOTE: May be confused with megakaryocytes.



A

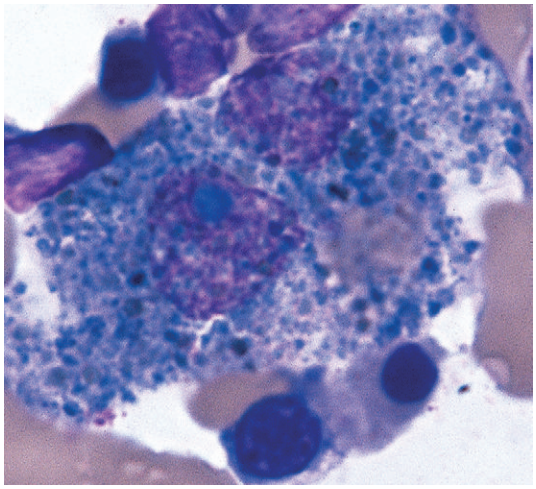
FIGURE 22–4A Metastatic tumor (BM ×100).



B

FIGURE 22–4B Metastatic tumor (BM ×500).

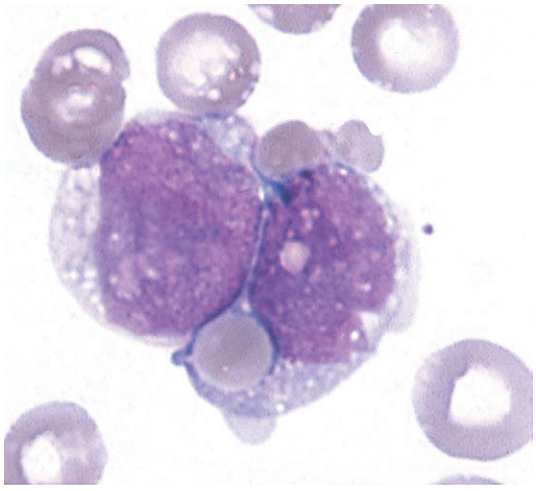
DESCRIPTION: Tumor cell clusters may be recognized during the ×100 scan of bone marrow, especially at or near the edge of the coverslip or glass slide. Characteristics of the tumor cells are more easily observed at ×1000 magnification. Cells are variable in size and shape within the same tumor clump. Nuclei vary in size and staining characteristics. Nucleoli are usually visible. It is sometimes difficult to distinguish one cell from another because of "molding" of cells.



A

FIGURE 22–5A Sea blue histiocyte (BM ×1000).

DESCRIPTION: Macrophage 20 to 60 μm in diameter with eccentric nucleus; cytoplasm contains varying numbers of prominent blue-green granules; may be seen in diseases with rapid cell turnover, such as myeloproliferative neoplasms

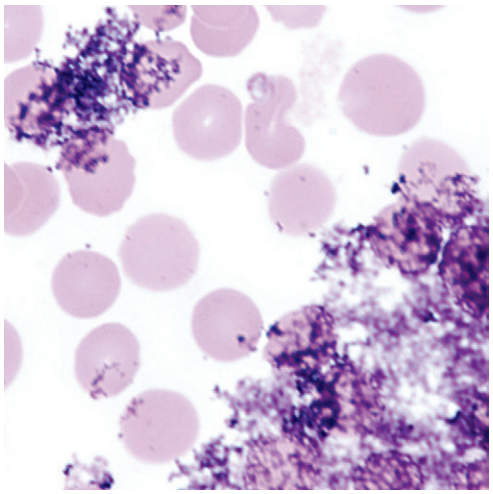


B

FIGURE 22–5B Erythrophagocytosis (BM ×1000).

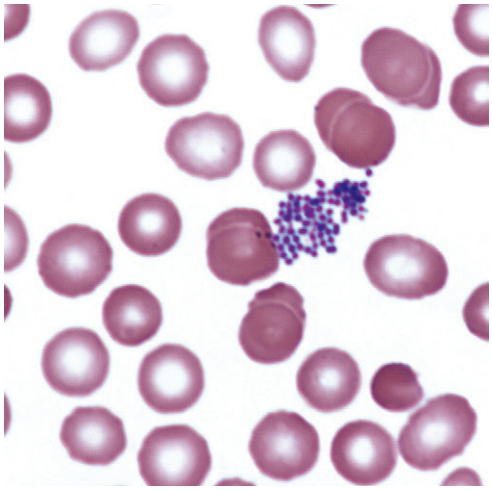
DESCRIPTION: Monocyte or macrophage with engulfed erythrocyte; may be seen in some transfusion reactions

ARTIFACTS IN PERIPHERAL BLOOD SMEARS



A

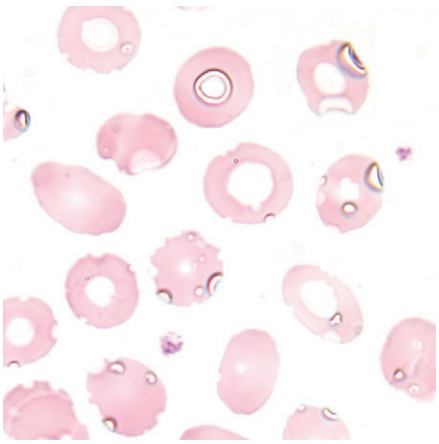
FIGURE 22–6A Precipitated stain (PB ×1000).



B

FIGURE 22–6B Bacteria in peripheral blood for comparison with precipitated stain.

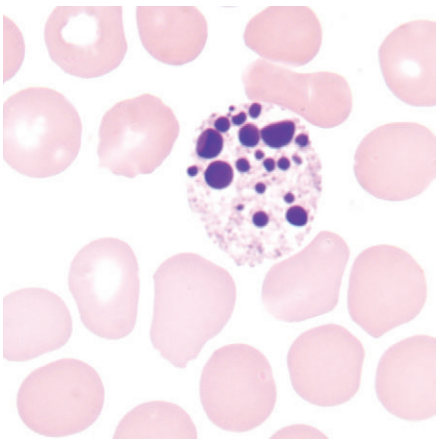
DESCRIPTION: Precipitate is in focus, but the cells are not. If bacteria are present within a cell, both cell and bacteria should be in focus at the same time.



C

FIGURE 22–6C Drying artifact in RBCs (PB ×1000).

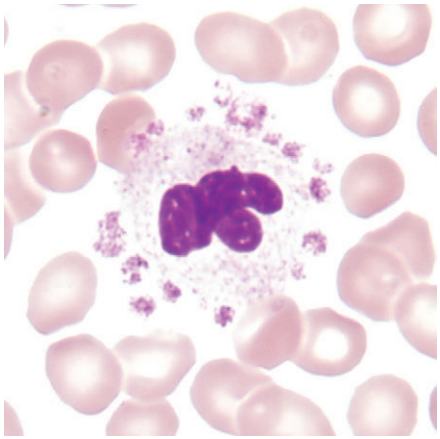
DESCRIPTION: Highly refractile areas because of slow drying of blood film



D

FIGURE 22–6D Necrotic cell (PB ×1000).

DESCRIPTION: Nuclear degeneration; note lack of chromatin pattern and nuclear filaments



E

FIGURE 22–6E Platelet satellitism (PB ×1000).

DESCRIPTION: Platelets adhering to neutrophils; in vitro phenomenon in blood collected in ethylenediaminetetraacetic acid in rare individuals which may cause falsely decreased platelet counts. May be resolved by collecting blood in sodium citrate.*

*Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.

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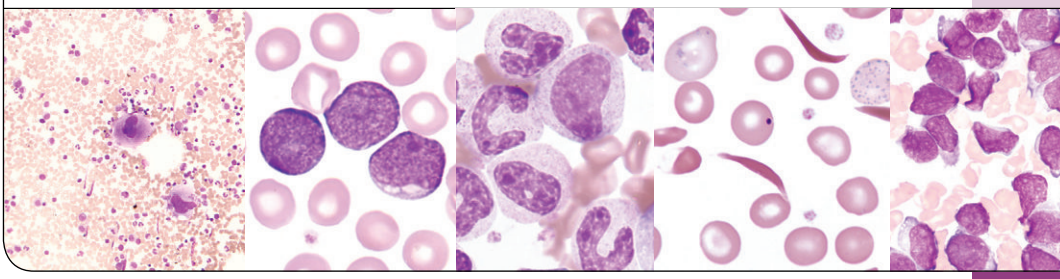
23

NORMAL NEWBORN PERIPHERAL BLOOD MORPHOLOGY



In the healthy, full-term newborn, peripheral blood collected within the first 12 hours of birth has distinctive morphology. Some morphological changes persist for up to 3 to 5 days after birth. These changes should be recognized as physiological and not pathological. For a fuller discussion of hematology in the newborn, refer to a hematology textbook such as *Hematology: Clinical Principles and Applications*[★] or a pediatric hematology text such as *Nathan and Oski's Hematology of Infancy and Childhood*.[†]

Entire books have been written to address abnormal hematology in neonates and especially in the premature infant. This chapter does not attempt to address those disorders but rather depicts morphological changes commonly seen in the healthy newborn.

Erythrocyte morphology demonstrates macrocytes, with a mean cell volume of 110 ± 15 fL, which declines dramatically after the first 12 hours. Up to 3 to 10 orthochromic normoblasts (nucleated red blood cells) may be seen per 100 white blood cells and should disappear by day 5. Polychromasia reflects the erythropoietic activity of the newborn. Anisocytosis is reflected in the red blood cell distribution width index, which ranges from 15.2% to 18.0%.

Occasional spherocytes are common, varying from one every two fields to one or more in every field.

Newborn total leukocyte counts are higher than for adults, and newborns have more segmented and band neutrophils than at any other time in childhood.[‡] An occasional metamyelocyte may be seen without evidence of infection. Monocyte morphology is similar to that of the adult.

Lymphocyte morphology is pleomorphic, spanning the range from reactive to mature. The presence of a nucleolus is not uncommon; however, the chromatin pattern is coarse and not as fine as seen in blasts. Hematogones (immature B cells) are occasionally seen in bone marrow and peripheral blood of newborns. Caution must be exercised to correctly differentiate hematogones from blasts that may indicate a pathologic condition.

[★]Rodak BF, Fritsma GA, Keohane EM, editors: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.

[†]Orkin SH, Nathan DG, Ginsburg D et al: *Nathan and Oski's hematology of infancy and childhood*, ed 7, St. Louis, 2009, Saunders.

[‡]Quinn CT, Buchanan GR: Hematopoiesis and hematologic diseases. In McMillan JA, Feigin RD, DeAngelis C, Jones MD, editors: *Oski's pediatrics*, Philadelphia, 2006, Lippincott Williams & Wilkins.

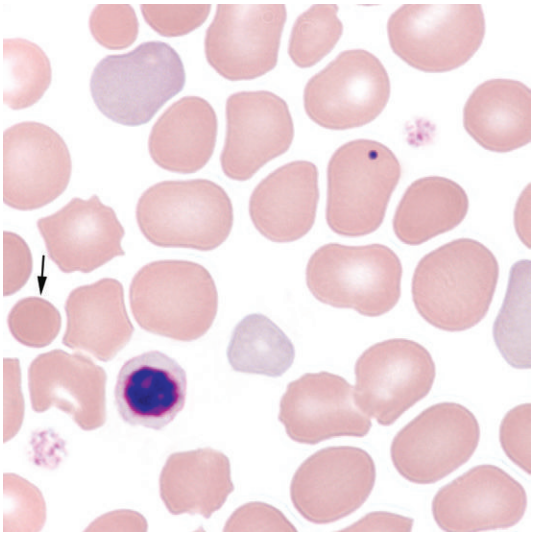


FIGURE 23–1 Peripheral blood from a neonate demonstrating macrocytes, polychromasia, nucleated red blood cell, Howell-Jolly body, and one spherocyte (*arrow*) (PB ×1000).

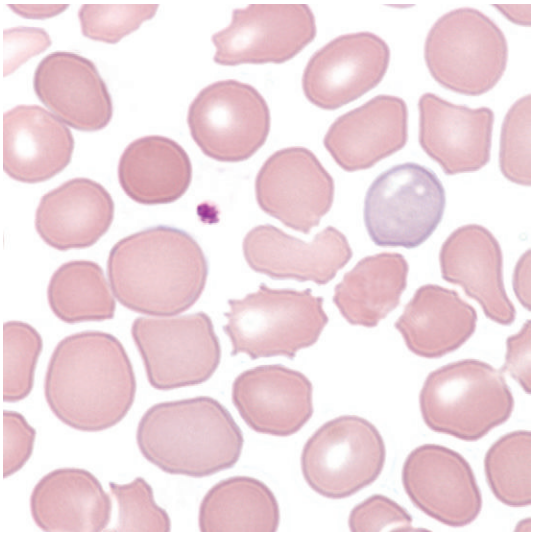


FIGURE 23–2 Peripheral blood from a neonate demonstrating polychromasia, anisocytosis, echinocytes, and spherocytes (PB ×1000).

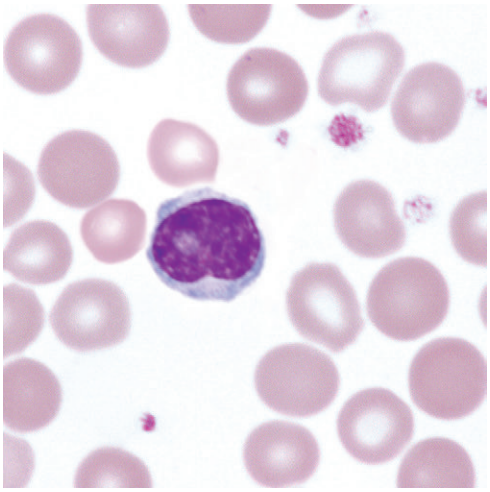


FIGURE 23–3 Lymphocyte from neonate blood. Although there appears to be a nucleolus, the chromatin pattern is coarse (PB × 1000).

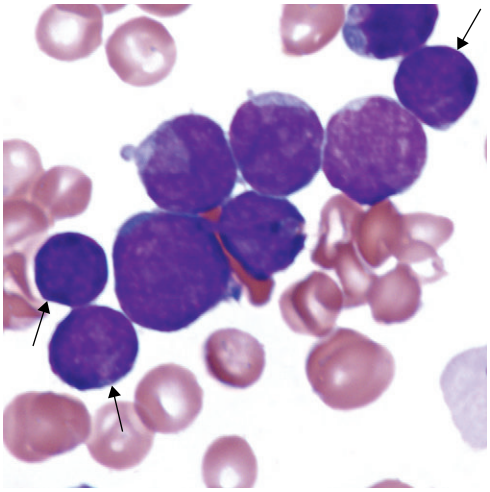


FIGURE 23–4 Bone marrow from neonate with acute lymphoblastic leukemia, demonstrating hematogones and lymphoblasts. Hematogones vary in size. Nucleus is round to oval with condensed, smudged chromatin. Nucleoli are absent or indistinct. Cytoplasm is indiscernible to scant. *Arrows* point to hematogones. Most of the other cells are blasts (BM ×1000). See chapter 16 for comparison with blasts.

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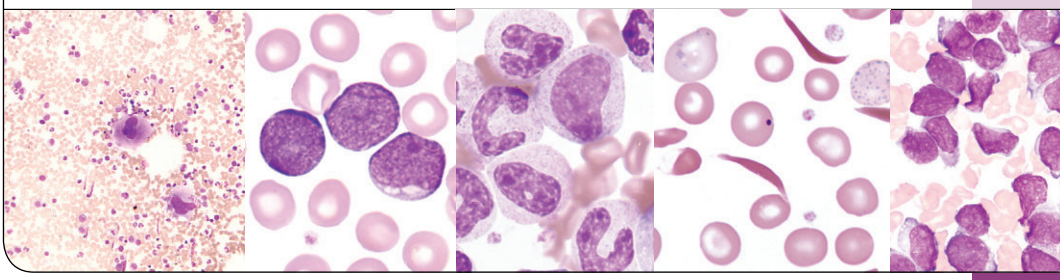
24

BODY FLUIDS



Fluid in the cavities that surround organs may serve as a lubricant or shock absorber, provide circulation of nutrients, or function for collection of waste. Evaluation of body fluids may include total volume, gross appearance, total cell count, differential cell count, identification of crystals, biochemical analysis, microbiological examination, immunological studies, and cytological examination. The most common body fluid specimens received in the laboratory are cerebrospinal fluid (CSF); pleural, peritoneal, and pericardial fluids (together known as *serous* fluids); and synovial fluids. Under normal circumstances, the only fluid that is present in an amount large enough to sample is CSF. Therefore, when other fluids are present in detectable amounts, disease is suspected.

This atlas addresses primarily the elements of fluids that are observable through a microscope. For a more detailed explanation of body fluids, consult a hematology or urinalysis textbook that includes a discussion of body fluids, such as *Hematology: Clinical Principles and Applications*★ or *Fundamentals of Urine and Body Fluid Analysis*.†

Because the number of cells in fluids is often very small, a concentrated specimen is preferable for performing the morphological examination. Preparation of slides using a cytocentrifuge is the method commonly used. This centrifuge spins at a low rate of speed to minimize distortion of cells, concentrating the cells into a "button" on a small area of the glass slide. The three elements of the cytocentrifuge are a cytofunnel, filter paper to absorb excess fluid, and a glass slide. These elements are clipped together in a clip assembly, and the entire apparatus is then centrifuged slowly. Excess fluid is absorbed by the filter paper, leaving a monolayer of cells in a small button on the slide. When the cytospin slide is removed from the centrifuge, it should be dry. If the cell button is still wet, the centrifugation time may need to be extended.

When preparing cytocentrifuge slides, a consistent amount of fluid should be used to generate a consistent yield of cells. Usually two to six drops of fluid are used depending on the nucleated cell count. Five drops of fluid will generally yield enough cells to perform a 100-cell differential if the nucleated cell count is at least 3/mm^3. For very high counts, a dilution with normal saline may be made. The area of the slide where the cell button will be deposited should be marked with a wax pencil in case the number of cells recovered is small and difficult to locate (Figure 24-1). Alternatively, specially marked slides can be used.

There may be some distortion of cells as a result of centrifugation or when cell counts are high. Dilutions with normal saline should be made before centrifugation to minimize distortion when nucleated cell counts are high. When the red blood cell (RBC) count is extremely high (more than 1 million), the slide should be made in the same manner as the peripheral blood smear slide (see Chapter 1). However, the examination of the smear should be performed at the end of the slide rather than the battlement pattern used for

★Rodak BF, Fritsma GA, Koehane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.

†Brunzel NA: *Fundamentals of urine and body fluid analysis*, ed 2, St. Louis, 2004, Saunders.

A

FIGURE 24–1A Wright-stained cytocentrifuge slide demonstrating a concentrated button of cells within the marked circle.

B

FIGURE 24–1B Wright-stained cytocentrifuge slide from a cerebrospinal fluid containing few cells, demonstrating the importance of marking the cell concentration area.

blood smears. This is because the larger, and usually more significant, cells are likely to be pushed to the end of the slide.

When examining the cytospin slide, the entire cell button should be scanned under the 10× objective to search for the presence of tumor cells. The 50× or 100× oil immersion lens should be used to differentiate the white blood cells. For the performance of the differential, any area of the cell button may be used, but if the cell count is low, a systematic pattern starting at one end of the side of the button and working toward the other is recommended.

Any cell that is seen in the peripheral blood may be found in a body fluid in addition to cells specific to that fluid (e.g., mesothelial cells, macrophages, tumor cells). However, the cells look somewhat different than in peripheral blood, and some in vitro degeneration is normal. The presence of organisms, such as yeast and bacteria, should also be noted (see Figures 24-12 to 24-14).

CELLS COMMONLY SEEN IN CEREBROSPINAL FLUID

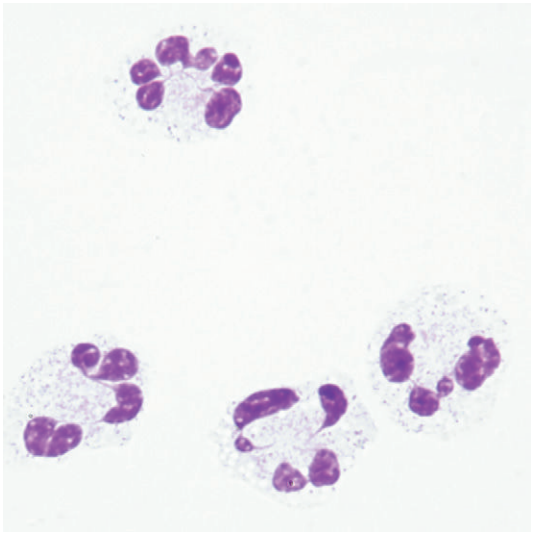


FIGURE 24–2 Segmented neutrophils (CSF ×1000).

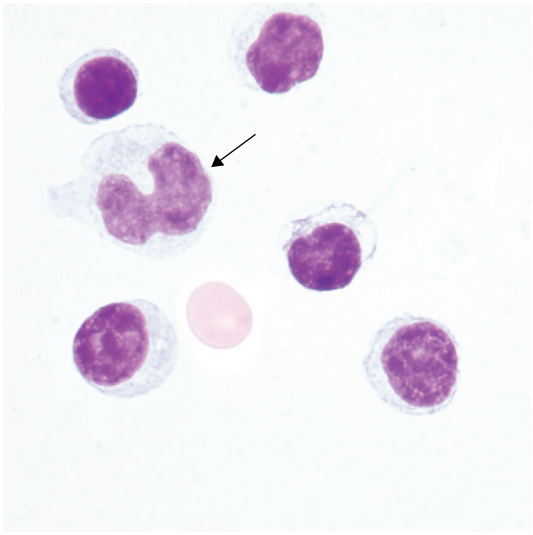


FIGURE 24–3 Lymphocytes and monocyte (*arrow*) (CSF ×1000).

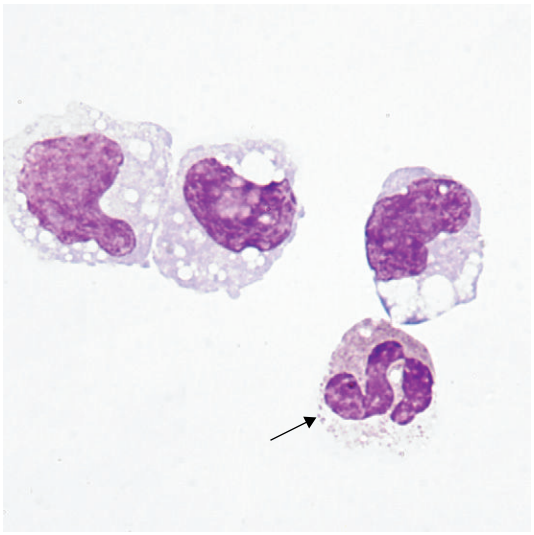


FIGURE 24–4 Monocytes and segmented neutrophil (*arrow*) (CSF ×1000).

COMMENTS: Small numbers of neutrophils, lymphocytes, and monocytes may be seen in normal CSF.

Increased numbers of neutrophils are associated with bacterial meningitis; early stages of viral, fungal, and tubercular meningitis; intracranial hemorrhage; intrathecal injections; central nervous system (CNS) infarct; malignancy; or abscess.

Increased numbers of lymphocytes and monocytes are associated with viral, fungal, tubercular and bacterial meningitis, and multiple sclerosis.

CELLS SOMETIMES FOUND IN CEREBROSPINAL FLUID

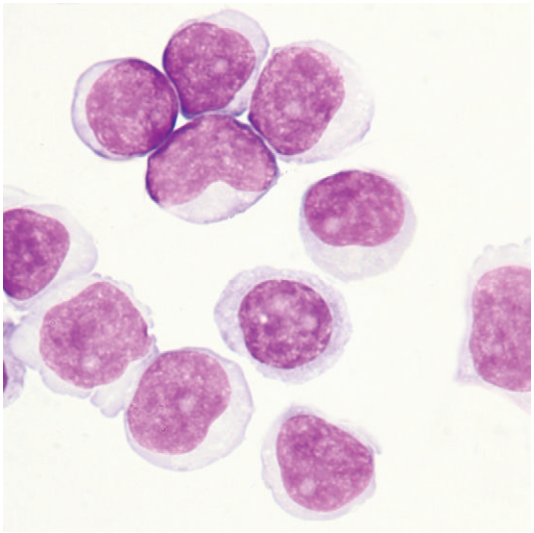


FIGURE 24–5 Reactive lymphocytes (CSF ×1000).

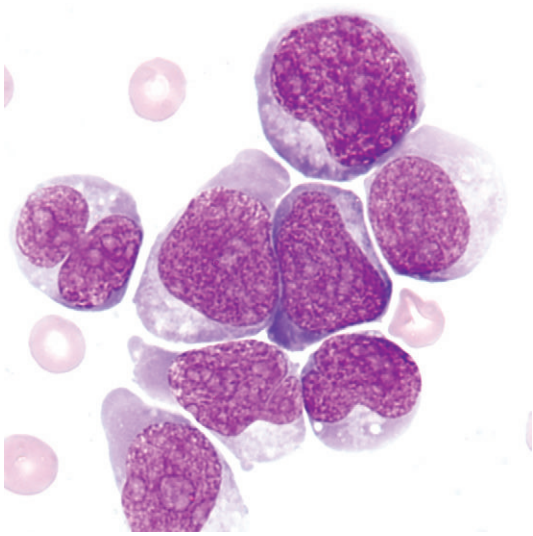


FIGURE 24–6 Acute lymphoblastic leukemia (CSF ×1000).

Reactive lymphocytes (Figure 24-5) are associated with viral meningitis and other antigenic stimulation. The cells will vary in size; nuclear shape may be irregular and cytoplasm may be scant to abundant with pale to intense staining characteristics. (See description of reactive lymphocytes, Figure 14-7.)

Blasts in the CSF may have some of the characteristics of the acute lymphoblastic leukemia (ALL) blasts seen in the peripheral blood (Figure 24-6; see Chapter 16). It is not unusual for ALL to have CNS involvement, and blasts may be present in the CSF before being observed in the peripheral blood.

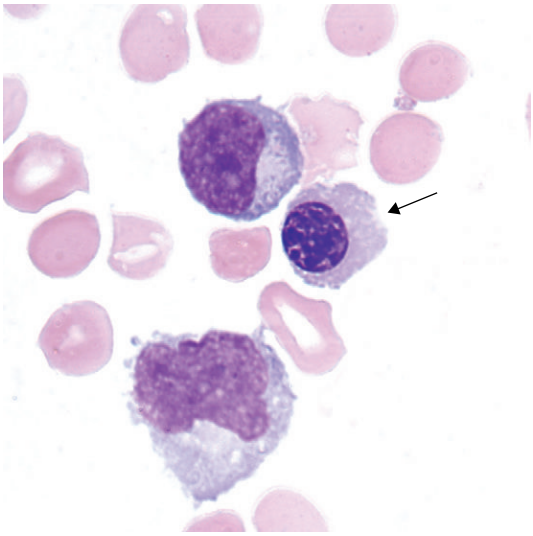


FIGURE 24–7 Nucleated red blood cell (CSF × 1000).

Associated with: Traumatic lumbar tap in premature infants, blood dyscrasias with circulating nucleated RBCs, and bone marrow contamination of CSF

CELLS SOMETIMES FOUND IN CEREBROSPINAL FLUID AFTER CENTRAL NERVOUS SYSTEM HEMORRHAGE

The following sequence of events is a typical reaction to intracranial hemorrhage or repeated lumbar punctures:

1. Neutrophils and macrophages—appear within 2 to 4 hours
2. Erythrophages—identifiable from 1 to 7 days
3. Hemosiderin and siderophages—observable from 2 days to 2 months
4. Hematoidin crystals—recognizable in 2 to 4 weeks

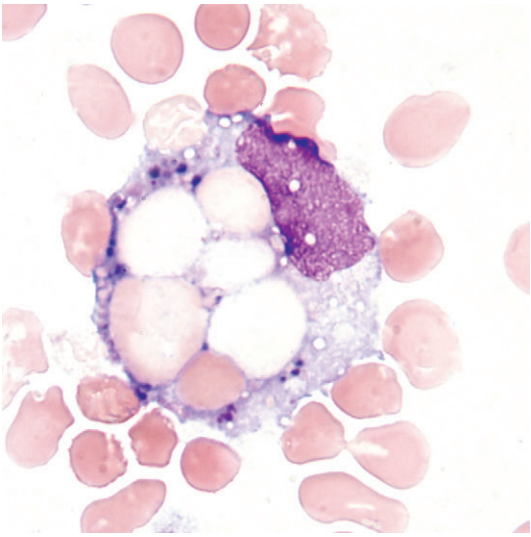


FIGURE 24–8 Erythrophage (CSF ×1000).

Macrophage with engulfed RBCs. RBCs are digested by enzymatic activity within the macrophage. The digestion process causes the RBCs to lose color and to appear as vacuoles within the cytoplasm of some macrophages.

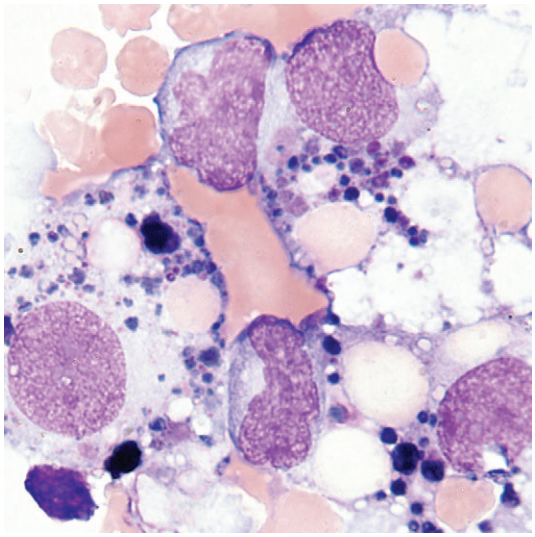


FIGURE 24–9 Hemosiderin (CSF ×1000).

Blue to black granules that contain iron, resulting from the degradation of hemoglobin, may be present in CSF for up to 2 months after intracranial hemorrhage. The cellular inclusions can be positively identified with an iron stain.

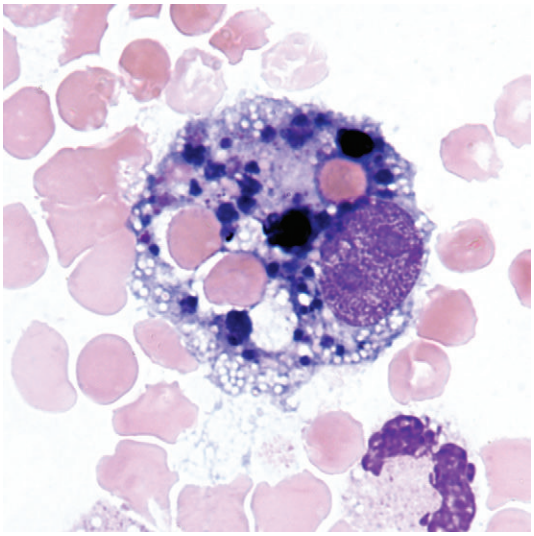


FIGURE 24–10 Siderophage (CSF ×1000).

Macrophage containing hemosiderin.

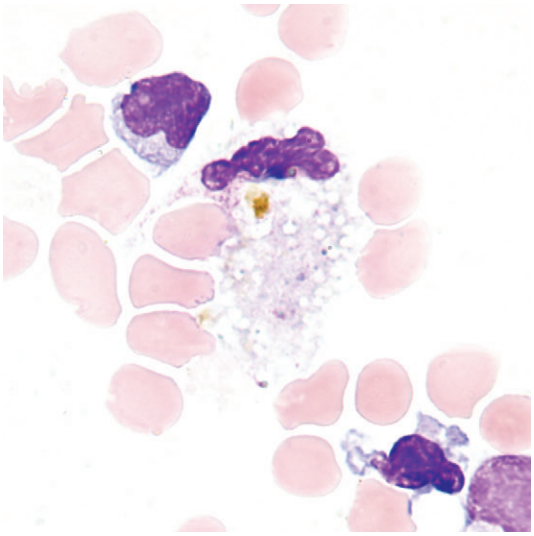


FIGURE 24–11 Hematoidin within macrophage (CSF ×1000).

Gold intracellular crystals composed of bilirubin. Hematoidin is the result of the catabolism of hemoglobin and may be present for several weeks after CNS hemorrhage.

NOTE: Macrophages may display the presence of a variety of particles within one cell. For example, one macrophage may contain hemosiderin and hematoidin.

ORGANISMS SOMETIMES FOUND IN CEREBROSPINAL FLUID

CSF is a sterile body fluid. Following are examples of some organisms that have been seen in CSF, but it is far from an all-inclusive list of possibilities (Figures 24-12 to 24-14). Note that organisms may be intracellular, extracellular, or both.

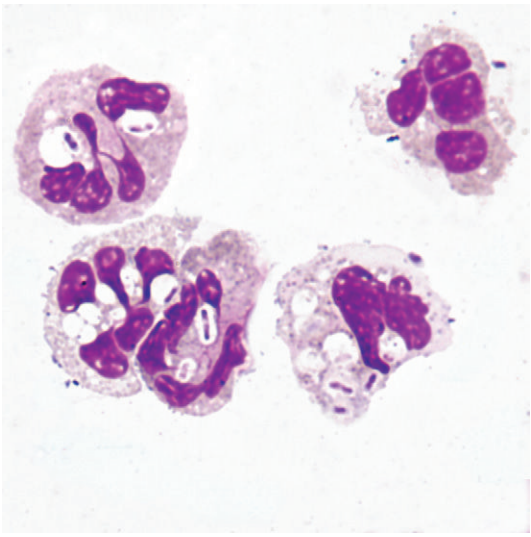


FIGURE 24–12 Bacteria engulfed by neutrophils (CSF ×1000).

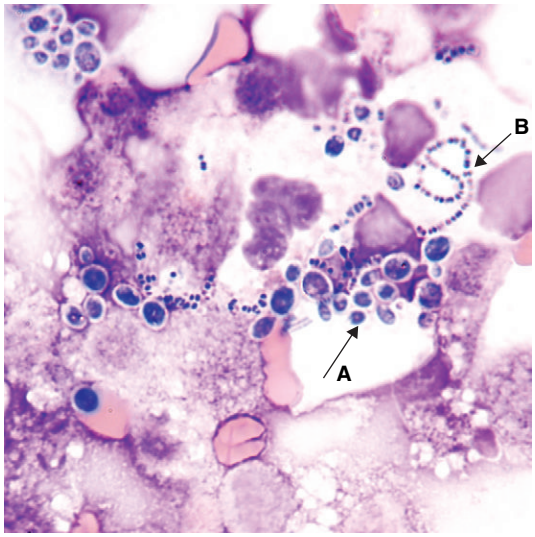


FIGURE 24–13 *Histoplasma capsulatum* (*A*) within macrophage (CSF ×1000). Note the presence of bacteria in chains (*B*).

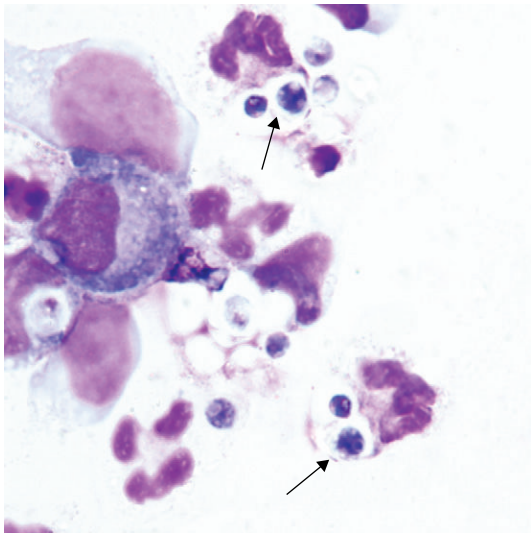


FIGURE 24–14 *Cryptococcus neoformans* inside neutrophil (CSF ×1000).

CELLS SOMETIMES FOUND IN SEROUS BODY FLUIDS (PLEURAL, PERICARDIAL, AND PERITONEAL)

NOTE: Any of the cell types found in the peripheral blood may be found in serous fluids.

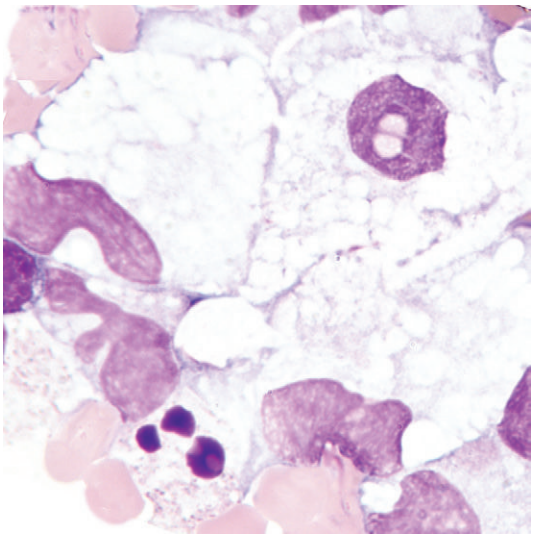


FIGURE 24–15 Macrophages (pleural fluid ×1000).

DESCRIPTION: Large cells with eccentric nuclei and vacuolated cytoplasm may be present in all body fluids. They may be seen with or without inclusions, such as RBCs, siderotic granules, or lipids.

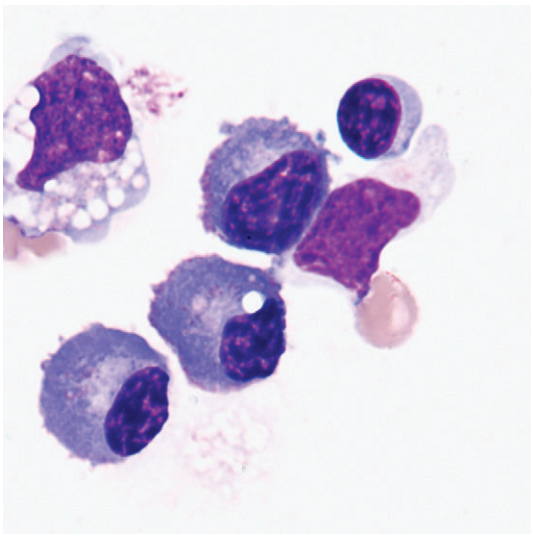


FIGURE 24–16 Plasma cells (pleural fluid ×1000).

DESCRIPTION Round to oval cell with eccentric nuclei, dark blue cytoplasm, perinuclear hof 8-29 μm in diameter.

Associated with: Rheumatoid arthritis, malignancy, tuberculosis, and other conditions that exhibit lymphocytosis

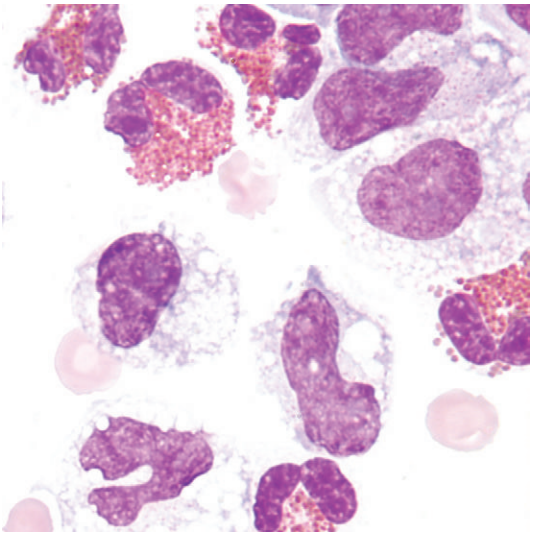


FIGURE 24–17 Eosinophils and macrophages (pleural fluid ×1000).

Associated with: Allergy, air, and/or foreign matter within the body cavity, parasites

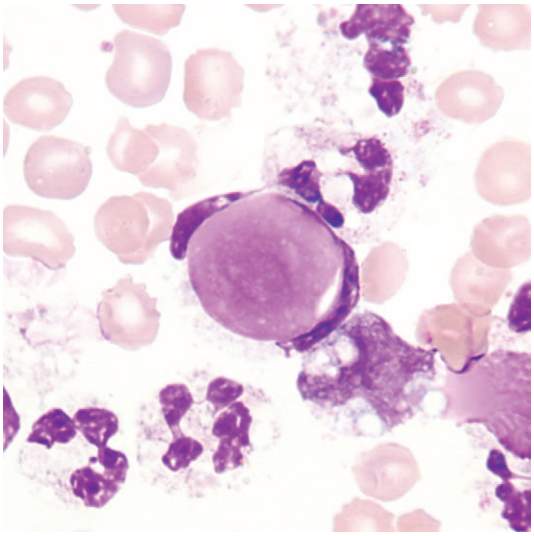


FIGURE 24–18 Lupus erythematosus cell (pleural fluid ×1000).

Intact neutrophil with engulfed homogenous mass. The mass displaces the nucleus of the neutrophil and is composed of degenerated nuclear material. Lupus erythematosus (LE) cells are formed in vivo and in vitro in serous fluids. LE cells may also form in synovial fluids.

Associated with: Lupus erythematosus

MESOTHELIAL CELLS

Mesothelial cells are shed from membranes that line body cavities and are often found in serous fluids.

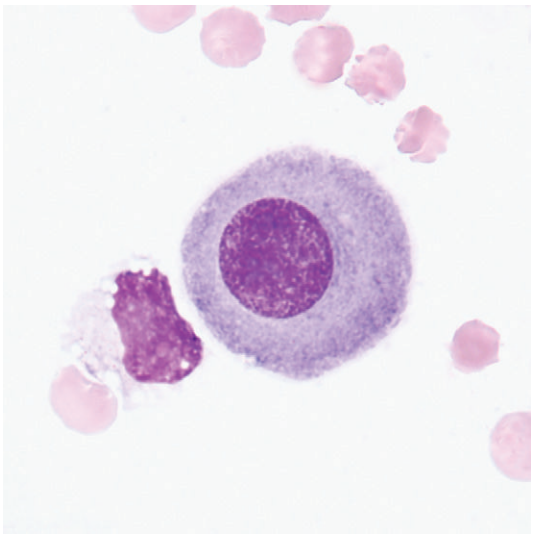


FIGURE 24–19 Mesothelial cell with pale blue cytoplasm (pleural fluid ×1000).

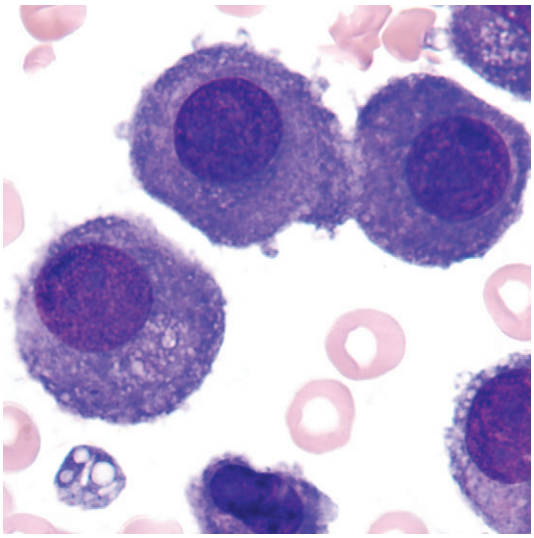


FIGURE 24–20 Mesothelial cells with deeply basophilic cytoplasm (pleural fluid ×1000).

SHAPE: Pleomorphic
SIZE: 12-30 μm
NUCLEUS: Round to oval with smooth nuclear borders; nucleus may be eccentric or multinucleated, making the distinction between the mesothelial and plasma cell difficult at times
Nucleoli: 1-3, uniform in size and shape
Chromatin: Fine, evenly distributed
CYTOPLASM: Abundant, light gray to deeply basophilic
Vacuoles: Occasionally
NOTE: Mesothelial cells may appear as single cells in clumps or sheets. The clumping of cells to one another and the variability of appearance require careful observation to accurately differentiate mesothelial cells from malignant cells. Three characteristics can aid in this determination:

1. Mesothelial cells on a smear tend to be similar to one another.
2. The nuclear membrane appears smooth by light microscopy.
3. Mesothelial cells maintain cytoplasmic borders. When appearing in clumps, there may be clear spaces between the cells. These spaces are often referred to as "windows."

MULTINUCLEATED MESOTHELIAL CELLS

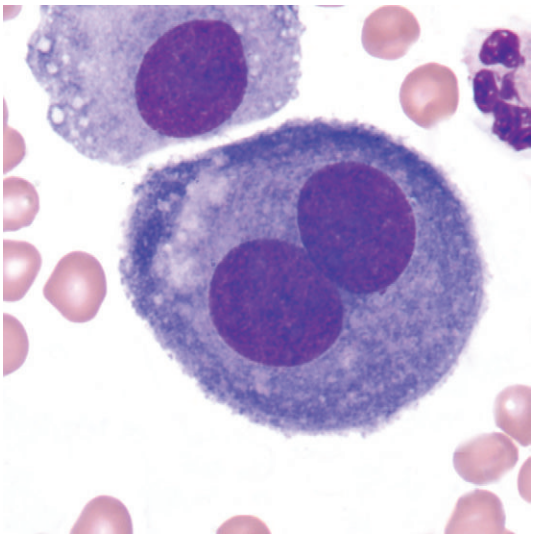


FIGURE 24–21 Binucleated mesothelial cell (pleural fluid ×1000).

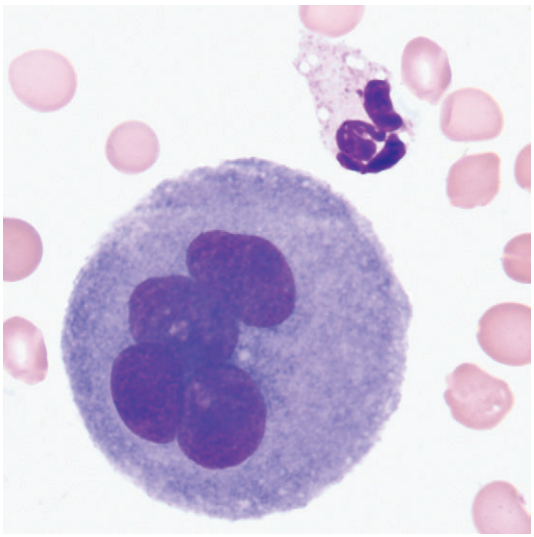


FIGURE 24–22 Multinucleated mesothelial cell (pleural fluid ×1000).

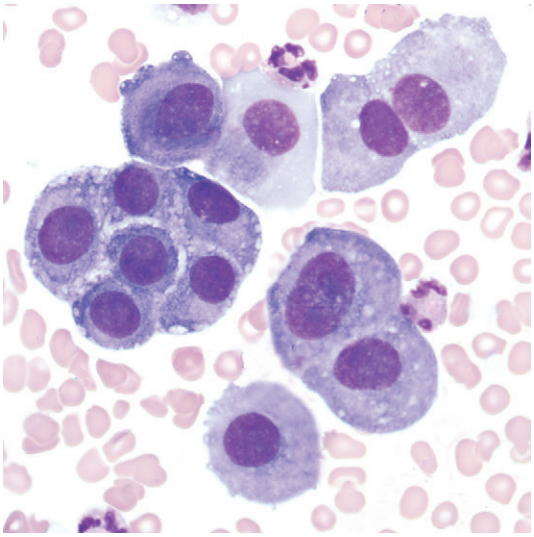


FIGURE 24–23 Clump of mesothelial cells. Note "windows" in the large clump (pleural fluid ×500).

TABLE 24-1 Characteristics of Benign and Malignant Cells

Benign	Malignant
Occasional large cells	Many cells may be very large
Light to dark staining	May be very basophilic
Rare mitotic figures	May have several mitotic figures
Round to oval nucleus; nuclei are uniform size with varying amounts of cytoplasm	May have irregular or bizarre nuclear shape
Smooth nuclear edge	Edges of nucleus may be indistinct and irregular
Nucleus intact	Nucleus may be disintegrated at edges
Nucleoli are small, if present	Nucleoli may be large and prominent
In multinuclear cells (mesothelial), all nuclei have similar appearance (size and shape)	Multinuclear cells have varying sizes and shapes of nuclei
Moderate to small N:C ratio	May have high N:C ratio
Clumps of cells have similar appearance among cells, are on the same plane of focus, and may have "windows" between cells	Clumps of cells contain cells of varying sizes and shapes, are "three-dimensional" (have to focus up and down to see all cells), and have dark staining borders; no "windows" between cells

From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.
N:C, Nuclear:cytoplasmic.

It is not always possible to distinguish malignant cells from mesothelial cells with the sole use of the light microscope. The following criteria for malignant cells may aid in this distinction.

NUCLEUS: High N:C ratio, membrane irregular
Nucleoli: Multiple, large with irregular staining
Chromatin: Hyperchromatic with uneven distribution
CYTOPLASM: Irregular membrane

NOTE: Smears with cells displaying one or more of the above characteristics should be referred to a qualified cytopathologist for confirmation. See Table 24-1 for a comparison of benign and malignant features. Malignant cells tend to form clumps with cytoplasmic molding. The boundaries between cells may be indistinguishable.

MALIGNANT CELLS SOMETIMES SEEN IN SEROUS FLUIDS

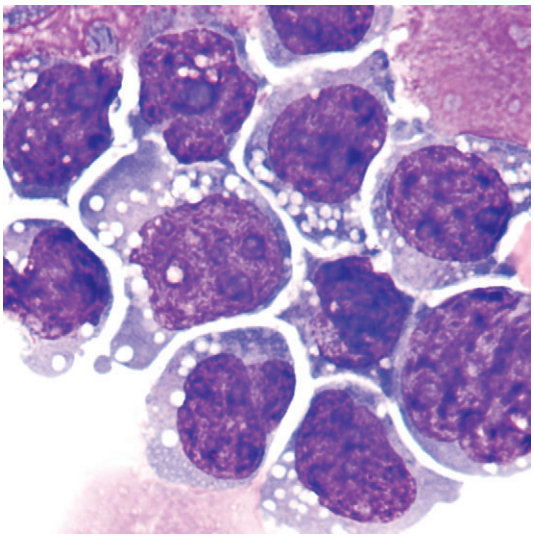


FIGURE 24–24 Non-Hodgkin lymphoma (pleural fluid ×1000).

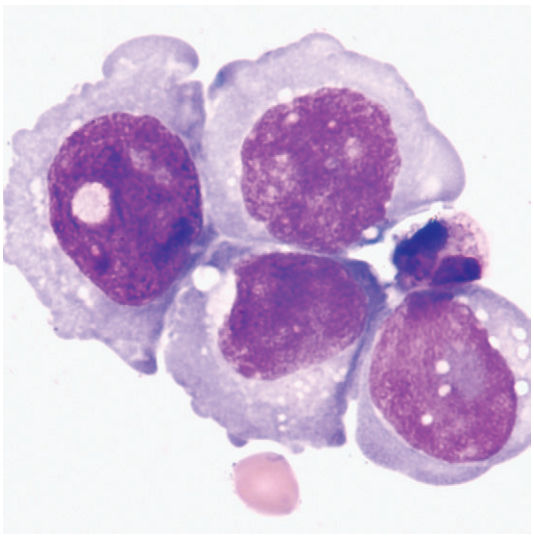


FIGURE 24–25 Breast tumor metastases (pleural fluid ×1000).

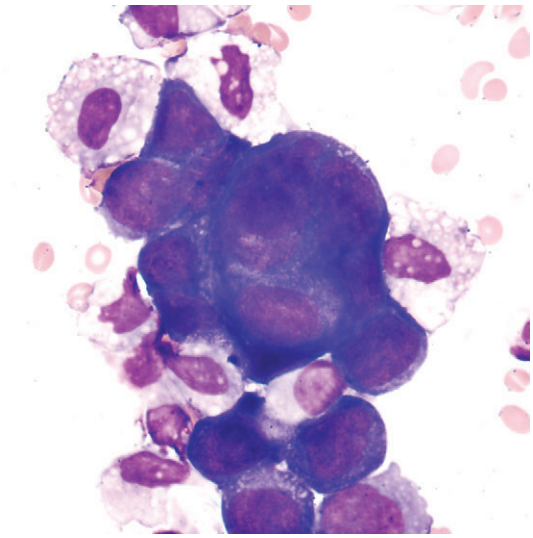


FIGURE 24–26 Malignant tumor (pleural fluid × 500). Note molding of cytoplasm (no "windows" between cells).

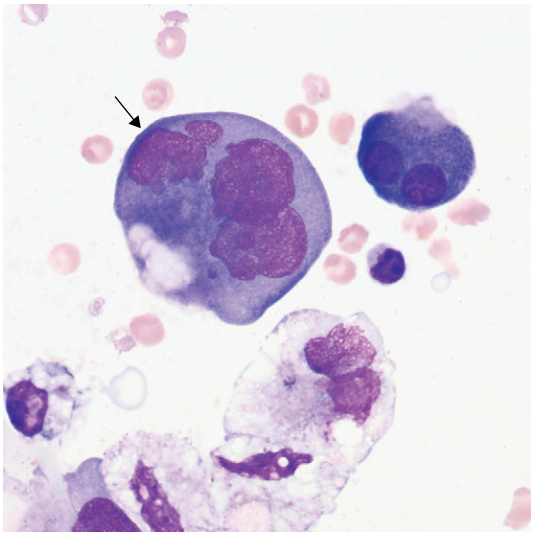


FIGURE 24–27 Adenocarcinoma, metastases from uterine cancer (pleural fluid ×500). Note irregular nuclear membranes.

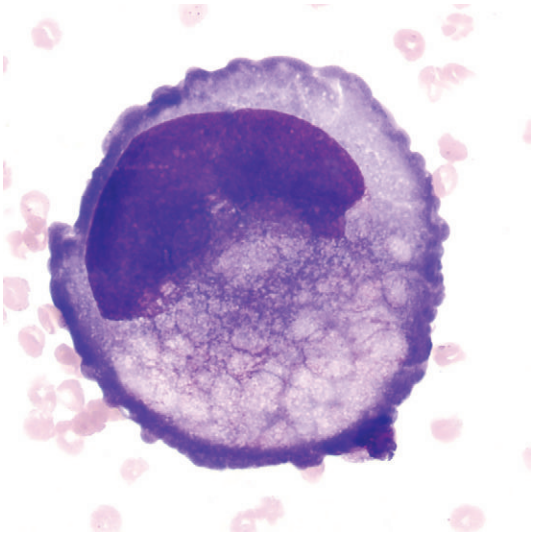


FIGURE 24–28 Malignant tumor (pleural fluid × 500).

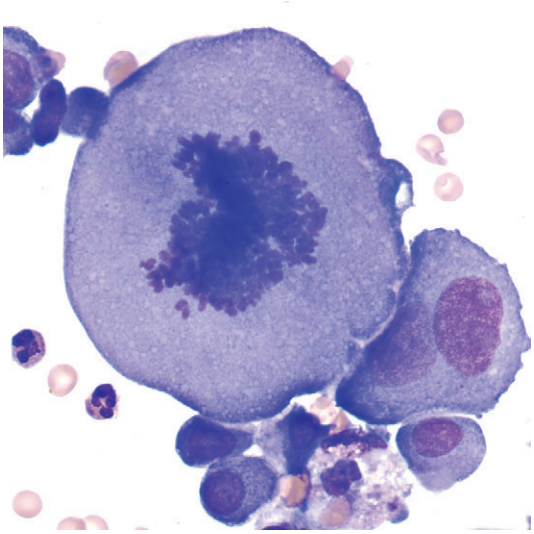


FIGURE 24–29 Mitotic figure in malignancy (pleural fluid ×500).

Mitotic figures may be found in normal fluids and are not necessarily an indication of malignancy. The size of this mitotic figure, however, is quite large, and malignant cells were easily found.

CRYSTALS SOMETIMES FOUND IN SYNOVIAL FLUID

Cells that may be found in normal synovial fluids include lymphocytes, monocytes, and synovial cells. Synovial cells, which line the synovial cavity, resemble mesothelial cells (see Figure 24-19) but are smaller and less numerous. Increased numbers of polymorphonuclear neutrophils may be seen in bacterial infection and acute inflammation. When neutrophils are seen, a careful search for bacteria should be performed. Tumor cells are possible but quite rare. LE cells may also be seen (see Figure 24-18).

It is important to perform a careful evaluation for crystals in synovial fluid. Although it is not necessary to use a stain, Wright stain is sometimes used. A polarizing microscope with a red compensator should always be used for confirmation. The most common crystals are monosodium urate, calcium pyrophosphate, and cholesterol.

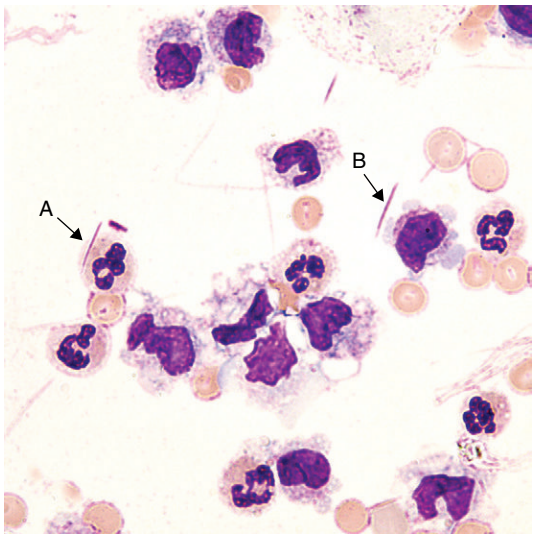
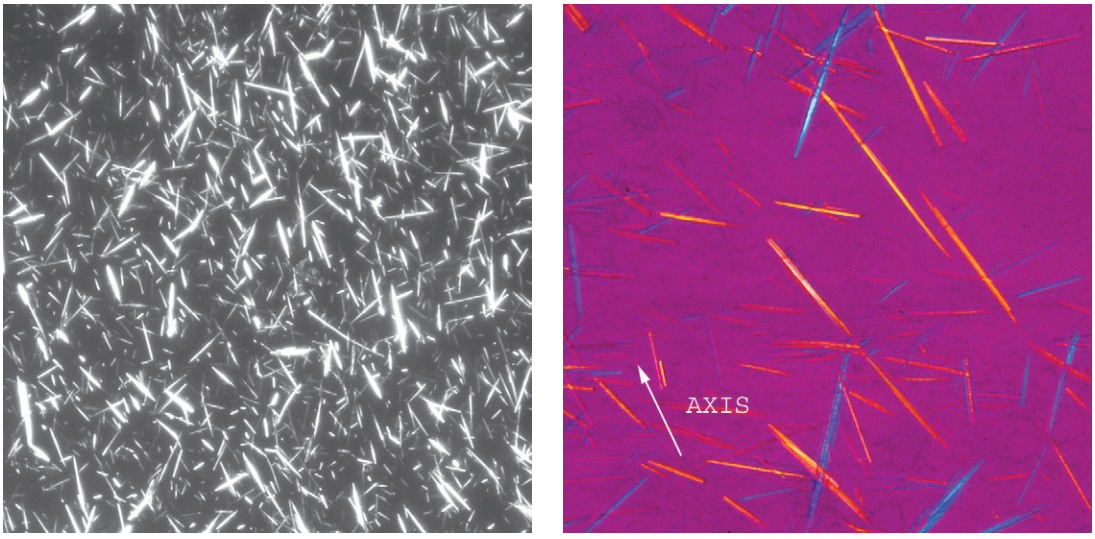


FIGURE 24–30 Monosodium urate crystals (synovial fluid ×1000; Wright stain). Needlelike crystals with pointed ends may be intracellular (*A*), extracellular (*B*), or both.

Associated with: Gout

FIGURE 24–31 Monosodium urate crystals (synovial fluid ×1000; unstained). (Courtesy George Girgis, MT [ASCP], Indiana University Health.)



A B

FIGURE 24–32 Monosodium urate crystals, polarized light microscopy **(A)** and with red compensator **(B)** (synovial fluid ×1000). (Courtesy George Girgis, MT [ASCP], Indiana University Health.)

Note the orientation of the crystals and corresponding colors. Crystals appear yellow when parallel to the axis of slow vibration and blue when perpendicular to the axis.

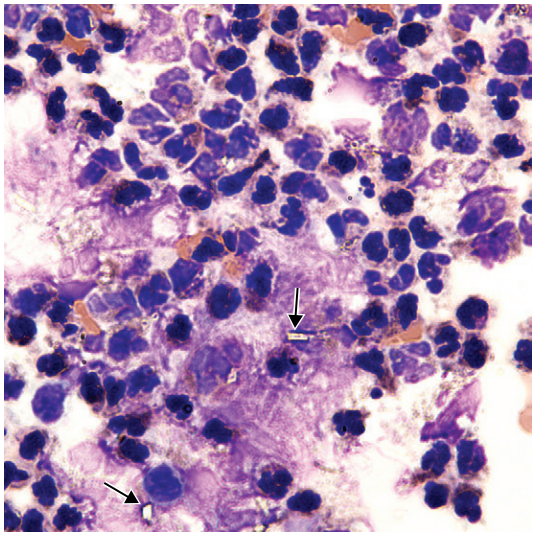
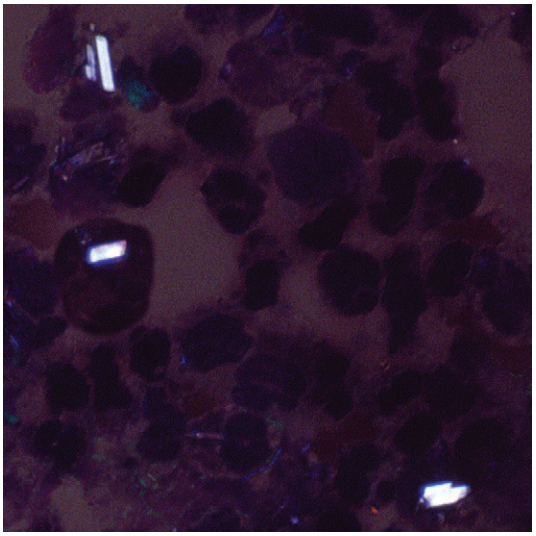


FIGURE 24–33 Calcium pyrophosphate crystals (synovial fluid ×1000; Wright stain).

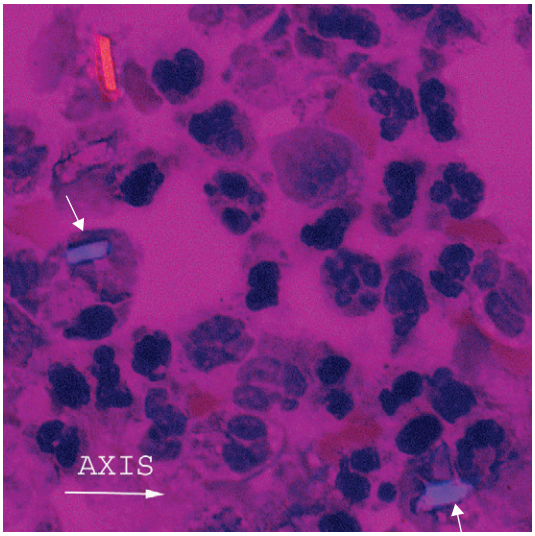
Rhomboid, rod-like chunky crystals may be intracellular, extracellular, or both.

Associated with: Pseudogout or pyrophosphate gout



A

FIGURE 24–34A Calcium pyrophosphate crystals, polarized light microscopy (synovial fluid ×1000). (Courtesy George Girgis, MT [ASCP], Indiana University Health.)



B

FIGURE 24–34B Calcium pyrophosphate crystals, polarized with red compensator (synovial fluid ×1000). (Courtesy George Girgis, MT [ASCP], Indiana University Health.)

Note the orientation of the crystals and corresponding colors. Crystals appear blue when parallel to the axis of slow vibration or yellow when perpendicular to the axis. Calcium pyrophosphate is only weakly birefringent, so that the colors are not as bright as monosodium urate crystals (see Figure 24-32).

FIGURE 24–35 Cholesterol crystals (synovial fluid ×500; unstained).

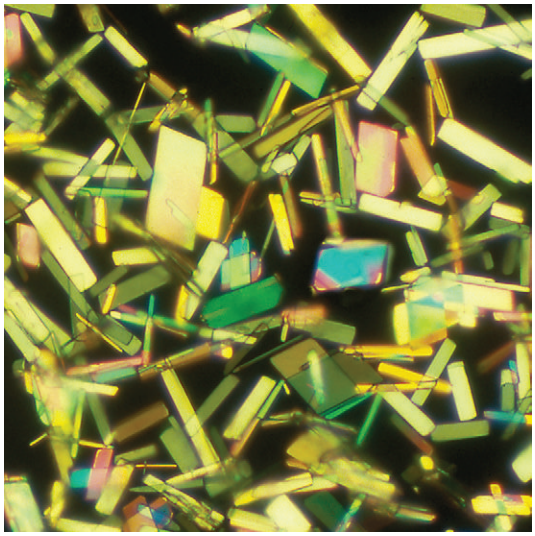


FIGURE 24–36 Cholesterol crystals (synovial fluid × 500; polarized light microscopy). (Courtesy George Girgis, MT [ASCP], Indiana University Health.)

Large, flat rectangular plates with notched corners.

Associated with: Chronic inflammatory conditions and considered a nonspecific finding

It is necessary to use polarized light for confirmation of cholesterol crystals, but it is not necessary to use a red compensator.

OTHER STRUCTURES SOMETIMES SEEN IN BODY FLUIDS

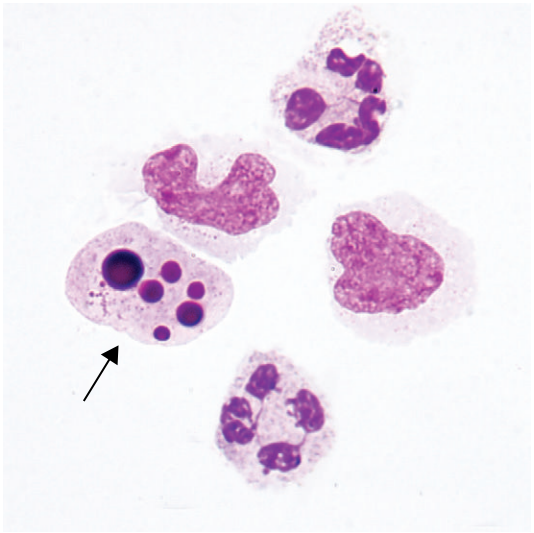


FIGURE 24–37 Necrosis (pleural fluid ×500).

Intracellular nuclear degeneration appearing as darkly stained mass(es) (*arrow*), compared with two segmented neutrophils. Contrary to necrosis seen in peripheral blood, necrotic figures in body fluids can develop in vivo.

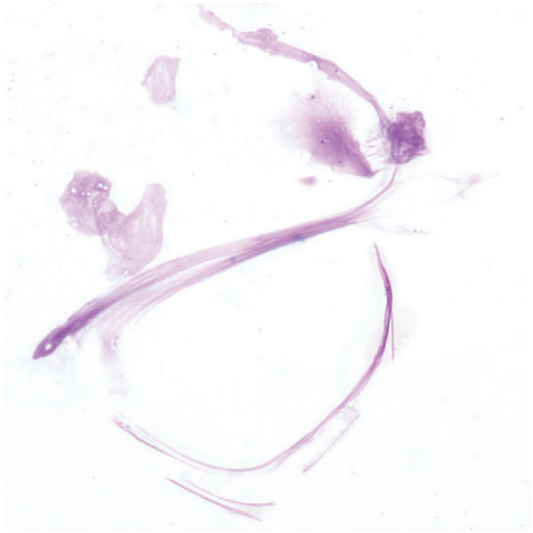


FIGURE 24–38 Artifact (pleural fluid ×500).

Fibers from the filter paper may appear near the edges of the slide. Fibers may be birefringent but lack the sharp pointed ends of monosodium urate crystals.

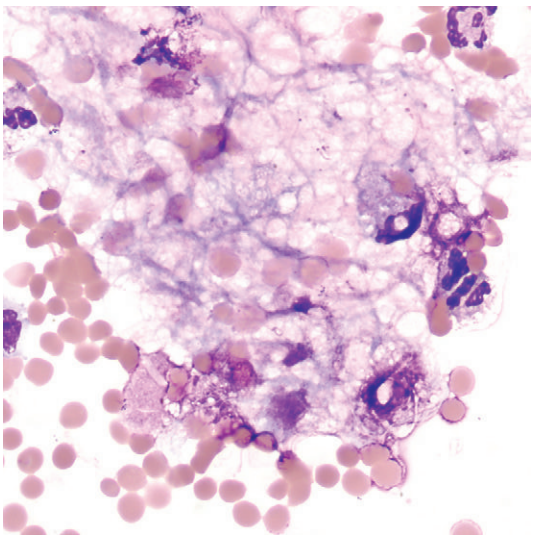


FIGURE 24–39 Brain tissue (CSF ×500).

The specimen in Figure 24-39 is from a patient who experienced head trauma.

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