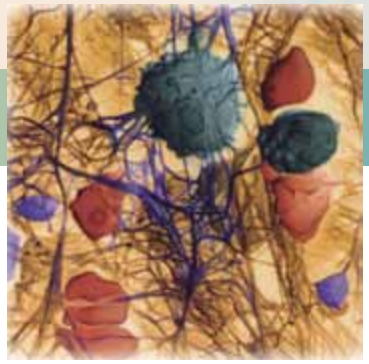

Chapter 5

Evaluation of Cell Morphology and Introduction to Platelet and White Blood Cell Morphology

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OBJECTIVES

At the end of the chapter, the learner should be able to:

1. Define the terms *flagged* and *reflex test* as they pertain to automated hematology results.
2. Describe the importance of maintaining competency in morphological identification.
3. List justifications for performing a manual morphology review.
4. List the steps in the performance of a peripheral blood smear examination.
5. Identify normal red blood cell morphology on a peripheral smear.
6. List the terms referring to abnormal red cell distribution, variation in red cell size, and variations in red cell color/hemoglobin content, being able to identify each abnormality on a peripheral smear and specify the particular clinical conditions associated with these abnormalities.
7. Define *anisocytosis* and *poikilocytosis* and list clinical conditions in which they may be reported.
8. Define the terms *normochromic*, *hypochromic*, *microcytic*, and *macrocytic* as they relate to red cell indices.
9. Correlate red cell indices with red cell morphology and the diagnosis of anemia.
10. Define the following terms: target cells, spherocytes, ovalocytes, elliptocytes, stomatocytes, and be able to identify these cells on a peripheral smear.
11. List diseases that may show fragmented red cells and describe their pathophysiology.
12. Describe the most common red blood cell inclusions and their composition, relating each inclusion to clinical conditions in which they may be found.
13. Correlate pathophysiology of clinical conditions associated with abnormal appearance of red cells noted on the peripheral blood smear.
14. Describe normal platelet morphology and specify some platelet abnormalities seen in pathologic conditions.
15. List conditions showing leukocyte cytoplasmic as well as nuclear changes.

Introduction

Hematology, as a laboratory discipline, has changed dramatically over the last three decades, primarily as a direct result of automation and enhanced technology. Multichanneled automated hematology analyzers provide a reliable and accurate complete blood count (CBC). The analyzers have evolved from basic direct current impedance enumeration of cells, to very complex instruments involving multiple technologies in one instrument. These various technologies have significantly enhanced the ability of automation to reduce actual personnel time spent in performing and reporting CBC analysis. An example of such technology is flow cytometry, which actually produces qualitative as well as quantitative differential cell counts and analysis. With this technology, abnormalities are *flagged*, which is an indication to the technologist the instrument has identified results outside of the established parameters of the laboratory and some form of review must be completed before reporting test results. The instrument, in most cases, will specify the result with an indicator such as a symbol (i.e., an asterisk) or a high (H) or low (L) designation depending on the abnormality. The required review may include comparing current patient results with prior history or having set parameters that would automatically request further testing referred to as *reflex testing*. Reflex testing of an abnormal or flagged result would include a slide review of abnormal morphology (usually scanning 8 to 10 oil immersion fields) and if necessary, a complete white cell differential count. The proportion of required manual morphology reviews are determined by the parameters set through laboratory policies which are based on financial and regulatory standards as well as medical considerations. In comparison with the procedure for an automated CBC, the examination of a peripheral blood smear is labor intensive, and therefore a relatively expensive investigation and must be used judiciously.

From the educational perspective, it is essential that students be trained to recognize cellular abnormalities, given that the majority of the blood smears they review will be abnormal. With the development of sophisticated automated blood-cell analyzers, the proportion of CBC samples that require a blood smear has steadily diminished and in many clinical settings is 10 to 15 percent or less, depending on facility patient population.[1] Even with the wealth of information that may be derived from an analyzer, the blood smear remains a crucial diagnostic aid, and maintaining technologist competency in the identification of cellular abnormalities should be a priority in the laboratory. There are morphologic abnormalities that are critical in the differential diagnosis of anemia that can still be determined only from a blood smear. Because of financial constraints of educational programs, students may have little or no training on sophisticated automation common in laboratories today. Regardless, students should have a strong background in manual white blood cell (WBC) differential counting as well as red cell and platelet morphology assessment.

This chapter guides the student in interpretation of red cell, platelet, and white cell morphology by first defining what is considered normal. Evaluation of abnormalities is discussed in terms of basic assessment techniques of the cellular morphology with particular emphasis on recognizing a distinct morphology and relating it to the clinical condition. Physiologic mechanisms are explained to give the reader a better understanding of an abnormality and how it relates to different disease states. This description of blood cell morphology maximizes the ability of each slide reviewer to recognize and correlate the blood morphology to the clinical pathology and abnormal results. A careful and thorough examination via light microscopy in the optimal area on a well made, well stained peripheral smear provides an experienced observer with valued information about morphology normal or abnormal.[2]

Examination of the Peripheral Blood Smear

A blood smear examination may be performed for a variety of reasons. It may be requested by the physician in response to perceived clinical features or to an abnormality discovered in a previous CBC. The examination may also be initiated by the technologist as a result of an abnormality in the CBC or in response to a *flagged* result reported from the hematology analyzer. A flagged result is an indication that a particular result has not met established laboratory criteria and must be reviewed. All laboratories should have a documented protocol for the examination of a laboratory-initiated blood smear examination. This protocol may be derived from studies performed in the facility or may be based on consensus standards published by nationally recognized organizations.

The microscopic examination of a peripheral blood smear provides a wealth of information to the clinician. It is used to detect or verify abnormalities and subsequently may provide the physician with information from which they may be able to make a differential diagnosis. Various forms of anemia may actually be diagnosed from abnormal red cell morphology reported on a blood smear examination. The report of abnormal white cell morphology may in fact indicate what additional testing may be required. Abnormal platelet morphology may detect a platelet function deficiency even when sufficient numbers of platelets have been reported from the analyzer.

The examination of the blood smear should include evaluation of the red cell, white cell, and platelet morphology. To evaluate the smear thoroughly the technologist should review at least 8 to 10 oil immersion fields (OIF). The red cell morphology evaluation should include examination for deviations in size, shape, distribution, concentration of hemoglobin, color, and the appearance of inclusions. The white cell morphology evaluation should consist of differentiation of the white blood cells and their overall appearance including nuclear abnormalities, cytoplasmic abnormalities, and the presence of abnormal inclusions that may denote a disease process. Platelet counts should be verified, and in addition the smear should be reviewed for platelet shape and size abnormalities and for clumping.

When abnormal morphology is identified on the smear, the technologist must determine if the abnormality is possibly artifactual and not pathological. For instance, refractile artifacts may be the result of water contamination and should not be confused with red cell inclusions. Echinocytes or crenated cells may also be artifacts if practically every cell in the thin portion of the film has a uniformly spiculed membrane.

The following describes the necessary steps in the examination of the peripheral blood smear.

Low-Power (10×) Scan

1. Determine the overall staining quality of the blood smear.
2. Determine if there is a good distribution of the cells on the smear.
   - Scan the edges and center of the slide to be sure there are no clumps of RBCs, WBCs, or platelets.
   - Scan the edges for abnormal cells.
3. Find an optimal area for the detailed examination and enumeration of cells.
   - The RBCs should not quite touch each other.
   - There should not be areas containing large amounts of broken cells or precipitated stain.
   - The RBCs should have a graduated central pallor.

High-Power (40×) Scan

1. Determine the WBC estimate.
   - The WBC estimate is performed under high power (400× magnification). WBCs are counted in ten fields and averaged. The estimate is reported according to the values given in Table 5–1.
   - The WBC estimate can also be performed using a factor which is based on the fact that each WBC seen in 400x magnification (high power field) is equivalent to approximately 2000 cells per µL of blood. For example, if the average number of WBCs counted per high power field was 5, the WBC estimate would be 5 × 2000 or 10,000/µL.
2. Correlate the WBC estimate with the WBC counts per mm^3 from the automated instruments.
3. Evaluate the morphology of the WBCs and record any abnormalities, such as toxic granulation or Döhle bodies

Oil Immersion (100×) Examination

1. Perform a 100 WBC differential count.
   - Counting should be performed by moving in a zig-zag manner on the smear (Fig. 5–1).
   - All WBCs are to be included until a total of 100 have been counted.
2. Evaluate the RBCs for anisocytosis, poikilocytosis, hypochromasia, polychromasia, and inclusions.
3. Perform a platelet estimate and evaluate platelet morphology.
   - Count the number of platelets in 10 OIFs.
   - Divide by 10.
   - Multiply by 15,000/mm^3 if the slide was prepared by an automatic slide spinner; multiply by 20,000/mm^3 for all other blood smear preparations.
4. Correct any total WBC count per mm^3 that has greater than 10 nucleated red blood cells (NRBCs) per 100 WBCs.
   - When performing the WBC differential, do not include NRBCs in your count, but report them as the number of NRBCs per 100 WBCs.
   - Use the following formula to correct a WBC count:

$$\text{Corrected WBCs/mm}^3 = \frac{\text{WBC/mm}^3 \times 100}{100 + \text{No. of NRBCs/100 WBCs}}$$

The examination of the peripheral blood smear is performed as part of the hematologic laboratory workup called the CBC.

The Normal Red Blood Cell

To identify abnormal morphology, one must be competent in normal morphology identification, and more importantly, capable of differentiating them from abnormal cells, so we

Table 5–1 Estimation of Total WBC Count from the Peripheral Blood Smear

No./High Power Field	Estimated Total WBC Count/mm³
2–4	4000–7000
4–6	7000–10,000
6–10	10,000–13,000
10–20	13,000–18,000

begin this section with a description of *normal* red blood cell (RBC) morphology. The mature erythrocyte (RBC, normocyte, discocyte) has a remarkable structure in that it lacks a nucleus and organelles, and yet all components necessary for survival and function are present. It is described as a biconcave disc with a survival time of approximately 120 days. On a Romanowsky (i.e., Wright's, Giemsa) stained blood smear, this mature red cell has a reddish-orange appearance. The RBC has an average diameter of 7 to 8 μm and an average volume of 90 fL. The area of central pallor is approximately 2 to 3 μm in diameter (Fig. 5–2), and the size variation of red cells from a normal patient is approximately 5%. The primary function of the red cell is the transportation of O_2 to the tissues of the body and transportation of CO_2 back to the lungs for expulsion. Fundamental to the red cell is the formation of hemoglobin, which is ultimately responsible for binding the oxygen molecule for transport. The oxygen-carrying capacity of each erythrocyte is dependent on the production of adequate amounts of functional hemoglobin. Also fundamental to the red cells functionality is the maintenance of the cellular membrane. The red cell membrane is composed of equal weighted portions of lipids and proteins and is responsible for sustaining a constant surface-to-volume ratio. Maintaining the integrity of this membrane is essential to the cells shape and deformability which allows the RBC to traverse through the microvasculature of the body. An alteration of this membrane may result in the inability of the red cell to function efficiently and ultimately may lead to the cell's early demise.

Assessment of Red Cell Abnormality

A well-stained and well-made blood smear with an even distribution of RBCs in the area to be examined is essential for any peripheral blood smear review. If these criteria are achieved, the reviewer must make a general assessment of whether the morphological abnormality is due to shape change (*poikilocytosis*) or size change (*anisocytosis*) or a change in color. Most assessments of anisocytosis are performed in concert with the red cell indices and the red cell distribution width (RDW) rating obtained from the hematology analyzer. In assessing the smear, the reviewer takes into account the percentage of cells that vary in size in at least 10 OIFs. For example, if the mean corpuscular volume (MCV) was 65 fL (80 to 100 for adults), the reviewer would expect to see a large percentage of small cells. If the MCV were 105 fL, the reviewer would expect to see primarily larger cells. More information on red cell indices, including calculations, may be found in Chapter 31 of this text.

The majority of laboratories use either qualitative remarks (*few* or *marked*) or a numerical grading (*1+* to *4+*) based on percentage of variation and to describe the type of cell or cells that have caused the variation from the normal. With this method, a reviewer can present to the clinician a series of objective ratings that can translate to a visual impression of a patient's peripheral smear. This assessment may be critical to the physician's differential diagnosis of certain forms of anemia. Reviewers are urged to avoid the use of terms that are vague (e.g., the term *present*) owing to the wide variations in the implication it may have to clinicians. See Table 5–2 for an example of guidelines in grading anisocytosis and poikilocytosis. Please note that the assessment of RBC morphologic abnormalities remains a manual task that is inherently subjective, and it is imperative that laboratories establish guidelines based on their own patient and physician population. It is essential to patient care that the laboratory and the clinician have similar interpretations of the results reported for all RBC morphology. Figure 5–3 is a composite chart of normal and abnormal red cell morphology.

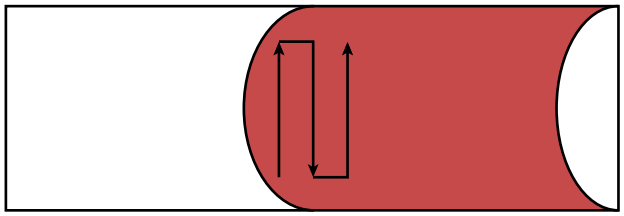

Figure 5-1 ■ Blood smear.

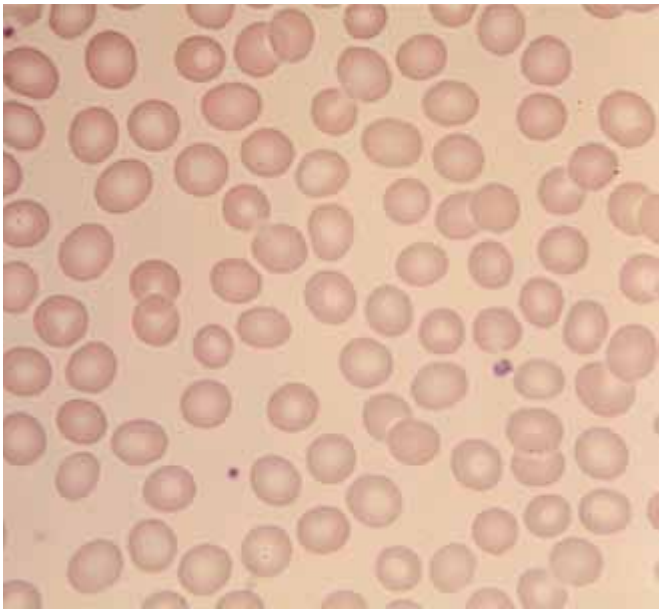

Figure 5-2 ■ Normal red blood cells.

Table 5–2 Grading Scale for Red Cell Morphology (Anisocytosis/Poikilocytosis)

Percentage of Cells that Differ in Size or Shape from Normal RBCs	
Normal	5%
Slight	5%–10%
1+	10%–25%
2+	25%–50%
3+	50%–75%
4+	>75%
Sample Situations	
2+ Microcytes	Few schistocytes
1+ Macrocytes	Few burr cells
	1+ Target cells
3+ Anisocytosis	2+ Poikilocytosis

Included in this chapter are flowcharts that correlate the abnormal morphology with a possible pathology. This scheme should enable the learner to more easily associate an abnormal morphology with the clinical condition.

Variations in Red Cell Distribution

Normal Distribution

The area of smear that is reviewed for morphologic abnormalities is of the utmost importance. The area to be reviewed should be in the thin portion of the smear where the red cells are slightly separated from one another or at most, barely touching, with no overlap. The thin area should represent at least one-third of the entire film.[2] The reviewer should avoid the thicker portion of the slide where cells are overlapping and the edges of smear where cells may be artifactually distorted in size, shape, and color. An exception is to be made when scanning for platelet clumping.

Abnormal Distribution

AGGLUTINATION

Agglutination is an aggregation of red cells into random clusters or masses. Agglutination is the result of an antigen–antibody reaction within the body, and in cases of autoagglutination the reaction is actually with the patient's own cells and the patient's serum or plasma. Such is the case with cold antibody syndromes, for example, *cold hemagglutination* disease and *paroxysmal cold hemoglobinuria* (PCH). Agglutination occurs at room temperature during sample preparation and appears as interspersed areas of clumping throughout the peripheral smear (Fig. 5–4). The use of saline will not disperse these agglutinated areas; however, warming the sample to 37°C helps to break up the agglutinins, allowing for the possibility of normal slide preparation for morphology review. The MCHC and MCV from these specimens are usually falsely elevated in response to the agglutinin formation. Other forms of autoagglutination may also occur spontaneously but are more likely to be seen in connection with certain hemolytic anemias, atypical pneumonia, staphylococcal infections, and trypanosomiasis. Agglutination is not to be confused with *rouleaux* which is described next.

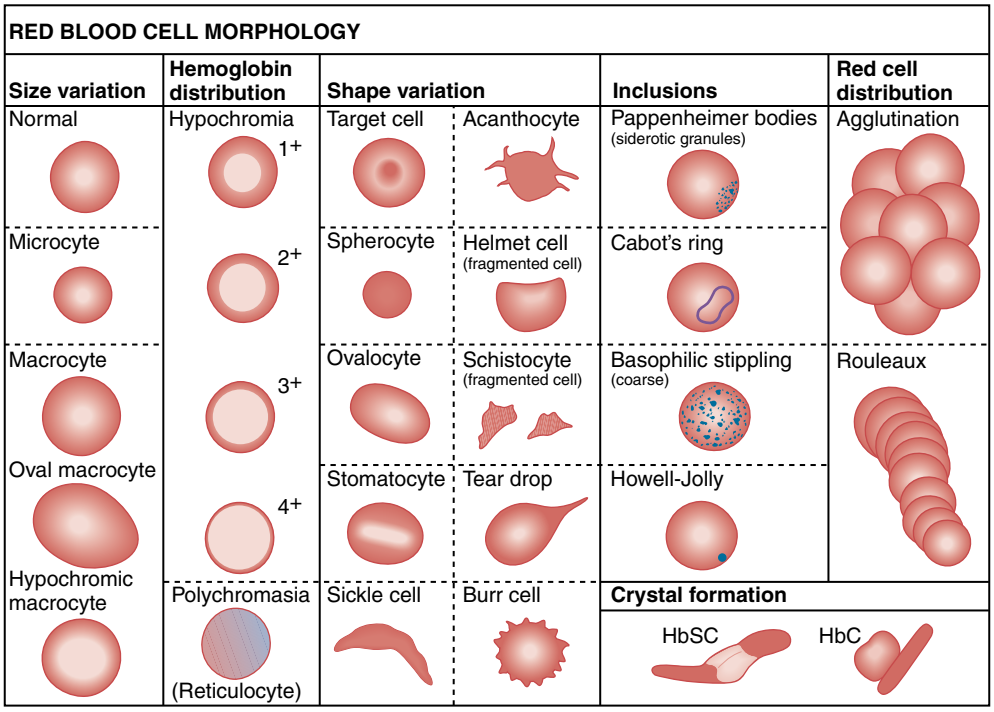

Figure 5-3 ■ Normal and abnormal red blood cell morphology.

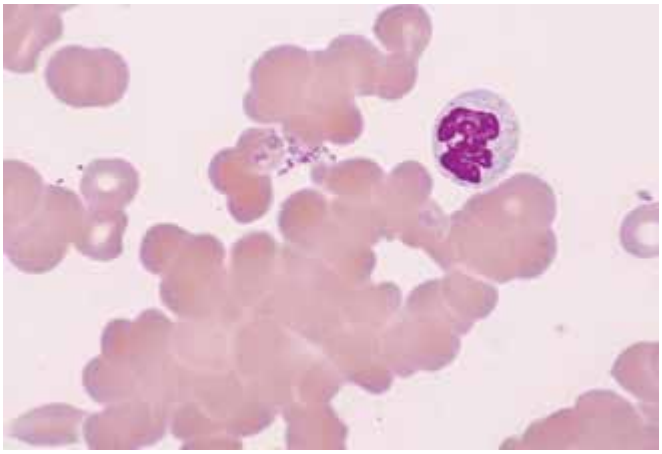

Figure 5-4 ■ Note the agglutination on the smear from a patient with cold hemagglutinin disease.

ROULEAUX

Rouleaux is a condition in which red cells appear as stacks of coins on the peripheral smear. The stacks may be short or long, but regardless of the length, the red cells appear stacked on one another. These stacks are rather evenly dispersed throughout the smear. Rouleaux formation is the result of elevated globulins or fibrinogen in the plasma where the red cells have been more or less "bathed" in this abnormal plasma which gives them a sticky consistency. This lowers the zeta (ζ) potential, thus facilitating the stacking effect (Fig. 5–5). The use of a saline dilution of the serum disperses rouleaux. Rouleaux formation correlates well with a high erythrocyte sedimentation rate.

Rouleaux is seen in patients with *hyperproteinemias* such as multiple myeloma and Waldenstrom's macroglobulinemia. It may also be seen in chronic inflammatory disorders, and some lymphomas. It is important to note that in cases of severe rouleaux it may be impossible to evaluate cell size or shape.

Peripheral smears reviewed in the thick portions of the smear and entire smears made too thick may appear to exhibit rouleaux. This is considered artifactual and should not be reported until it is verified in the thin portion of the smear or a new slide is prepared.

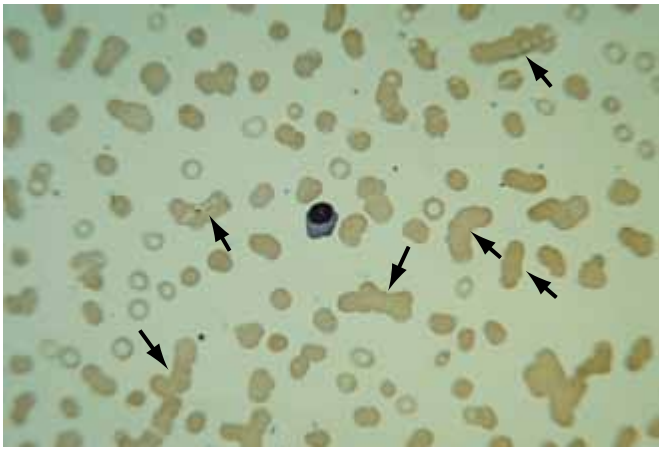

Figure 5-5 ■ Peripheral blood showing marked rouleaux formation. Note the "stacked coin" appearance of the red cells.

Variations in Size

Anisocytosis

Any significant variation is size is known as *anisocytosis.* This size variation is frequently found in the leukemias and in most forms of anemia. The severity of the variation should also correspond to an increased RDW. Anisocytosis results from abnormal cell development, and typically results from a deficiency in the raw materials (i.e., iron, vitamin B_{12}, folic acid) needed to manufacture them or by a congenital defect in the cell's structure. Cell size may deviate, from measuring smaller than the normal 7 μm, to being larger than normal. The terms used to describe these abnormalities are *microcyte* (≤6 μm) and *macrocyte* (≥9 μm). These terms are used in conjunction with the terms microcytosis and macrocytosis and should also correlate with the red cell indice results. Anisocytosis is graded in most facilities as 1+ to 4+ (see Table 5–2). When reporting anisocytosis, it is important to describe the morphology picture in terms of microcytosis or macrocytosis, or in cases of a dimorphic population there may be the appearance of both (Fig. 5–6).

Normocytes

The average size of the erythrocyte is indicated by the measurement of the MCV, a result generated by the automated hematology analyzer. The MCV is considered an integral part of a CBC. Observation of red cell morphology on the blood smear provides a quality control check on the electronic MCV, as well as the other two red cell indices, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).[2] A "normal" MCV would correspond to the MCV reference range (80 to 100 fL for adults). Subsequent review of the blood smear should yield no significant

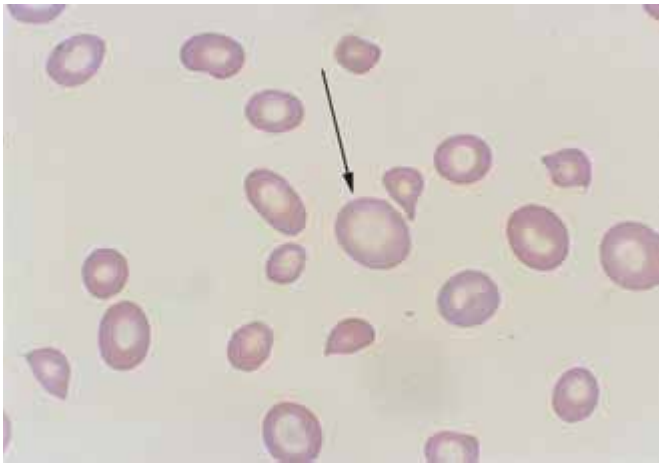

Figure 5-6 ■ Note the different size (anisocytosis) and shape (poikilocytosis) of the red cells. Compare the largest (macrocytic) cell below the arrow in the center of the field with the smaller (microcytic) cells.

size variation from the normal 7- to 8-µm red cell. This scenario is referred to as *normocytic* and the red cells are referred to as normocytes. This information would prove useful to the physician in the diagnosis of anemia. In the case of a normal MCV and a high RDW (normal RDW is 11.5% to 14.5%), the reviewer would expect to see a mixture of large and small cells.[3] This scenario is referred to as a dimorphic population and may be the result of a recent blood transfusion, or possibly the patient may be in the recovery stages of anemia. Patient history plays an important role in this situation.

Macrocytes

Macrocytes are cells that are approximately 9 µm or larger in diameter, having an MCV of greater than 100 fL.[4] Anemias associated with these cells are referred to as *macrocytic*. These cells may appear in the peripheral circulation by several mechanisms. One mechanism is impaired deoxyribonucleic acid (DNA) synthesis, which results in *megaloblastic* erythropoiesis leading to a decreased number of cellular divisions, and consequently a larger cell. This form of erythropoiesis produces a *megaloblastic anemia* and may be the result of B_{12} or folate deficiency, chemotherapy, or any process producing a nuclear maturation defect. Macrocytes with an oval shape (macroovalocytes), neutrophilic hypersegmentation, as well as MCV values exceeding 120 fL are typically seen in this type of anemia.

The most common cause of *nonmegaloblastic* macrocytosis is accelerated erythropoiesis which results from conditions such as acute blood loss or alcoholism. The cells are released prematurely from the marrow, are non-nucleated, and appear larger than a mature erythrocyte. On a Wright-stained smear the cells will appear as round polychromatophilic macrocytes and on a supravitally (i.e., new methylene blue) stained smear they appear as reticulocytes. Neutrophilic hypersegmentation is not typically seen in this form of macrocytosis.[5]

Macrocytosis may result from other conditions, such as hypothyroidism and various bone marrow disorders, as well as occur in neonatal blood, postsplenectomy, and in cases where excess plasma cholesterol may be taken up by the red cell which subsequently leads to an increase in the surface area of the cell. However, this last mechanism may not be reflective of a "true" macrocytosis (obstructive liver disease). Macrocytes should be evaluated for shape (oval versus round) as shown in Figure 5–6, color (red versus blue), pallor (if present), and the presence or absence of inclusions. The conditions in which macrocytes may be seen are listed in Figure 5–7.

Microcytes

A microcyte is a small cell having a diameter of less than 7 µm and an MCV of less than 80 fL. Anemias associated with microcytes are said to be *microcytic*. The hemoglobin content of these cells may be normal to decreased. A consequence of any defect that results in impaired hemoglobin synthesis may produce a microcytic, hypochromic (MCHC <32% and cells with increased central pallor) blood picture. When erythroid cells are deprived of any of the essential elements in hemoglobin synthesis (see Chap. 6), the result is an increase in cellular divisions and consequently a smaller cell in the peripheral blood. This form of abnormal hemoglobin synthesis is seen in iron deficiency, deficiency of heme synthesis (sideroblastic anemia), deficiency of globin synthesis (thalassemia), and chronic disease states. In the case of iron deficiency, microcytosis will not be visually apparent until iron stores in the body have been completely exhausted and iron deficient erythropoiesis takes place, as in *iron deficiency anemia* (IDA). Iron deficiency is the most common cause of anemia, affecting some 30% of the world population and accounting for up to 500 million cases worldwide, which also makes it the most common microcytic/hypochromic anemia in the world.[6] It is especially common in women of childbearing age. The causes of this deficiency may vary depending on the age and sex of the patient and it is important to determine what instigated the deficiency before treatment begins.

Decreased or defective globin synthesis also presents as a microcytic/hypochromic anemia, but in most cases this results from a genetic abnormality producing a hereditary

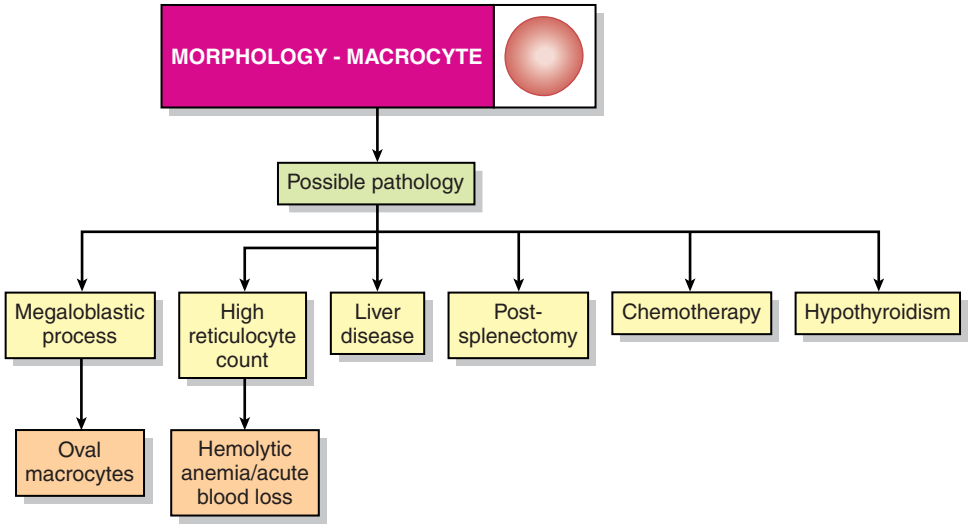

Figure 5-7 ■ Correlation of macrocytes to pathologic processes.

anemia known as *thalassemia*. This microcytic/hypochromic anemia is rare and in the homozygous form it may result in a severe anemia with a high rate of mortality. In the milder heterozygous form this anemic picture may be confused with IDA. The appearance of target cells, family studies, as well as additional hematological testing may be needed for a differential diagnosis.

It is important to note that other disease processes such as sideroblastic anemia and lead poisoning may produce significant numbers of microcytic red cells, in most cases without hypochromia. Clinical conditions in which microcytes may be seen as the predominant cell morphology are illustrated in Figure 5–8.

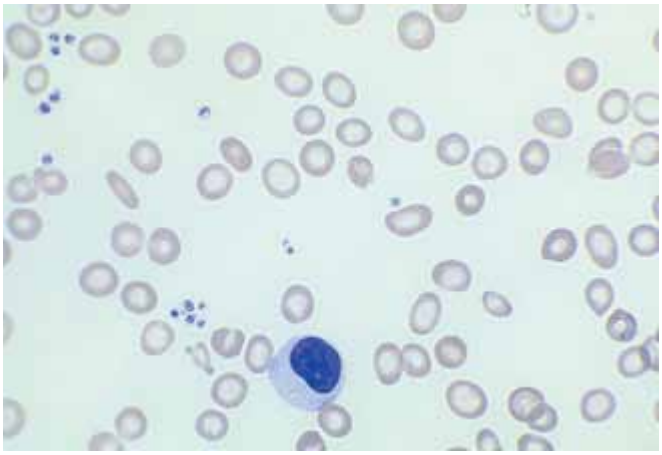

Figure 5-9 ■ Note the large central pallor in many of the red cells depicting hypochromia.

Hemoglobin Content—Color Variations

Normochromia

The term normochromic indicates the red cell is essentially normal in color. A normochromic erythrocyte has a well hemoglobinized cytoplasm with a small but distinct zone of central pallor. The area of pallor does not exceed 3 µm when measured linearly. In a well stained peripheral smear, the normal red cells will appear reddish-orange in color. The term *normochromic* is used to describe an anemia with a normal MCHC and MCH. When used in conjunction with a normal MCV, the anemia would be described as a *normochromic/normocytic* anemia (see Fig. 5–2).

Hypochromia

Any RBC having a central area of pallor of greater than 3 µm is said to be *hypochromic*. There is a direct relationship between the amount of hemoglobin deposited in the red cell and the appearance of the red cell when properly stained. The term hypochromia literally means "low color" and indicates that the cells have less than the normal amount of hemoglobin. Typically any irregularity in hemoglobin synthesis will lead to some degree of hypochromia (Fig. 5–9).

Most clinicians choose to assess hypochromia based on the mean corpuscular hemoglobin concentration (MCHC), which by definition measures hemoglobin content in a given volume of red cells (100 mL). When the MCHC is <32% the anemic process is described as being hypochromic and the slide reviewer should scan the peripheral smear for the presence of red cells with increased central pallor or hypochromia. A lower MCHC result typically correlates with a larger central pallor in the affected red cells. In general, this is very reliable; however, it does not take into account the situation in which a true hypochromia is observed in the presence of a normal MCHC. In many cases, the MCHC will not be concordant with what is observed on the peripheral smear. The morphologist should not be unduly influenced by the RBC indices in the evaluation of hypochromia. True hypochromia will appear as a delicate shaded area of pallor as opposed to pseudohypochromia (the water artifact), in which the area of pallor is distinctly outlined. It is important to note that not all hypochromic cells are microcytic. Target cells possess some degree of hypochromia, and there are macrocytes and normocytes that can be distinctly hypochromic.

The most common condition manifesting hypochromia is IDA. In severe cases of IDA, red cells exhibit an inordinately thin band of hemoglobin. Patients with iron deficiency may have many hypochromic cells, depending on the magnitude of the deficiency. In addition to large numbers of hypochromic cells, there may be large numbers of microcytes as well. Iron deficiency anemia is commonly referred to as a microcytic/hypochromic anemia. Hypochromia in the alpha (α) and beta (β) trait thalassemia syndromes is much less pronounced. However red cells in the α–thalassemias and β–thalassemia homozygous states show significant amounts of pallor.[7] Sideroblastic anemias show a prominent

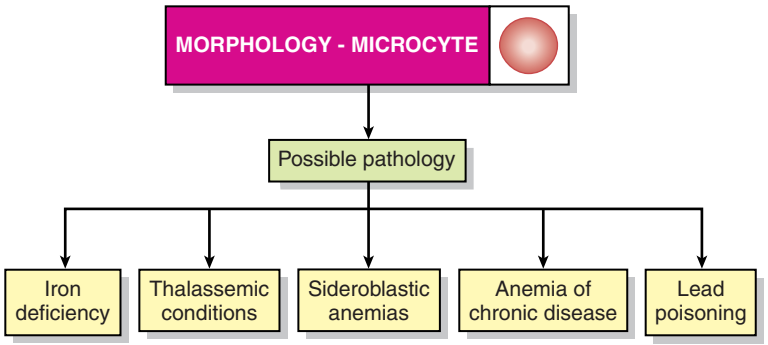

Figure 5-8 ■ Correlation of microcytes to pathologic processes.

Table 5–3 Hypochromia Grading

1+ Area of central pallor is one-half of cell diameter
2+ Area of pallor is two-thirds of cell diameter
3+ Area of pallor is three-quarters
4+ Thin rim of hemoglobin

dimorphic blood picture—macrocytic, normocytic, and microcytic cells together, only some of which show true hypochromia. Some hypochromic cells may be seen in patients with lead poisoning. Refer to Table 5–3 for a guideline to grading hypochromia.

Hyperchromia

Red cells with a decreased surface-to-volume ratio and a decreased or absent central pallor may be described as *hyperchomic*. True hyperchromia exists when the MCHC is >36% and may be seen in the peripheral smears of patients with hemolytic anemias, including hemolysis caused by burns. Even though true hyperchromia does exist it is not reported as such. It is reported in terms of the cell abnormalities resulting from the increased volume of hemoglobin and the decreased surface area. The cell produced from these phenomena appears as a solid reddish-orange disc with no central pallor and is referred to as a *spherocyte*, and is discussed later in this chapter.

Polychromasia

When RBCs are delivered to the peripheral circulation prematurely, their appearance in the Wright-stained smear is distinctive. These red cells are described as *polychromatophilic* (diffusely basophilic) and are gray-blue in color and usually larger than normal red cells (Fig. 5–10). The basophilic color of the red cell is the result of the residual RNA involved in hemoglobin synthesis. Polychromatophilic macrocytes, as seen on a Wright's stained smear, are actually reticulocytes; however, the reticulum cannot be visualized without supravital staining.

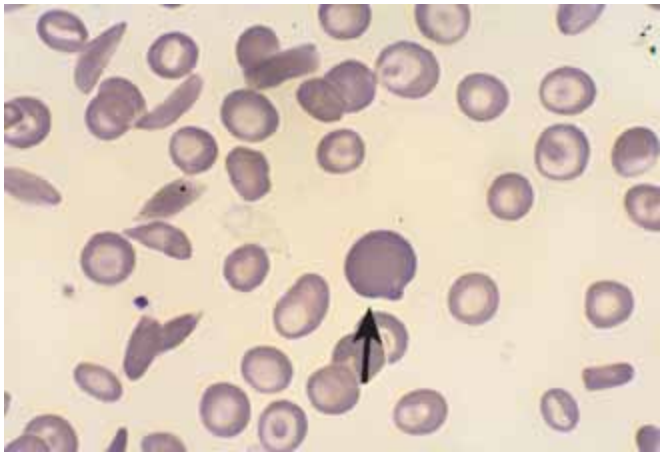

Figure 5-10 ■ Note polychromasia in the cell with the arrow.

It is not uncommon to find a few polychromatophilic cells in a normal peripheral blood smear, because regeneration of red cells is a dynamic process. The reticulocyte count should reflect the degree of polychromasia. In the blood smear, polychromatophilic red cells appear in varying shades of blue. Any clinical condition in which the marrow is stimulated, particularly RBC regeneration, will produce a polychromatophilic blood picture. This represents effective erythropoiesis as well as an assessment of bone marrow function. Examples of several conditions in which polychromasia is noted include acute and chronic hemorrhage, hemolysis, and any regenerative red cell process. The degree of polychromasia is an excellent indicator of therapeutic effectiveness when a patient is given iron or vitamin therapy as a treatment for anemia. Refer to Table 5–4 for a guideline to polychromasia grading.

Variations in Shape

Poikilocytosis

Poikilocytosis is the term used to describe a variation in red cell shape. Normal erythrocytes vary only slightly from the concise round shape of a biconcave disc, so even a slight variation in significant numbers may prove to be important. These *poikilocytic* cells may take on such peculiar shapes as teardrops, pencils, and sickles. The differential diagnosis of anemia cannot be determined from a reported poikilocytosis. The term should be used in conjunction with more descriptive terminology which would specify the particular morphological abnormality observed. Examples of specific poikilocytes are sickle cells, which result from abnormal hemoglobin, and spherocytes, which result from a red cell membrane abnormality as many of the poikilocytic cells do. The differential diagnosis of some forms of anemia may be determined by identification of a specific morphological abnormality.

The term poikilocytosis refers to the entire red cell morphology in the scanned area of a peripheral smear and is graded as 1+ to 4+ (see Table 5–2). Many labs consider the term poikilocytosis as a "catch all" phrase for abnormal red cells and in lieu of grading the smear for poikilocytosis opt only to grade the specific types of morphologically abnormal cells seen. In these cases the particular cells should be reported in terms of few, moderate, and many.

Table 5–4 Polychromasia Grading

	Percentage of Red Cells that are Polychromatophilic
Slight	1%
1+	3%
2+	5%
3+	10%
4+	>11%

Target Cells (Codocytes)

Target cells appear on the peripheral blood as a result of an increase in RBC surface membrane. They are artificially induced on the smear and their true circulating form, as seen with an electron microscope, is a bell-shaped cell. The name *codocyte* is from the Greek word *kodon* meaning bell. In air-dried smears, however, they appear as "targets," with a large portion of hemoglobin displayed at the rim of the cell and a portion of hemoglobin that is central, eccentric, or banded (Fig. 5–11). As the name implies, the cell actually resembles a target and is sometimes referred to as a "*bull's eye*" cell as well as a "*Mexican hat*" cell.

The mechanism of targeting is related to excess membrane cholesterol and phospholipid and decreased cellular hemoglobin. This is well documented in patients with liver disease, in whom the cholesterol/phospholipid ratio is altered. Mature red cells are unable to synthesize cholesterol and phospholipid independently. As cholesterol accumulates in the plasma, as seen in liver dysfunction, the red cell is expanded by increased membrane lipid, resulting in increased surface area. Consequently, the osmotic fragility is also decreased (see Chap. 31). Target cells are seen in many types of anemia (Fig. 5–12); however, they are most prominent in the hemoglobinopathies, thalassemias and liver disease.

Spherocytes

Spherocytes have a reduced surface-to-volume ratio that results in a cell with no central pallor. Because of their density (intense color) and smaller size, they are easily distinguished in a peripheral smear. Their shape change is irreversible and may also be seen as *microspherocytes*. They are considered the most common form of the erythrocyte morphological disorders stemming from an abnormality of the cell membrane. This abnormality may be hereditary or acquired and may be produced by a variety of mechanisms affecting the red cell membrane. Perhaps the most detailed mechanism for sphering is the congenital condition known as *hereditary spherocytosis* (HS). This is an inherited, autosomal dominant condition and is due to a deficiency of, or a dysfunction in, the membrane proteins spectrin, ankyrin, band 3 and/or protein 4.2.[8] The membrane cytoskeleton is dependent on these particular proteins to maintain the shape, deformability, and elasticity of the red cell. The deficiency and/or dysfunction of any one these membrane components will destabilize the cytoskeleton, resulting in abnormal red cell morphology and a shorter lifespan for the affected red cells in circulation.[8] Spherocytes are typically seen in large numbers in peripheral smears from these patients. Premature destruction of these abnormal erythrocytes in the spleen may produce a mild to severe hemolytic anemia depending on the severity of the abnormality. Erythrocytes from patients with hereditary spherocytosis have a mean influx of sodium twice that of normal cells. Because these spherocytes have increased ability to metabolize glucose, they can handle the excessive intracellular sodium while in the plasma, but when they reach the microenvironment of the spleen, the active–passive transport system is unbalanced with increased sodium and decreased glucose resulting in swelling and hemolysis of these cells (see Chap. 9). In more than one-half of these patients, the MCHC is greater than 36%. Yet, for individuals not exhibiting an increased MCHC, a careful observation of their peripheral smear is the key to diagnosis.[9] Figure 5–13 depicts a blood smear from a patient with hereditary spherocytosis.

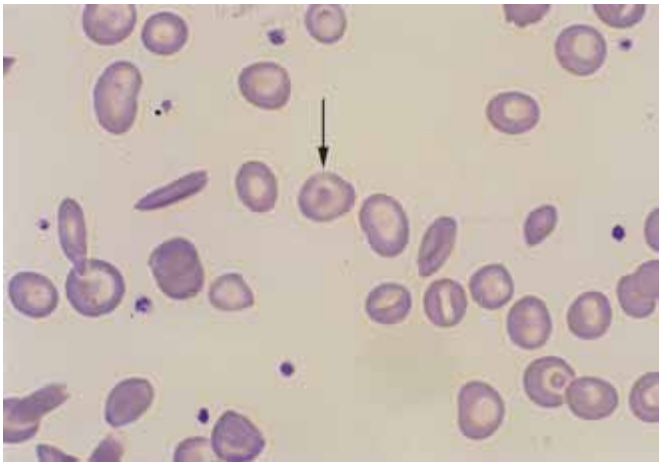

Figure 5-11 ■ Note the target cell at the arrow.

The acquired forms of spherocytosis share the mutual defect with HS in that there is a loss of membrane. In the normal aging process of red cells they gradually lose their functionality through loss of cellular lipids, proteins, etc.; thus, spherocytes are produced as a final stage before senescent red cells are detained in the spleen and trapped by the reticuloendothelial system. This natural process does not typically result in anemia. Another mechanism of producing sperocytes that may result in a mild to severe anemia is autoimmune hemolytic anemia. The coating of the red cells with antibodies and the detrimental effect of complement activation results in the membrane loss of cholesterol accompanied by a loss of surface area without hemoglobin loss producing spherocytes. The reduced surface-to-volume ratio of all spherocytes renders them abnormally susceptible to osmotic lysis; consequently, they have an increased osmotic fragility. Hemolysis is known to result from membrane abnormalities; therefore, other hemolytic processes may also produce spherocytes. They may also be seen as *microspherocytes* in the peripheral smears of burn patients. Figure 5–14 lists the more common pathologic conditions in which spherocytes are seen.

Stomatocytes

The word *stomatocyte* is derived from the Greek word *stoma,* which means mouth. They have a central pallor which is said to be slit-like or mouth-like on peripheral blood smears. These red cells are of normal size, but are not biconcave, and in wet preparations appear bowl-shaped (Fig. 5–15). The abnormal morphology resulting in the stomatocyte is thought to be the result of a membrane defect. Stomatocytosis is associated with abnormalities in red cell cation permeability that lead to

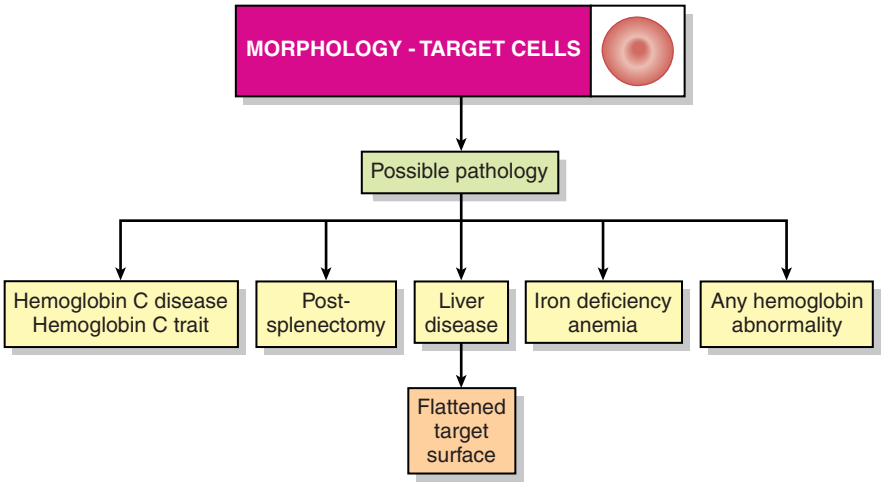

Figure 5-12 ■ Correlation of target cells to pathologic processes.

changes in red cell volume, which may be either increased (hydrocytosis) or decreased (xerocytosis), or is some cases, near normal.[9] Hydrocytosis and xerocytosis represent the extremes of a spectrum of red cell permeability defects.[9] The exact physiologic mechanism of stomatocytic shape is poorly understood and the molecular basis of this disorder is unknown. Stomatocytosis may be acquired or congenital. As with hereditary spherocytosis, stomatocytes are seen in significant numbers in the hereditary form known as *hereditary stomatocytosis* and in smaller numbers in the acquired form. Many chemical agents can induce stomatocytosis in vitro (phenothiazine and chlorpromazine); however, these changes are reversible.[10] Stomatocytes are known to have an increased permeability to sodium; consequently, their osmotic fragility is increased.

Stomatocytes are more often artifactual than a true manifestation of a particular pathophysiologic process. The artifactual stomatocyte has a distinct slit like area of central pallor, whereas the area of pallor in the genuine stomatocyte appears shaded. Several of the associated disease states in which stomatocytes may be found are hereditary spherocytosis (the stomatospherocyte is best viewed in wet preparations); hereditary stomatocytosis (which is usually a benign condition or at most a mild normochromic/normocytic anemia), hemolytic anemia, alcoholic cirrhosis, and acute alcoholism. Stomatocytosis is also present on peripheral blood smears of patients with Rh deficiency syndrome, also known as Rh null disease, in which erythrocytes from these rare individuals have either absent (Rh_{null}) or markedly reduced (Rh_{mod})Rh antigen expression. This may result in a mild to moderate hemolytic anemia, and mutations in the *Rh30* and *RhAG* genes have been associated with this syndrome.[9]

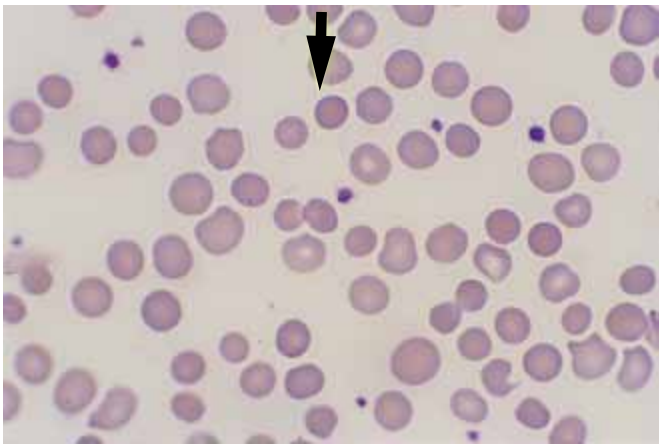

Figure 5-13 ■ Note the spherocyte at arrow in a blood smear from a patient with hereditary spherocytosis.

Ovalocytes and Elliptocytes

Many investigators consider the terms *ovalocyte* and *elliptocyte* to be interchangeable; however, for the purposes of this discussion, they are viewed as distinct and separate. This morphological abnormality is thought to be the result of a mechanical weakness or fragility of the membrane skeleton and may be acquired or congenital. The pathogenesis of the formation of either of these cells is unknown. Ovalocytes may be considered as more egg-shaped and have a greater tendency to vary in their hemoglobin content. They can appear normochromic or hypochromic, normocytic or macrocytic. Megaloblastic anemia is characterized by oval macrocytes (macroovalocytes) that may be 9 μm or more in diameter and lack central pallor (Fig. 5–16).[2]

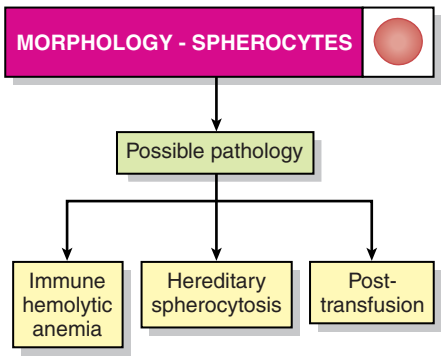

Figure 5-14 ■ Correlation of spherocytes to pathologic processes.

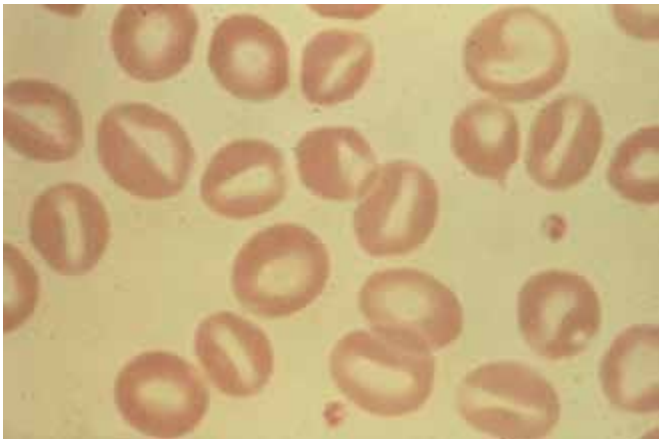

Figure 5-15 ■ Stomatocytes in peripheral blood.

Elliptocytes, on the other hand, are pencil, rod, or cigar-shaped and hemoglobin appears to be concentrated on both ends of the cell. They are invariably not hypochromic, exhibiting a normal central pallor. Hereditary elliptocytosis (HE) is an inherited condition with anywhere from 25% to 90% of all cells demonstrating the elliptical appearance. The erythrocytes in HE, in most cases, have a normal survival time; patients are typically asymptomatic and are diagnosed incidentally during testing for unrelated conditions.[9] In approximately 10% of cases where red cell survival time is shortened, patients' symptoms may vary from a mild to severe transfusion-dependent hemolytic anemia. Mutations in the red cell membrane protein α-spectrin account for a majority of cases of HE, with the remaining cases arising from mutations in β-spectrin or protein 4.1R (Fig. 5–17).[11]

Ovalocytes/elliptocyte may be seen in association with several disorders in addition to those already mentioned such as microcytic/hypochromic anemia, myelodysplastic syndromes, and myelophthistic anemia.[2] Refer to Figure 5–18 for a description of pathologic processes associated with ovalocytes and elliptocytes (see Chap. 9).

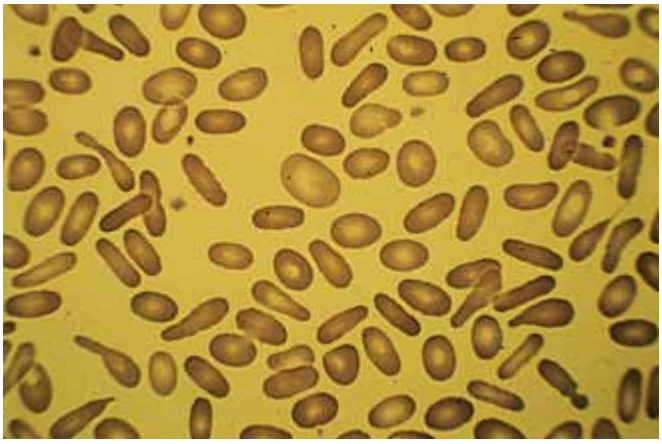

Figure 5-16 ■ Note the high percentage of elliptocytes in this blood smear from a patient with hereditary elliptocytosis.

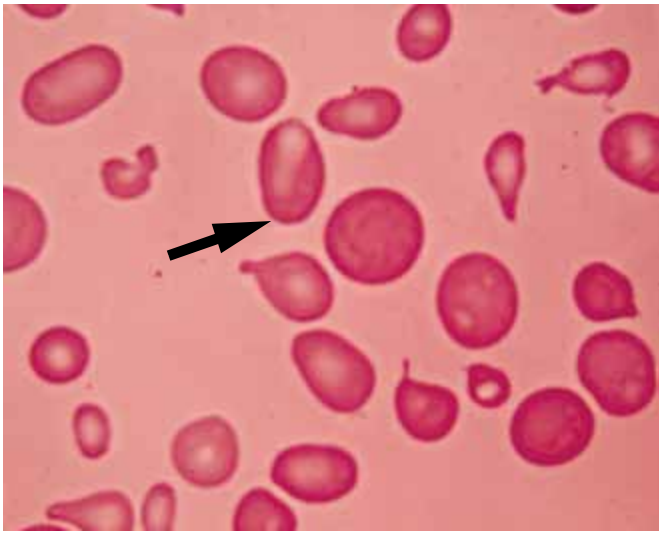

Figure 5-17 ■ Note the oval macrocyte (microovalocyte) at the arrow. Smear from a patient with pernicious anemia.

Sickle Cells (Drepanocytes)

Depranocytes or *sickle* cells are typically crescent or sickle shaped with pointed projections at one or both ends of the cell. These cells have been transformed by hemoglobin polymerization into rigid, inflexible cells no longer resembling the normal biconcave disc (Fig. 5–19). Patients may be homozygous or in some cases heterozygous for the presence of the abnormal hemoglobin, hemoglobin S. In the homozygous patient, physiologic conditions of low oxygen tension (in vivo or in vitro) cause the abnormal hemoglobin to polymerize, forming tubules that line up in bundles to deform the cell. The surface area of the transformed cell is much greater, and the normal elasticity of the cell is severely restricted. These cells have lost their ability to deform and in many cases are unable to negotiate the microvasculature of the tissues, which leads to oxygen depravation in those areas (see Chap. 11).

Most sickled cells possess the ability to revert to the discocyte shape when oxygenated; however, approximately 10% are incapable of reverting to their normal shape. These irreversibly sickled cells (ISCs) are the result of repeated sickling episodes. On the peripheral smear, they appear as crescent-shaped cells with long projections. When reoxygenated, the ISCs may undergo fragmentation. During a symptomatic period, the percentage of ISCs varies tremendously, and, consequently, it does not correlate with symptomatology. Sickle cells are not usually seen in the peripheral smears of individuals who are heterozygous (Hgb AS), and are only rarely seen in conjunction with other abnormal hemoglobins (i.e., Hgb C_{Harlem} Hgb $S_{Memphis}$). Classically, sickled cells are best seen in wet preparations. Many of the cells observed on the Wright–Giemsa stain are the oat cell-shaped form of the sickled cell (Fig. 5–20). In this form, the projections are much less pronounced and the central area of the cell is fairly broad. This shape is reversible.[12] The more prominent pathologic conditions in which sickle cells may be observed are listed in Figure 5–21. In this figure, a morphological distinction is made between ISCs and reversibly sickled cells.

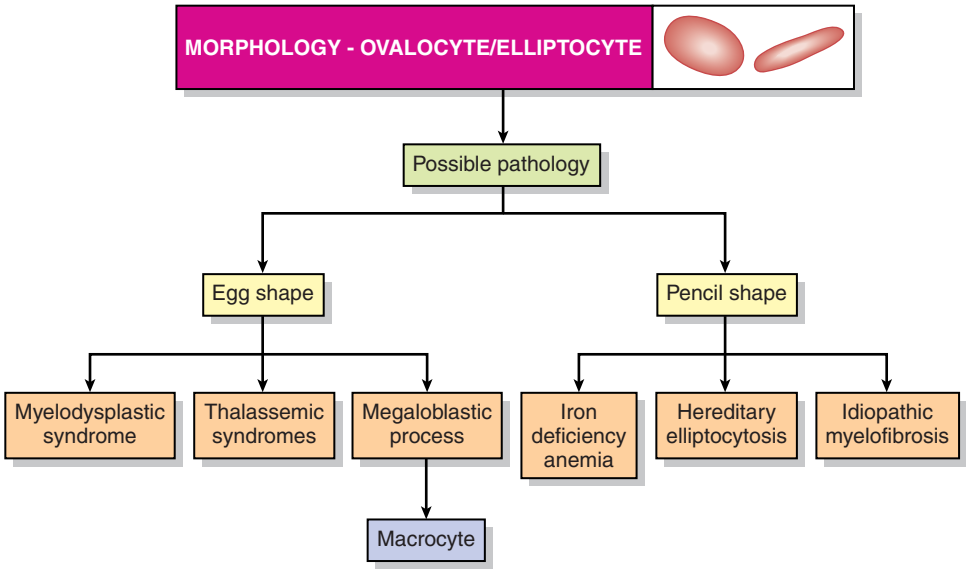

Figure 5-18 ■ Correlation of ovalocytes and elliptocytes to pathologic processes.

Fragmented Cells (Schistocytes, Helmet Cells, Keratocytes)

Schistocytes are split, cut, or cloven cells resulting from some form of trauma to the cell membrane. It is recognized that not all membrane alterations occur pathologically. However, there are certain triggering events in disease that invariably lead to fragmentation such as alteration of normal fluid circulation. Examples of fluid alterations are the development of fibrin strands, damaged endothelium, or a damaged heart valve prosthesis. The flow of blood in the circulation may actually sweep the erythrocytes through the fibrin strands, splitting the red cell. The shapes of these cells vary based on the shear forces and presentation of the red cells as they are cut by the fibrin. Intrinsic defects of the red cell make it less deformable and, therefore, more likely to be fragmented as it traverses the microvasculature of the spleen. Examples such as antibody-altered red cells and red cells containing inclusions have significant alterations that increase their likelihood of being fragmented, consequently decreasing their survival time.

Schistocytes are the extreme form of red cell fragmentation (Fig. 5–22). Whole pieces of red cell membrane appear to be missing, and bizarre red cells are apparent. Schistocytes may occur in patients with microangiopathic hemolytic anemia, disseminated intravascular coagulation (DIC), heart valve surgery, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura,[13] renal graft rejection, vasculitis, in severe burn cases, as well as *march hemoglobinuria* (a form of hemoglobinuria seen in soldiers and long-distance runners).

Keratocytes are red cells that have been caught on fibrin strands in circulation, and rather than splitting, the cell hangs over the fibrin fusing two sides of the cell together, creating a vacuole. Once the cell escapes from the fibrin strand it appears in the peripheral blood as a red cell with a vacuole in one end resembling a blister and is called a *blister* cell. It also is said to resemble a women's handbag and may be called a *pocket-book* cell (see Fig. 5–22). Once the vacuole ruptures, the resulting cell appears to have two horns. This "horned" cell also resembles a helmet and is sometimes reported as such, but is actually a keratocyte (Greek for *keras*, horn).[2] The

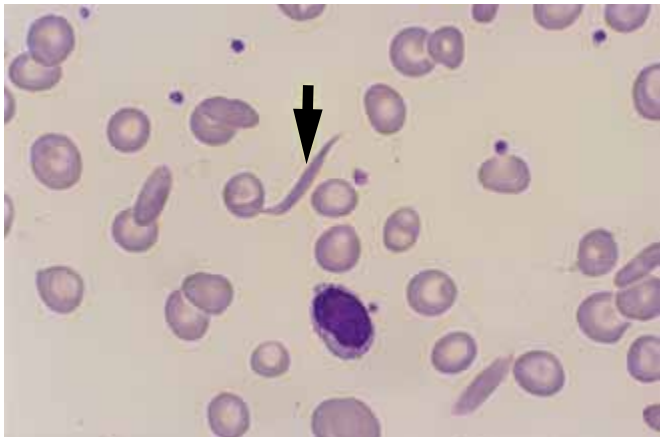

Figure 5-19 ■ Irreversibly sickled cells.

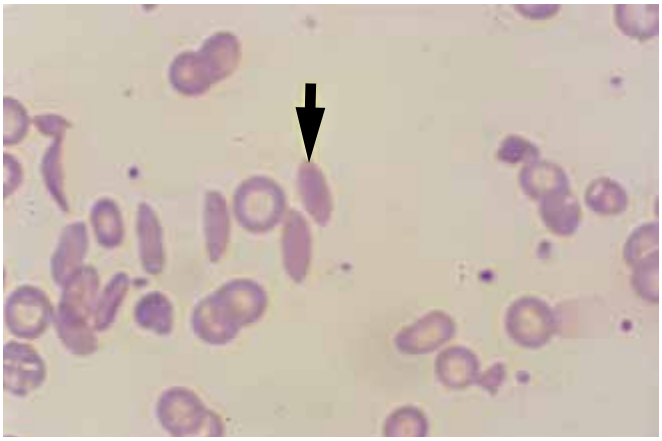

Figure 5-20 ■ Reversible, oat-shaped sickle cell.

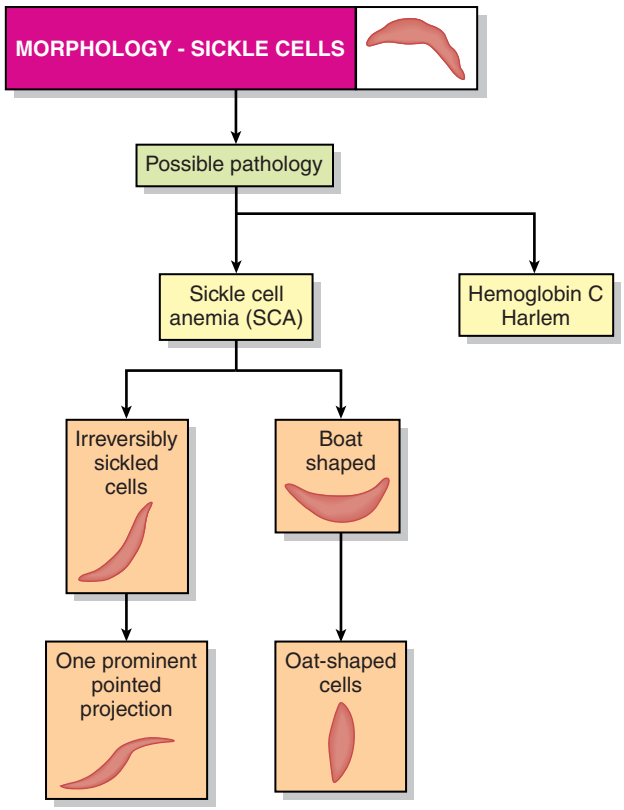

Figure 5-21 ■ Correlation of sickle cells to pathologic processes.

primary difference in the two cells is not in their appearance, but in their formation.

The *helmet* cell also has distinctive projections, usually two, surrounding an empty area of the red cell membrane. Helmet cells are seen in hematological conditions in which large inclusion bodies are formed (Heinz bodies, Howell–Jolly bodies). Fragmentation occurs by the pitting mechanism of the spleen. This pitting mechanism removes the inclusion from the cell, giving the appearance of having taken a "bite out of the cell" and is sometimes referred to as a *bite* cell (Fig. 5–23).

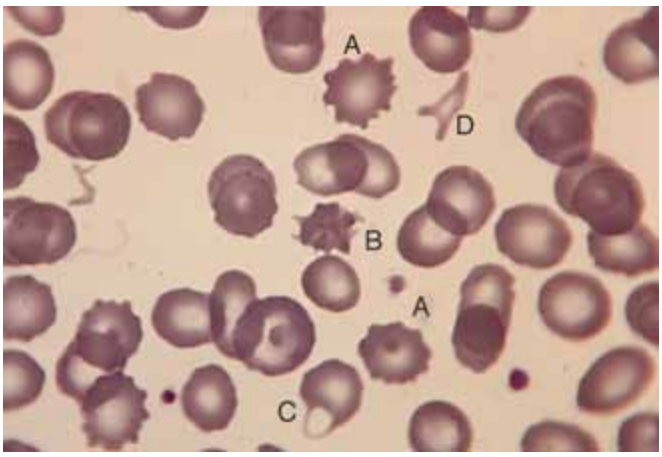

Figure 5-22 ■ Peripheral blood from a patient with renal disease. Note the presence of fragmented cells: *A.* burr cells; *B.* acanthocyte; *C.* blister/pocketbook cells; *D.* schistocyte.

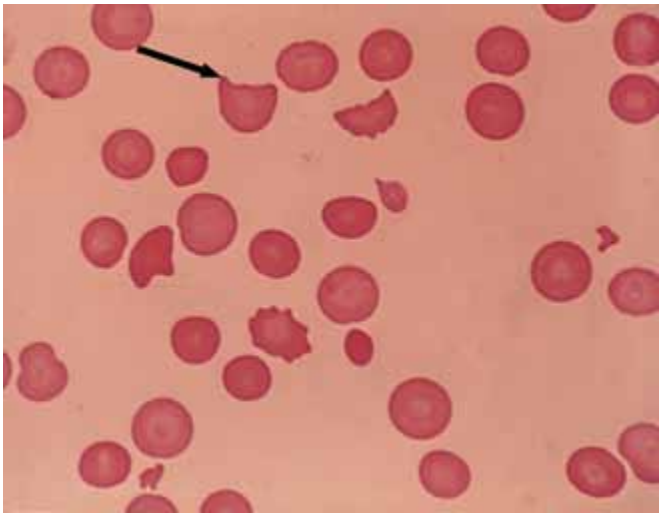

Figure 5-23 ■ Note the bite cell at the arrow.

A helmet cell and a bite cell are, therefore, one and the same. The helmet cells may also be seen in patients with pulmonary emboli, myeloid metaplasia and DIC. All fragmented red cells are considered fragile and their survival time is diminished significantly to days, if not hours, owing to splenic sequestration.

Please note that all laboratories may not report fragmented red cells in the same manner (i.e., all fragmented red cells reported as schistocytes) owing to the similarities in their origins. Regardless of the specificity of the terminology used, it is imperative that the morphologists give a qualitative estimate of the abnormality seen in all fields. Especially in significant numbers, the appearance of fragmented red cells will provide physicians with important information on the condition of their patients.

Refer to Figure 5–24 for a flowchart correlation of the fragmented cells matched to the pathologic processes in which they may be observed.

Burr Cells (Echinocytes)

Burr cells (echinocytes) are red cells with approximately 10 to 30 rounded spicules evenly placed over the surface of the red cells (see Fig. 5–22). They are normochromic and normocytic, for the most part. They may be observed as an artifact, usually as a result of specimen contamination, in which case they will appear in large numbers and will present with evenly dispersed smooth projections and may be referred to as crenated. The terms crenated cell and echinocyte may be used interchangeably by some reviewers and therefore are not reported. "True" burr cells occur in small numbers and appear irregularly sized with unevenly spaced spicules. They may be seen in uremia, heart disease, cancer of the stomach, bleeding peptic ulcer, immediately following an injection of heparin, and in a number of patients with untreated hypothyroidism. In general, they may occur in situations that cause a change in tonicity of the intravascular fluid (e.g., dehydration and azotemia). Burr cells may be considered pathologic and should be reported.

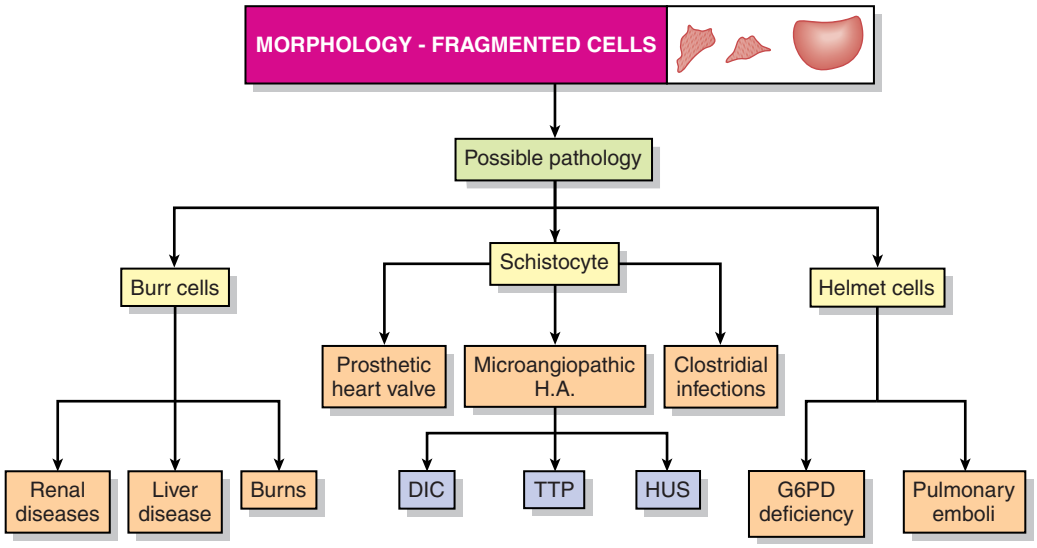

Figure 5-24 ■ Correlation of fragmented cells to pathologic processes. HA = hemolytic anemia; DIC = disseminated intravascular coagulation; HUS = hemolytic uremic syndrome; TTP = thrombotic thrombocytopenic purpura.

Acanthocytes (Thorn Cells, Spur Cells)

An acanthocyte is defined as a cell of normal or slightly reduced size, possessing 3 to 12 spicules of uneven length distributed along the periphery of the cell membrane. The uneven projections of the acanthocyte are blunt rather than pointed, and the acanthocyte can easily be distinguished from the peripheral smear background because it appears to be saturated with hemoglobin. It appears essentially as a spherocyte with thorns. The MCHC is, however, always in the normal range (Fig. 5–25).

Specific mechanisms relating to the formation of acanthocytes are unknown; however, some details about these peculiar cells are of interest. Acanthocytes contain an excess of cholesterol and have an increased cholesterol-to-phospholipid ratio; consequently their surface area is increased. The lecithin content of acanthocytes is decreased. The only inherited condition in which acanthocytes are seen in high numbers is the rare condition *abetalipoproteinemia*. Most cases of acanthocytosis are acquired, such as the deficiency of lecithin-cholesterol acyltransferase, which has been well documented in patients with severe hepatic disease. This enzyme is synthesized by the liver and is directly responsible for esterifying free cholesterol; when this enzyme is deficient, cholesterol is increased in the plasma. Acanthocytes may also be seen in myeloproliferative disorders, microangiopathic hemolytic anemia (MAHA), and autoimmune hemolytic anemias. The presence of acanthocytosis in peripheral blood smears remains the hallmark of the clinical diagnosis of most neuroacanthocytosis syndromes, such as chorea-acanthocytosis (ChAc) and McLeod syndrome.[14]

The red cell responds to this excess cholesterol in one of two ways, depending on the balance of other lipids in the membrane. It will become a target cell or an acanthocyte. Once an acanthocyte is formed, it is very liable to splenic sequestration and fragmentation, and the fluidity of the membrane is directly affected. The most prominent pathologies in which acanthocytes may be observed are listed in Figure 5–26.

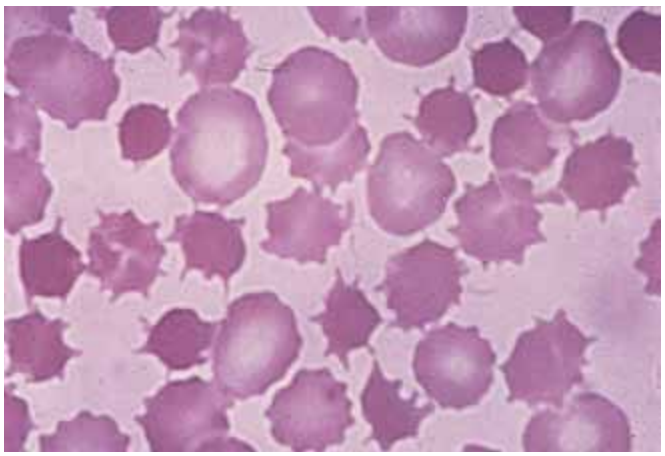

Figure 5-25 ■ Note the acanthocytes on this peripheral smear.

Teardrop Cells (Dacrocytes)

Teardrop cells appear in the peripheral circulation as tear-shaped or pear-shaped red cells (Fig. 5–27). The extent to which a portion of the red cells form tails is variable, and these cells may be normal, reduced, or increased in size. The exact physiologic mechanism is unknown, yet teardrop formation from inclusion-containing red cells is well documented. As cells containing large inclusions attempt to pass through the microcirculation, the portion of the cells containing the inclusion cannot pass through and consequently gets pinched, leaving a tailed end. For some reason, the red cell is unable to maintain the discocyte shape once this has occurred.

Teardrop cells are seen most prominently in idiopathic myelofibrosis with myeloid metaplasia. This type of morphological finding can also be seen in patients with the thalassemia syndromes, in drug-induced Heinz body formation, in iron

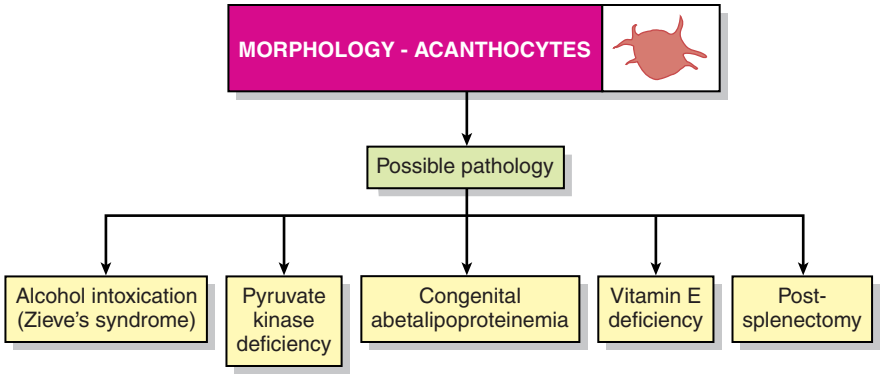

Figure 5-26 ■ Correlation of acanthocytes to pathologic processes.

deficiency, and in conditions in which inclusion bodies are formed. They may also be seen in megaloblastic processes as large tear-shaped cells (macroteardrops). Refer to Figure 5–3 for a composite of abnormal red cell morphology.

Red Cell Inclusions

Howell–Jolly Bodies

Howell–Jolly bodies (Fig. 5–28) are nuclear remnants containing DNA. They are 1 to 2 μm in size and may appear singly or doubly in an eccentric position on the periphery of the cell membrane. They are thought to develop in periods of accelerated or abnormal erythropoiesis. They may be seen in Romanowsky, i.e., Wright's, Giemsa, or supravitally stained peripheral smears.

A fragment of the chromosome becomes detached and is left floating in the cytoplasm after the nucleus has been extruded. Under ordinary circumstances, the spleen effectively pits these nondeformable bodies from the cell. However, during periods of erythroid stress, the pitting mechanism cannot keep pace with inclusion formation.

Howell–Jolly bodies may be seen after surgical splenectomy, congenital absence of the spleen, or splenic atrophy after multiple infarctions. They may also be seen in patients with thalassemic syndromes, sickle cell anemia as well as other hemolytic anemias, and in megaloblastic anemias.

Basophilic Stippling

Red cells that contain ribosomes can potentially form stippled cells; however, it is thought that the actual stippling is the result of the drying of cells in preparation for microscopic examination. Coarse, diffuse, or punctate basophilic stippling may occur and consist of ribonucleoprotein and mitochondrial remnants (Fig. 5–29). These aggregates of ribosomes result from an alteration in the biosynthesis of hemoglobin.

Diffuse basophilic stippling appears as a fine blue dusting, whereas coarse stippling is much more clearly outlined and easily distinguished. Punctate basophilic stippling is a coalescing of smaller forms and is very prominent and easily identifiable.

Stippling may be found in any condition showing defective or accelerated heme synthesis, such as alcoholism, thalassemia syndromes, megaloblastic anemias, and arsenic intoxication. It is also considered a characteristic feature in the diagnosis of lead poisoning. Basophilic stippling may be seen on a Romanowsky or supravitally stained peripheral smear. It is important for the reviewer not to confuse stippling with Pappenheimer bodies. The primary differentiation factors are that stippling appears

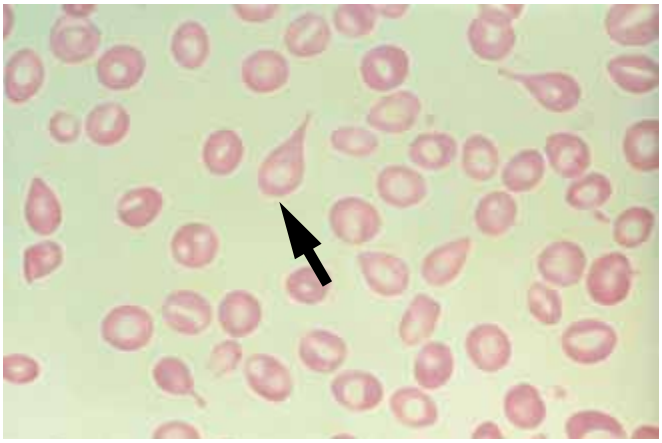

Figure 5-27 ■ Teardrop cells (peripheral blood).

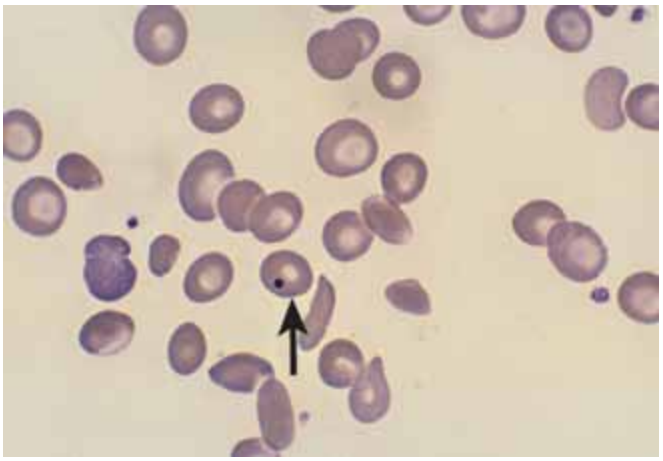

Figure 5-28 ■ Howell–Jolly body.

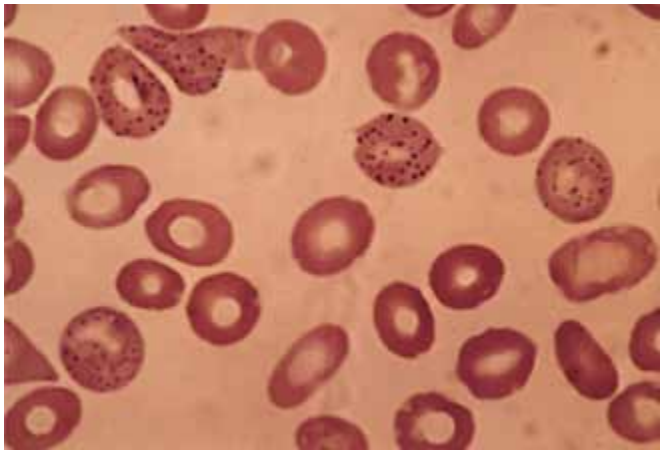

Figure 5-29 ■ Note the cells with red cell inclusions: basophilic stippling seen on a peripheral smear in a patient with lead poisoning.

homogeneously over the cell, whereas Pappenheimers tend to appear as clusters in the cells periphery.

Pappenheimer Bodies and Siderotic Granules

Pappenheimer bodies (*siderotic granules*) are small, irregular magenta inclusions seen along the periphery of red cells. They usually appear in clusters, as if they have been gently placed on the red cell membrane. Their presence on a Wright's or a supravital stained peripheral smear is presumptive evidence for the presence of iron. However, the Prussian blue stain is the confirmatory test for determining the presence of these inclusions. These bodies/granules in RBCs are nonheme iron, resulting from an excess of available iron throughout the body. Even though Pappenheimers and siderotic granules are the same inclusion, they are designated differently depending on the stain used. The inclusions are termed *Pappenheimer bodies* when seen in a Wright-stained smear (Fig. 5–30) and *siderotic granules* when seen in Prussian blue or other kinds of iron stain. The explanation for the difference in terminology is that Romanowsky stains visualize Pappenheimer bodies by staining the protein matrix of the granule, whereas Prussian blue stain is responsible for staining the iron portion of the granule.

Once the presence of siderotic granules has been confirmed by iron stains, the cells in which they are found are termed siderocytes. Siderocytes containing a nucleus are described as sideroblasts and are commonly seen in sideroblastic anemias. Sideroblasts exhibiting numerous siderotic granules found within the mitochondria forming a ring around at least one-third of the nucleus, are labeled as pathologic ringed sideroblasts. Siderocytes are seen in any condition in which there is iron overloading such as hemochromatosis or hemosiderosis. They may also be seen in the hemoglobinopathies (e.g., sickle cell anemia and thalassemia) and in patients following splenectomy.

Heinz Bodies

Heinz bodies are formed as a result of denatured or precipitated hemoglobin. They are large (0.3 to 2 μm) inclusions that are rigid and severely distort the cell membrane. They can be formed for visualization in vitro by incubation with phenylhydrazine (a strong oxidizing agent). On initial exposure to phenylhydrazine, small crystalline bodies appear, coalesce, and migrate to an area beneath the cell membrane. This procedure is used before staining with crystal violet or brilliant cresyl blue where the presence of Heinz bodies may be seen on the peripheral smear. Heinz bodies cannot be visualized with Romanowsky stains (Fig. 5–31).

Heinz bodies may be seen in the α-thalassemic syndromes, glucose-6-phosphate dehydrogenase (G6PD) deficiency under oxidant stress, and in any of the unstable hemoglobin syndromes (i.e., hemoglobin Köln, hemoglobin Zurich). They may also be seen in red cell injury resulting from chemical insult.

Cabot Rings

The exact physiologic mechanism in Cabot ring formation has yet to be elucidated. This structure may represent a part of

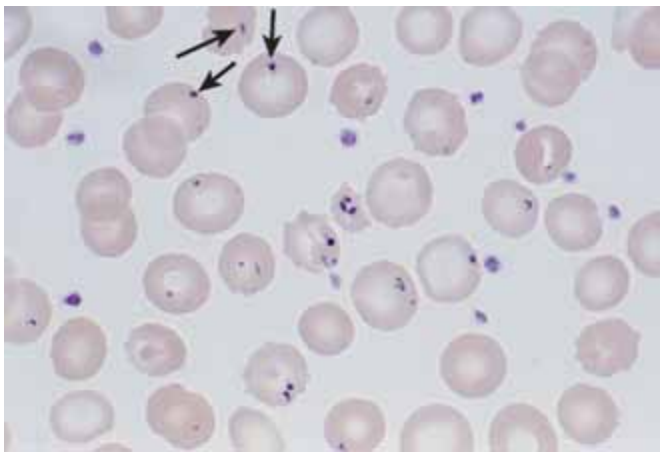

Figure 5-30 ■ Pappenheimer bodies (Wright stain).

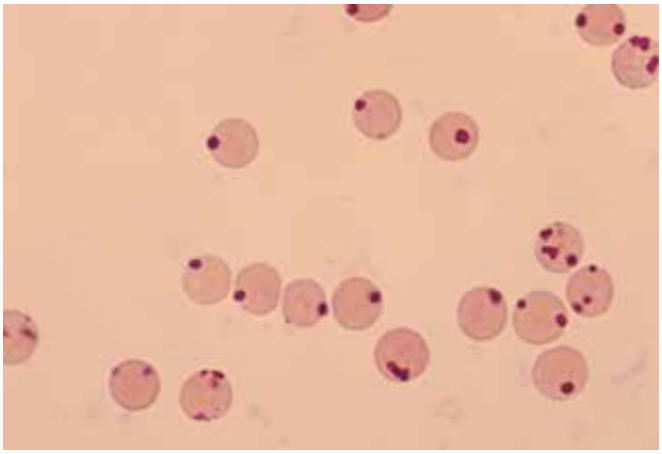

Figure 5-31 ■ Heinz body prep; note the appearance of Heinz body inclusions.

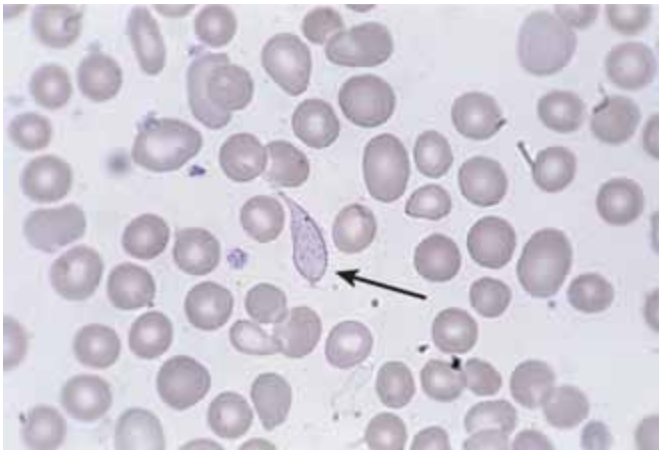

Figure 5-32 ■ Note the appearance of a Cabot's ring in the cell at the arrow.

the mitotic spindle, remnants of microtubules, or a fragment of the nuclear membrane. Cabot rings are found in heavily stippled cells and appear in a figure-of-eight conformation similar to the beads of a necklace (Fig. 5–32). Cabot rings may be found in megaloblastic anemias, dyserythropoiesis, homozygous thalassemia syndromes, and postsplenectomy.

Table 5–5 summarizes abnormal red cell morphologies and associated disease states and RBC inclusions.

Hemoglobin CC Crystals

Hemoglobin (Hb) C crystals may be found in hemoglobin CC disease. HbC disease is a mild chronic hemolytic anemia in which the patient is homozygous for the abnormal hemoglobin C.[15] HbCC crystals are formed by the crystallization of the abnormal hemoglobin into one end of the red cell membrane. The crystal forms in a hexagonal shape with blunt ends, leaving the remainder of the cell with the appearance of being empty. These crystals tend to stain dark red and are said to resemble a "bar of gold" and may be referred to as such (Fig. 5–33).

HbCC crystals may not always be demonstrated in HbC disease, but their appearance has been found to increase after splenectomy. HbCC crystals are not seen in HbC trait (HbAC).

Hemoglobin SC Crystals

Hemoglobin SC (HbSC) crystals may be found on the peripheral smears of patients diagnosed with HbSC disease. SC disease is a chronic hemolytic disorder punctuated by acute

Table 5–5 Summary of Abnormal Red Cell Morphologies and Disease States That May Be Associated with These Abnormal Morphologies

Microcytes

- Iron-deficiency anemia
- Thalassemias
- Lead poisoning
- Sideroblastic anemia

Macrocytes

- Megaloblastic anemias
- High reticulocyte count
- Liver disease
- Myelodysplatic syndromes

Target Cells

- Liver disease
- Hemoglobinopathies
- Thalassemias
- Sideroblastic anemia

Spherocytes

- Hemolytic anemias
- Posttransfusion
- Hereditary spherocytosis

Elliptocytes

- Hereditary elliptocytosis
- Iron-deficiency anemia
- Thalassemias

Stomatocyte

- Acute alcoholism
- Malignancies

Sickle Cells

- Sickle cell anemia
- Sickle thalassemia

Acanthocytes

- Congenital abetalipoproteinemia
- Vitamin E deficiency
- Alcohol intoxication
- Postsplenectomy

Burr Cells

- Liver disease
- Renal disease
- Severe burns
- Bleeding gastric ulcers

Helmet Cells

- G6PD deficiency
- Pulmonary emboli

Schistocytes

- Disseminated intravascular coagulopathy (DIC)
- Thrombotic thrombocytopenic purpura (TTP)
- Hemolytic uremic syndrome

Teardrop Cells

- Severe anemias
- Myeloproliferative disorders
- Pernicious anemia

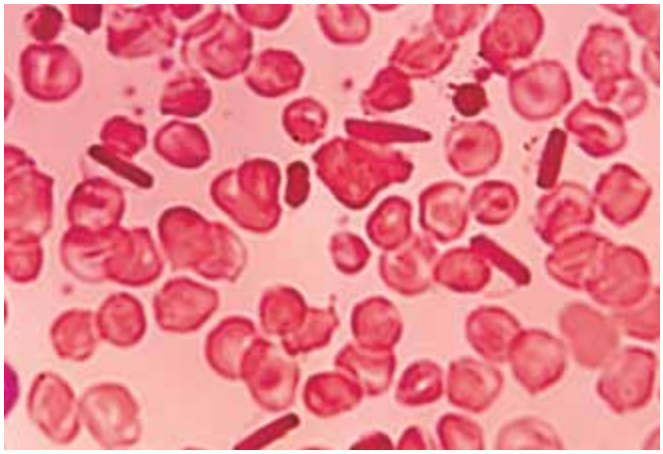

Figure 5-33 ■ Note the hexagonal shaped crystal inclusions in a peripheral smear from a patient with HbC disease. These HbC crystals leave the remainder of the cellular cytoplasm to appear as "empty."

painful crisis and diverse chronic organ damage, secondary to the presence of both HbS and HbC.[15] The pathophysiology of the disease is exacerbated by the presence of both hemoglobins, as they tend to exhibit traits that are common to each such as sickling from HbS and crystallization from HbC. The result of this combination is the formation of crystals with fingerlike blunt-pointed projections protruding from the cell membrane. The projections have been said to resemble the Washington Monument and consequently SC crystals may be referred to as "Washington Monument" crystals (Fig. 5–34).

Protozoan Inclusions

Two organisms are briefly discussed in this section because of their tendency to invade the red cells and the fact that their appearance on a peripheral blood smear is confirmation of infection by the organism. Although only an experienced reviewer would be expected to differentiate these organisms, it is important that all slide reviewers have knowledge of these organisms in order to recognize their appearance as an abnormality needing further review or testing.

All four species of the malaria parasite will invade RBCs. The species include *Plasmodium vivax, Plasmodium malaria, Plasmodium falciparum,* and *Plasmodium ovale* and are transmitted by the *Anopheles* mosquito. The parasite may appear in different forms (i.e., ring, troph) and although it is important to the physician for a differential diagnosis and treatment, all reviewers are not expected to be proficient in identification of the specific form. The primary concern is the recognition of the abnormality as a parasite and that it is not confused with normal morphology such as platelets superimposed over red cells.

Babesia microti is also an organism that invades red cells. It is transmitted by tick bites and may appear as ring forms resembling some forms of malaria. The distinguishing feature of *Babesia* is that it also invades blood circulation and on blood smears may appear in groups outside the erythrocyte. Patient symptoms and travel history are also useful in differentiating the two organisms (Fig. 5–35).

Examination of Platelet Morphology

The normal platelet has several distinctive morphological characteristics. This structure measures approximately 2 to 4 µm, with a discoid shape and even blue granules dispersed throughout a light-blue cytoplasm (Fig. 5–36). In pathologic states, platelets may appear as blue or gray agranular discs; they may be extremely large and may show tailing or streaming of the cytoplasm. In rare instances, one may see megakaryocytic fragments in the peripheral circulation.

A close and thorough examination of platelet morphology provides important information about the patient's hemostatic capability. Gross variation in platelet morphology may be seen in infiltrative disease of the bone marrow (e.g., idiopathic myelofibrosis or metastatic infiltrates). Large platelets may be seen in any disorder associated with increased platelet turnover, such as may occur with idiopathic thrombocytopenic purpura

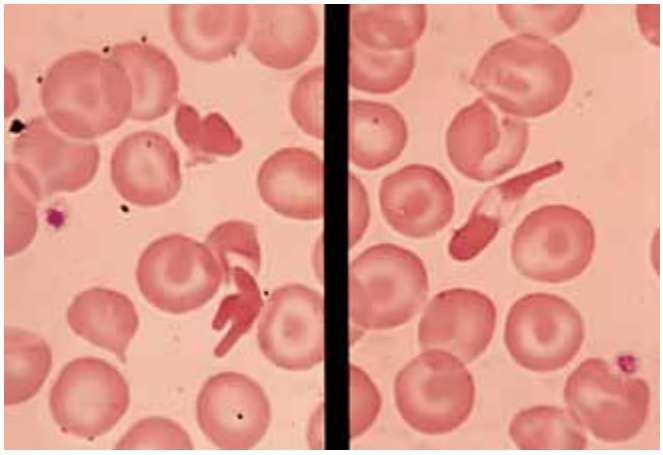

Figure 5-34 ■ Note the "fingerlike projections" in this peripheral smear from a patient with HbSC disease. These HbSC crystals are said to resemble the Washington Monument.

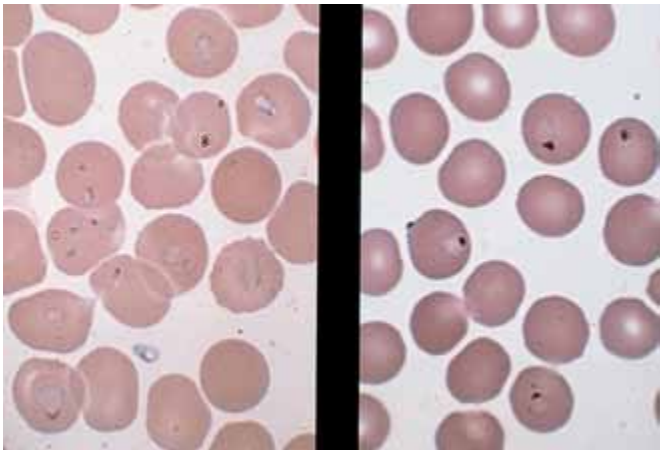

Figure 5-35 ■ Comparison of babesiosis (*left*) and malarial forms (*right*).

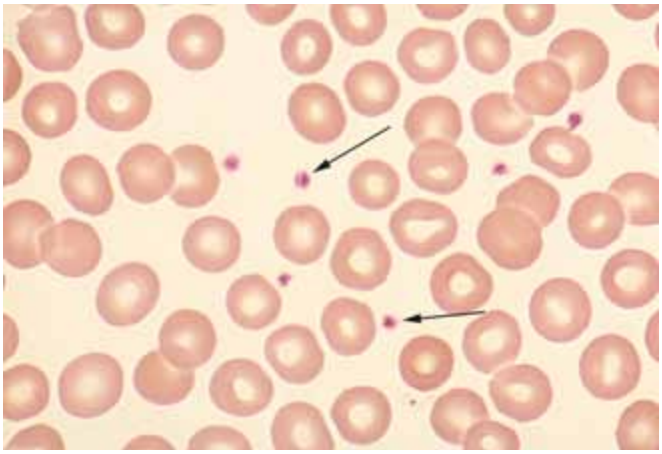

Figure 5-36 ■ Normal platelet at arrow.

or bleeding disorders. In addition to the elevated platelet count, morphological changes may also occur postsplenectomy.

White Blood Cell Morphology

Evaluation of white blood cells (WBCs, leukocytes) is performed primarily in response to abnormalities identified by the automated blood cell counter. As with the evaluation of red cell morphology, the technologist must be proficient in identification of normal WBC morphology in order to adequately identify morphologic abnormalities. Figure 5–37 includes a normal neutrophil as well as a normal lymphocyte. Slide reviewers must be able to recognize the appearance of normal cells, their general size, their shape, and their overall appearance. They must also distinguish between normal granularity and the presence of abnormal inclusions. If immature cells are present, a skilled reviewer should be able to identify the cell line and the stage of maturation.

The presence of immature cells is a significant finding, with the greater the immaturity, typically, the more severe the diagnosis. The presence of more immature forms such as promyelocytes or myeloblasts, in the absences of severe infection, strongly suggests direct marrow architectural involvement. This may indicate an infiltrative, neoplastic, or myeloproliferative process. Regardless of the reason for the appearance of the immature cells, the reviewer should be able to identify the cells accurately. Proper cellular identification at this point may be critical to the patient's diagnosis and subsequent prognosis.

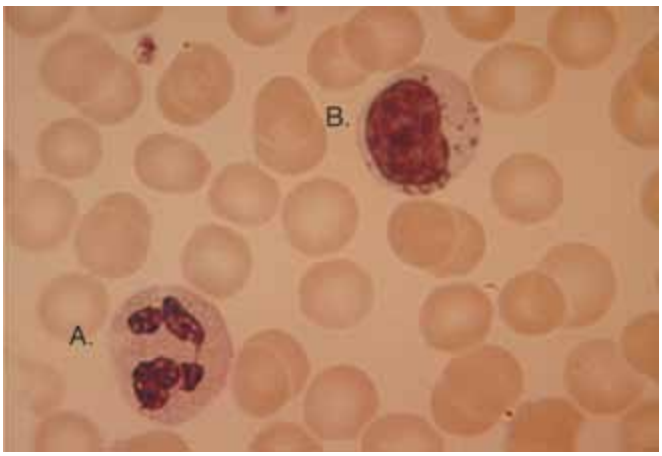

Figure 5-37 ■ *A,* Normal neutrophil, *B,* normal lymphocyte.

Mature white blood cells may exhibit several morphological changes. In the performance of a slide review/WBC differential the reviewer should take note of the appearance of the nucleus of the white cells as well as the cytoplasm. Neutrophils tend to exhibit a wider variety of morphologic changes than the other cell types with these changes originating in the cytoplasm in response to various pathologic processes. The specific alteration may involve the appearance or lack of cytoplasmic inclusions. Severe infections, inflammatory conditions, or other leukemoid reactions may be accompanied by toxic granulation, toxic vacuolization, or the presence of Dohle bodies (see Chap. 15). Toxic granulation and Dohle bodies are generally considered nonspecific reactive changes, whereas vacuolization strongly indicates a serious bacterial infection. This must also be noted with its importance determined by the physician based on the patient's condition.

Toxic granulation describes medium to large granules that are evenly scattered throughout the cytoplasm of segmented polymorphonuclear neutrophil leukocytes. These granules are seen in metabolically active neutrophilia and are composed of peroxidases and acid hydrolases. Although nonspecific, they may occur in patients with severe bacterial infections, toxemia of pregnancy, or vasculitis, or in patients receiving chemotherapy. Toxic vacuolization refers to the round, clear unstained areas that are dispersed randomly throughout the cytoplasm of neutrophils in patients with overwhelming infections. Additional cytoplasmic inclusions—Dohle bodies—are oval, blue, single or multiple inclusions originating in RNA, and are 1 to 3 μm in diameter. Dohle bodies may be seen in peripheral blood smears of patients with severe infections, in patients with severe burns, in pregnant women, and in patients receiving chemotoxic drugs. In these conditions, they represent toxic changes; however, Dohle body-like inclusions are also characteristically observed in certain congenital qualitative WBC disorders such as the May–Hegglin anomaly and Chédiak–Higashi disorder (see Chap. 15).

In addition to these morphological changes, severe bacterial infections are also commonly associated with a moderate leukocytosis and a shift to the left in granulocytes. Mild infections are characterized by a slight leukocytosis, with or without the shift to the left. "Shift to the left" implies a release of younger granulocytes—specifically bands and metamyelocytes—from the bone marrow storage pool. These particular cell populations may often be observed during an infection or inflammatory process. The degree of leukocytosis or neutrophilia is useful in discriminating among bacterial, viral, or fungal conditions. Leukocytosis commonly refers to an increase in peripheral blood leukocyte (WBC) concentration of greater than 10,000 cells/μL. Because acute infection can rapidly mobilize the neutrophilic nondividing marrow storage pool, the patient usually has a WBC count below 50,000 cells/μL (average is 25,000 cells/μL). A shift

to the left is seen in the peripheral blood smear; however, it is unusual to see cells as immature as myelocytes in the peripheral blood. Fungal infections may also be associated with neutrophilia and an increased WBC count, but a monocytosis is more commonly observed. Viral infections usually are not associated with neutrophilia but rather with lymphocytosis (see Chap. 15).

Leukemoid reactions are characterized by a peripheral neutrophilia that may resemble a chronic leukemia. The WBC count is between 50,000 and 100,000 cells/μL, with immaturity observed in one or more cell types. However, a high blast count is not part of the WBC differential picture, which can be helpful in eliminating leukemia as part of the differential diagnosis. Acute infections, chronic infections such as tuberculosis and chronic osteomyelitis, as well as severe metabolic inflammatory and neoplastic processes have all been associated with leukemoid reactions. Extremely elevated WBC counts (greater than 100,000 cells/μL) are more suggestive of a myeloproliferative process (see Chaps. 17 and 18), although exceptions have been reported.

The reviewer should also take note of the appearance of the nucleus, and in particular the number of segmentations. The appearance of increased segmentation (normal is three to five lobes) may indicate a megaloblastic process. This is referred to as *hypersegmentation* and is reported when neutrophils contain greater than five lobes or when significant numbers of neutrophils all contain at least five lobes or more. The decreased segmentation should also be noted, as it may be an indication of a benign hereditary condition known as Pelger–Huet anomaly or may actually be the result of a leukemic process. This decreased segmentation consists of neutrophils with two lobes or less and is described by the term *hyposegmentation*.

Physiologic leukocytosis is defined as an increased WBC count without a shift to the left or any associated morphological changes previously described for granulocytes. This transient condition may be associated with such stimuli as exercise, intense emotional stress, anesthesia, or the administration of epinephrine or glucocorticoids.

Case Study 1

A 1-year-old African American child is brought to the emergency department by her mother because the child had no appetite and had not eaten in the last 2 days. Additional information from the mother described a normally happy child who had recently become restless and irritable. She was learning to walk, but now would not even attempt standing. She has had a low-grade temp which is now elevated to 102° F. On examination there is a definite yellow tinge to the sclerae and the spleen is palpable. Also noted was a spindle-shaped deformity of two fingers on the right hand that were swollen and obviously painful to the touch. The CBC results were as follows:

continued

CBC Results	Patient Results	Reference Range
WBC	17.0×10^9/L	$6.0–17.5 \times 10^9$/L
RBC	2.4×10^{12}/L	$3.8–5.5 \times 10^{12}$/L
Hgb	7.5 g/dL	12–14.5 g/dL
Hct	22.0%	30%–43%
MCV	92 fL	80–100 fL
RDW	18%	11.5%–14.5%

WBC Differential		RBC Morphology	
40%	Granulocytes	Anisocytosis	3+
58%	Lymphocytes	Poikilocytosis	3+
2%	Monocytes	Hypochromia	1+
		Macrocytosis	1+
		Microcytosis	1+
		Schistocytes	2+
		Sickled cells	1+
		Targets	1+
		Polychromasia	1+

Additional Tests Ordered	
Reticulocyte count	13%
Sickledex	Positive
Hgb electrophoresis	Hgb SS pattern

DISCUSSION

Sickle cell disease is a hereditary disease that results in a chronic moderate to severe hemolytic anemia. The disease is characterized by the substitution of valine from the normal glutamic acid at the sixth position in the β-chain, resulting in an abnormal hemoglobin that polymerizes when exposed to low oxygen-tension conditions. This polymerization results in the formation of sickle-shaped cells that are capable of temporarily or permanently blocking microcirculation, and the resulting stasis may lead to hypoxia and ischemic infarcts of various organs.

The disease is not evident at birth and does not manifest itself until the gamma chains of the newborn are replaced by β^s-chains after 3 to 6 months of life.[15] Clinical manifestations may be divided into acute and chronic episodes. Acute problems result from a vaso-occlusive crisis termed "sickle cell crisis." This "crisis" typically includes an acute hemolytic episode as well. Patients in sickle cell crisis present with acute pain, fatigue, and possibly jaundice. All three of these symptoms were present in our case study, as was evidence of some form of anemic process.

Chronic manifestations of sickle cell disease usually appear after mid-childhood.[16] These include disturbances in growth and development, bone and joint disease, and organ damage involving mostly all of the organ systems of the body at some point during the process of the disease.

QUESTIONS

1. Are the morphological findings on the blood film compatible to the results from the analyzer?
2. Did the other tests ordered confirm a probable diagnosis?
3. Is the reticulocytes count useful?

continued

ANSWERS

1. (Note: manual differential and morphology review performed due to flagging of analyzer results, Fig. 5–38.) Morphology results correlate with the values from the instrument, for example, the RDW is elevated and this is reflected on the smear as 3+ anisocytosis. Many more abnormalities are detected by reviewing the peripheral smear. These abnormalities are typically seen in patients in crisis as was this child.

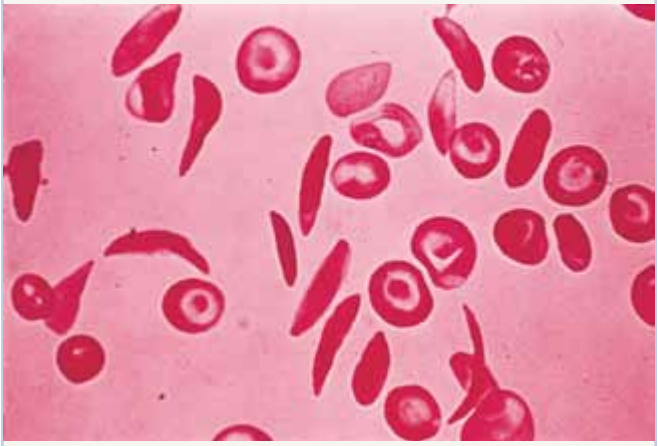

Figure 5-38 ■ Case study; note abnormal red cell morphology.

continued

2. Yes. The sickledex is a screening test performed on whole blood and is positive in both sickle cell disease and sickle cell trait. The confirmatory test is the hemoglobin electrophoresis, which will differentiate abnormal hemoglobin traits from abnormal hemoglobin disease. In this case the presence of the SS pattern is diagnostic of Hgb S disease which will result in sickle cell anemia.
3. Yes. The reticulocytes count is a valid indicator of bone marrow performance. In cases of sickle cell crisis, in which the nonfunctional sickle cells are being destroyed, a properly functioning marrow should accelerate the hematopoietic process. The 13% reticulocytes count is indicative of a proper response by the bone marrow to the hemolytic process the patient was experiencing.

Questions

1. A prominent morphologic clue when suspecting lead poisoning is the presence of which of the following on a peripheral smear?
   a. Heinz bodies
   b. Target cells
   c. Siderotic granules
   d. Basophilic stippling
2. In which of the following disease states would you expect to find oval macrocytes on the peripheral smear?
   a. Iron deficiency anemia
   b. Lead poisoning
   c. Megaloblastic anemia
   d. Hereditary spherocytosis
3. All but one of the following are possible mechanisms for the production of macrocytes:
   a. Liver disease
   b. Postsplenectomy status
   c. Pernicious anemia
   d. Thalassemia minor
4. An abnormal erythrocyte seen in liver disease and hemoglobinopathies and thalassemias and is characterized by the "bull's eye" area is known as a:
   a. Stomatocyte
   b. Target cell
   c. Schistocyte
   d. Hypochromic cell
5. Morphological abnormalities found in cases of severe burns, microangiopathic hemolytic anemias, and disseminated intravascular coagulation (DIC) are:
   a. Schistocytes
   b. Crenated cells
   c. Ovalocytes
   d. Stomatocytes
6. Oat-shaped cells may be associated with:
   a. Myelofibrosis
   b. Hereditary spherocytosis
   c. Burns
   d. Sickle cell anemia
7. How would a cell be classified that has a diameter of 9 μm and an MCV of 104 fL?
   a. Macrocytic
   b. Microcytic
   c. Normal
   d. Either normal or slightly microcytic
8. Abnormal platelet morphology may be observed most prominently in:
   a. Idiopathic myelofibrosis
   b. Anemia of chronic disorders
   c. Hereditary spherocytosis
   d. Septic shock

9. Which type of red cell inclusion is a DNA remnant?
   a. Heinz body
   b. Howell–Jolly body
   c. Pappenheimer body
   d. Cabot ring
10. Which of the following are considered microcytic/hypochromic anemias?
    a. Autoimmune hemolytic anemia
    b. Pernicious anemia
    c. Iron deficiency anemia
    d. Megaloblastic anemia
11. A hypersegmented neutrophil may be seen in which of the following anemias?
    a. Iron deficiency
    b. Megaloblastic
    c. Autoimmune hemolytic anemia
    d. Anemia of chronic disorders
12. Precipitates of denatured hemoglobin found primarily in patients with hemolytic anemia resulting from oxidant stress describe:
    a. Howell–Jolly bodies
    b. Heinz bodies
    c. Basophilic stippling
    d. Pappenheimer bodies
13. Pappenheimer inclusions are formed from:
    a. Excess α-chains
    b. Excess β-chains
    c. Excess iron
    d. Oxidant stress
14. RBC inclusions resulting from an acceleration in hemoglobin biosynthesis and consists of RNA:
    a. Howell–Jolly bodies
    b. Heinz bodies
    c. Basophilic stippling
    d. Pappenheimer bodies

The following answer pool is used for items 15 to 29 (Match)

a. Anisocytosis	f. Polychromasia	k. Schistocytes
b. Poikilocytosis	g. Microspherocytes	l. Rouleaux
c. Hypochromasia	h. Target cells	m. Acanthocytes
d. Microcytic	i. Stomatocytes	n. Dacrocytes
e. Depranocyte	j. Blister cells	o. Echinocytes

_____15. RBCs with a large area of central pallor
_____16. Variation in the size of the red blood cells
_____17. Also known as codacytes
_____18. RBCs appearing stacked on each other
_____19. MCV of 65%
_____20. RBCs with a mouthlike central pallor
_____21. RBCs without an area of central pallor
_____22. RBCs with evenly distributed spicules on the membrane
_____23. RBC fragments
_____24. RBCs appearing bluish in color
_____25. Variation in the shape of the RBCs
_____26. Congenital abetalipoproteinemia
_____27. The formation of a vacuole in an RBC "trapped" by fibrin
_____28. Formed when an RBC with an inclusion squeezes out of a tight space
_____29. Seen in HbS disease

See answers at the back of this book.

SUMMARY CHART

- A variation in cell size is termed *anisocytosis*.
- A variation in cell shape is termed *poikilocytosis*.
- On an automated cell counter, a flag is a signal that a significant abnormality may be present in the sample.
- Microcytes are associated with an MCV of less than 80 fL and are seen in IDA, thalassemias, sideroblastic anemias, and anemia of chronic disease.
- Macrocytes are associated with an MCV of more than 100 fL and are seen in megaloblastic and nonmegaloblastic processes.
- Macrocytes are associated with high reticulocyte counts and liver disease; oval macrocytes are associated with megaloblastic processes.
- Red cells that appear polychromatophilic on a Wright's stained smear would appear as reticulocytes on a supravital stain.
- Spherocytes are associated with hereditary spherocytosis and an MCHC that is greater than 36% in many cases.
- Spherocytes may also be seen in autoimmune hemolytic anemia (AIHA) and posttransfusion.
- There are two types of sickle cells, irreversible sickle cells (ISCs) and oat-shaped, reversible sickle cells that are both commonly seen in HbS disease.

(continued)

SUMMARY CHART – cont'd

- Any regenerative red cell process will result in inclusions such as Howell–Jolly bodies, basophilic stippling, and Pappenheimer bodies.
- Howell–Jolly bodies, Pappenheimer bodies, and basophilic stippling may be seen in peripheral smears stained with both Romanowsky type stain (i.e., Wright's, May–Grumwald) and supravital stain (i.e., new methylene blue, brilliant cresyl blue)
- Siderotic granules and Pappenheimer bodies are basically the same inclusion. The differentiating factor is that on an iron stain the inclusions are known as siderotic granules where on Wright's stain they are known as Pappenheimer bodies.
- Howell–Jolly bodies are seen in patients postsplenectomy.
- Helmet cells are seen in patients with G6PD syndrome and occur as a result of Heinz body formation.
- Heinz bodies cannot be seen on a Wright-stained peripheral smear.
- Abnormal platelet morphology includes lack of granulation, giant platelets, and megakaryocytic fragments.
- Abnormal white cell morphology includes the presence or absence of cytoplasmic granulation, or presence of cytoplasmic vacuolizaton, as well the appearance of the cellular nucleus.
- Hyposegmentation describes decreased neutrophil segmentation (≤2 lobes).
- Hypersegmentation describes increased neutrophil segmentation (≥5 lobes).

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