Although the lungs provide the means for gas exchange between the external and internal environments, it is the hemoglobin in the red blood cells that transports oxygen to the tissues. The red blood cells also function as carriers of carbon dioxide and participate in acid-base balance. The function of the red blood cells, in terms of oxygen transport, is discussed in Chapter 27, and acid-base balance in Chapter 32. This chapter focuses on the red blood cell, anemia, transfusion therapy, polycythemia, and age-related changes in the red blood cells.

The erythrocytes, 500 to 1000 times more numerous than other blood cells, are the most common type of blood cell. The mature red blood cell, the erythrocyte, is a non-nucleated, biconcave disk (Fig. 14-1). This unique shape contributes in two ways to the oxygen transport function of the erythrocyte. The biconcave shape provides a larger surface area for oxygen diffusion than would a spherical cell of the same volume, and the thinness of the cell membrane enables oxygen to diffuse rapidly between the exterior and the innermost regions of the cell (Fig. 14-2A).

Another structural feature that facilitates the transport function of the red blood cell is the flexibility of its membrane.

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**THE RED BLOOD CELL**

After completing this section of the chapter, you should be able to meet the following objectives:

- Trace the development of a red blood cell from erythroblast to erythrocyte.
- Discuss the function of iron in the formation of hemoglobin.
- Describe the formation, transport, and elimination of bilirubin.
- Explain the function of the enzyme glucose-6-phosphate dehydrogenase in the red blood cell.
- State the meaning of the red blood cell count, percentage of reticulocytes, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentration as it relates to the diagnosis of anemia.

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The erythrocytes, 500 to 1000 times more numerous than other blood cells, are the most common type of blood cell. The mature red blood cell, the erythrocyte, is a non-nucleated, biconcave disk (Fig. 14-1). This unique shape contributes in two ways to the oxygen transport function of the erythrocyte. The biconcave shape provides a larger surface area for oxygen diffusion than would a spherical cell of the same volume, and the thinness of the cell membrane enables oxygen to diffuse rapidly between the exterior and the innermost regions of the cell (Fig. 14-2A).

Another structural feature that facilitates the transport function of the red blood cell is the flexibility of its membrane.
FIGURE 14-1 • A highly magnified (x11,397) scanning electron micrograph of a number of red blood cells found enmeshed in a fibrinous matrix on the luminal surface of an indwelling vascular catheter. Note the biconcave shape of each erythrocyte, which increases the surface area of these hemoglobin-filled cells, thus promoting more effective gas exchange. (Centers for Disease Control and Prevention Public Images Library, Courtesy Janice Carr.)

The biconcave shape and flexibility of the red cell membrane are maintained by a complex network of fibrous proteins, especially one called spectrin (Fig. 14-3). Spectrin forms an attachment with another protein, called ankyrin, that resides on the inner surface of the membrane and is anchored to an integral protein that spans the membrane. This unique arrangement of proteins imparts elasticity and stability to the membrane and allows it to deform easily.

The function of the red blood cell, facilitated by the hemoglobin molecule, is to transport oxygen to the tissues. Because oxygen is poorly soluble in plasma, about 95% to 98% is carried bound to hemoglobin. The hemoglobin molecule is composed of two pairs of structurally different alpha (α) and beta (β) polypeptide chains (see Fig. 14-2B). Each of the four polypeptide chains consists of a globin (protein) portion and a heme unit, which surrounds an atom of iron that binds oxygen. Thus, each molecule of hemoglobin can carry four molecules of oxygen. Hemoglobin is a natural pigment; because of its iron content, it appears reddish when oxygen is attached and has a bluish cast when deoxygenated. The production of each type of globin chain is controlled by individual structural genes with five different gene loci. Mutations, which can occur anywhere in these five loci, have resulted in over 550 types of abnormal hemoglobin molecules.

### RED BLOOD CELLS

- The function of red blood cells, facilitated by the iron-containing hemoglobin molecule, is to transport oxygen from the lungs to the tissues.
- The production of red blood cells, which is regulated by erythropoietin, occurs in the bone marrow and requires iron, vitamin B₁₂, and folate.
- The red blood cell, which has a life span of approximately 120 days, is broken down in the spleen; the degradation products such as iron and amino acids are recycled.
- The heme molecule, which is released from the red blood cell during the degradation process, is converted to bilirubin and transported to the liver, where it is removed and rendered water soluble for elimination in the bile.

The two major types of normal hemoglobin are adult hemoglobin (HbA) and fetal hemoglobin (HbF). HbA consists of a pair of α chains and a pair of β chains. HbF is the predominant hemoglobin in the fetus from the third through the ninth months of gestation. It has a pair of gamma (γ) chains substituted for the α chains. Because of this chain substitution, HbF has a higher affinity for oxygen than adult hemoglobin. This affinity facilitates the transfer of oxygen across the placenta from the HbA in the mother's blood to the HbF in the fetus's blood. HbF is replaced within 6 months of birth with HbA.

![Plasma membrane](https://example.com/plasma.png)

![Hemoglobin](https://example.com/hemoglobin.png)

![Biconcave structure of the red blood cell](https://example.com/red-blood-cell.png)

![Hemoglobin molecule](https://example.com/hemoglobin-molecule.png)
Hemoglobin Synthesis

The rate at which hemoglobin is synthesized depends on the availability of iron for heme synthesis. A lack of iron results in relatively small amounts of hemoglobin in the red blood cells. The amount of iron in the body is approximately 2 g in women and up to 6 g in men. Body iron is found in several compartments. Most iron (approximately 80%) is complexed to heme in hemoglobin, with small amounts found in the myoglobin of muscle, the cytochromes, and iron-containing enzymes. The other 20% is stored in the bone marrow, liver, spleen, and other organs. Iron in the hemoglobin compartment is recycled. When red blood cells age and are destroyed in the spleen, the iron from their hemoglobin is released into the circulation and returned to the bone marrow for incorporation into new red blood cells or to the liver and other tissues for storage.

Dietary iron also helps to maintain body stores. Iron, principally derived from meat, is absorbed in the small intestine, especially the duodenum (Fig. 14-4). When body stores of iron are diminished or erythropoiesis is stimulated, absorption is increased. In iron overload, excretion of iron is accelerated. Normally, some iron is sequestered in the intestinal epithelial cells and is lost in the feces as these cells slough off. The iron that is absorbed enters the circulation, where it immediately combines with an β-globulin, apotransferrin, to form transferrin, which is then transported in the plasma. From the plasma, iron can be deposited in tissues such as the liver, where it is stored as ferritin, a protein–iron complex, which can easily return to the circulation. Serum ferritin levels, which can be measured in the laboratory, provide an index of body iron stores. Clinically, decreased ferritin levels usually indicate the need for prescription of iron supplements. Transferrin can also deliver iron to the developing red cell in bone marrow by binding to membrane receptors. This iron is taken up by the developing red cell, where it is used in heme synthesis.

Red Cell Production

Erythropoiesis refers to the production of red blood cells. After birth, red cells are produced in the red bone marrow. Until 5 years of age, almost all bones produce red cells to meet the growth needs of a child, after which bone marrow activity gradually declines. After 20 years of age, red cell production
takes place mainly in the membranous bones of the vertebrae, sternum, ribs, and pelvis. With this reduction in activity, the red bone marrow is replaced with fatty yellow bone marrow.

The red blood cells are derived from precursor cells called erythroblasts, which are formed continuously from the pluripotent stem cells in the bone marrow (Fig. 14-5). The red cell precursors move through a series of divisions, each producing a smaller cell as they continue to develop into mature red blood cells. Hemoglobin synthesis begins at the early erythroblast stage and continues until the cell becomes a mature erythrocyte. During its transformation from normoblast to reticulocyte, the red blood cell accumulates hemoglobin as the nucleus condenses and is finally lost. The period from stem cell to emergence of the reticulocyte in the circulation normally takes approximately 1 week. Maturation of reticulocyte to erythrocyte takes approximately 24 to 48 hours. During this process, the red cell loses its mitochondria and ribosomes, along with its ability to produce hemoglobin and engage in oxidative metabolism. Most maturing red cells enter the blood as reticulocytes. Approximately 1% of the body's total complement of red blood cells is generated from bone marrow each day, and the reticulocyte count therefore serves as an index of the erythropoietic activity of the bone marrow.

Erythropoiesis is governed for the most part by tissue oxygen needs. Any condition that causes a decrease in the amount of oxygen that is transported in the blood produces an increase in red cell production. The oxygen content of the blood does not act directly on the bone marrow to stimulate red blood cell production. Instead, the decreased oxygen content is sensed by the peritubular cells in the kidneys, which then produce a hormone called erythropoietin. Normally, about 90% of all erythropoietin is produced by the kidneys, with the remaining 10% formed in the liver. Although erythropoietin is the key regulator of erythropoiesis, a number of growth factors, including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and insulin-like growth factor-1 (IGF-1), are involved in the early stages of erythropoiesis.

Erythropoietin acts primarily in later stages of erythropoiesis to induce the erythrocyte colony-forming units to proliferate and mature through the normoblast stage into reticulocytes and mature erythrocytes. In the absence of erythropoietin, as in kidney failure, hypoxia has little or no effect on red blood cell production. Human erythropoietin can be produced by recombinant deoxyribonucleic acid (DNA) technology. It is used for the management of anemia in cases of chronic renal failure, for anemias induced by chemotherapy in persons with malignancies, and in the treatment of anemia in human immunodeficiency virus (HIV)-infected persons treated with zidovudine.

Because red blood cells are released into the blood as reticulocytes, the percentage of these cells is higher when there is a marked increase in red blood cell production. In some severe forms of anemia, the reticulocytes (normally about 1%) may account for as much as 30% of the total red cell count. In some situations, red cell production is so accelerated that numerous erythroblasts appear in the blood.

**Red Cell Destruction**

Mature red blood cells have a life span of approximately 4 months, or 120 days. As the red blood cell ages, a number of changes occur: metabolic activity in the cell decreases, enzyme activity declines, and adenosine triphosphate (ATP) decreases.
Membrane lipids become reduced and the cell membrane becomes more fragile, causing the red cell to self-destruct as it passes through narrow places in the circulation and in the small trabecular spaces in the spleen. The rate of red cell destruction (1% per day) normally is equal to the rate of red cell production, but in conditions such as hemolytic anemia, the cell’s lifespan may be shorter.

The destruction of red blood cells is facilitated by a group of large phagocytic cells found in the spleen, liver, bone marrow, and lymph nodes. These phagocytic cells recognize old and defective red cells and then ingest and destroy them in a series of enzymatic reactions. During these reactions, the amino acids from the globin chains and iron from the heme units are salvaged and reused (Fig. 14-6). The bulk of the heme unit is converted to bilirubin, the pigment of bile, which is insoluble in plasma and attaches to plasma proteins for transport. Bilirubin is removed from the blood by the liver and conjugated with glucuronide to render it water soluble so that it can be excreted in the bile. Excess elimination of bilirubin in the bile due to increased red cell destruction can lead to the development of bilirubin gallstones. The plasma-insoluble form of bilirubin is referred to as unconjugated bilirubin and the water-soluble form as conjugated bilirubin. Serum levels of conjugated and unconjugated bilirubin can be measured in the laboratory and are reported as direct and indirect, respectively. If red cell destruction and consequent bilirubin production are excessive, unconjugated bilirubin accumulates in the blood. This results in a yellow discoloration of the skin, called jaundice.

When red blood cell destruction takes place in the circulation, as in hemolytic anemia, the hemoglobin remains in the plasma. The plasma contains a hemoglobin-binding protein called haptoglobin. Other plasma proteins, such as albumin, can also bind hemoglobin. With extensive intravascular destruction of red blood cells, hemoglobin levels may exceed the hemoglobin-binding capacity of haptoglobin and other plasma proteins. When this happens, free hemoglobin appears in the blood (i.e., hemoglobinemia) and is excreted in the urine (i.e., hemoglobunuria). Because excessive red blood cell destruction can occur in hemolytic transfusion reactions, urine samples are tested for free hemoglobin after a transfusion reaction.

**Red Cell Metabolism and Hemoglobin Oxidation**

The red blood cell, which lacks mitochondria, relies on glucose and the glycolytic pathway for its metabolic needs (see Chapter 4). The enzyme-mediated anaerobic metabolism of glucose generates the ATP needed for normal membrane function and ion transport. The depletion of glucose or the functional deficiency of one of the glycolytic enzymes leads to the premature death of the red blood cell. An offshoot of the glycolytic pathway is the production of 2,3-diphosphoglycerate (2,3-DPG), which binds to the hemoglobin molecule and reduces the affinity of hemoglobin for oxygen. This facilitates the release of oxygen at the tissue level. An increase in the concentration of 2,3-DPG occurs in conditions of chronic hypoxia such as chronic lung disease, anemia, and residence at high altitudes.

The oxidation of hemoglobin—the combining of hemoglobin with oxygen—can be interrupted by certain chemicals (e.g., nitrates and sulfates) and drugs that oxidize hemoglobin to an inactive form. The nitrite ion reacts with hemoglobin to produce methemoglobin, which has a low affinity for oxygen. Large doses of nitrites can result in high levels of methemoglobin, causing pseudocyanosis and tissue hypoxia. For example, sodium nitrate, which is used in curing meats, can produce methemoglobin when taken in large amounts. In nursing infants, the intestinal flora is capable of converting significant amounts of inorganic nitrate (e.g., from well water) to nitrite. This inadvertent exposure to nitrates can cause serious toxic effects.

A hereditary deficiency of glucose-6-phosphate dehydrogenase (G6PD; to be discussed) predisposes to oxidative denaturation of hemoglobin, with resultant red cell injury and lysis. Hemolysis usually occurs as the result of oxidative stress generated by either an infection or exposure to certain drugs.

**Laboratory Tests**

Red blood cells can be studied by means of a sample of blood (Table 14-1). In the laboratory, automated blood cell counters rapidly provide accurate measurements of red cell content and cell indices. The red blood cell count measures the total number of red blood cells in a microliter (μL) of blood. The percentage

**FIGURE 14-6** - Destruction of red blood cells and fate of hemoglobin.
of reticulocytes (normally approximately 1%) provides an index of the rate of red cell production. The hemoglobin (grams per deciliter [dL] or 100 milliliters [mL] of blood) measures the hemoglobin content of the blood. The major components of blood are the red cell mass and plasma volume. The hematocrit measures the red cell mass in a 100-mL plasma volume. To determine the hematocrit, a sample of blood is placed in a glass tube, which is then centrifuged to separate the cells and the plasma. The hematocrit may be deceptive because it varies with the quantity of extracellular fluid, rising with dehydration and falling with overexpansion of extracellular fluid volume (Fig 14-7).

TABLE 14-1 Standard Laboratory Values for Red Blood Cells

<table>
<thead>
<tr>
<th>TEST</th>
<th>NORMAL VALUES</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell count (RBC)</td>
<td></td>
<td>Number of red cells in the blood</td>
</tr>
<tr>
<td>Men</td>
<td>4.2–5.4 x 10^6/μL</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>3.6–5.0 x 10^6/μL</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes</td>
<td></td>
<td>Rate of red cell production</td>
</tr>
<tr>
<td>Men</td>
<td>14–16.5 g/dL</td>
<td>Hemoglobin content of the blood</td>
</tr>
<tr>
<td>Women</td>
<td>12–15 g/dL</td>
<td>Volume of cells in 100 mL of blood</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td>Size of the red cell</td>
</tr>
<tr>
<td>Men</td>
<td>40%–50%</td>
<td>Concentration of hemoglobin in the red cell</td>
</tr>
<tr>
<td>Women</td>
<td>37%–47%</td>
<td>Red cell mass</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>85–100 fl</td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration</td>
<td>31–35 g/dL</td>
<td></td>
</tr>
<tr>
<td>Mean cell hemoglobin</td>
<td>27–34 pg/cell</td>
<td></td>
</tr>
</tbody>
</table>

Red cell indices are used to differentiate types of anemias by size or color of red cells. The mean corpuscular volume (MCV) reflects the volume or size of the red cells. The MCV falls in microcytic (small cell) anemia and rises in macrocytic (large cell) anemia. Some anemias are normocytic (i.e., cells are of normal size or MCV). The mean corpuscular hemoglobin concentration (MCHC) is the concentration of hemoglobin in each cell. Hemoglobin accounts for the color of red blood cells. Anemias are described as normochromic (normal color or MCHC) or hypochromic (decreased color or MCHC). Mean cell hemoglobin (MCH) refers to the mass of the red cell and is less useful in classifying anemias.

A stained blood smear provides information about the size, color, and shape of red cells and the presence of immature or abnormal cells. If blood smear results are abnormal, examination of the bone marrow may be indicated. Bone marrow commonly is aspirated with a special needle from the posterior iliac crest or the sternum. The aspirate is stained and observed for number and maturity of cells and abnormal types.

**IN SUMMARY**, the red blood cell provides the means for transporting oxygen from the lungs to the tissues. The biconcave shape of the red cell increases the surface area for diffusion of oxygen across the thin cell membrane. A complex cytoskeleton of proteins attached to the interior of the membrane maintains its shape and allows the cell to be deformed while passing through the small capillaries. The red cell contains hemoglobin, a molecule composed of two polypeptide chains each consisting of a globin (protein) portion and a heme unit, which surrounds an iron atom that combines reversibly with oxygen. Red cells develop from stem cells in the bone marrow and are released as reticulocytes into the blood, where they become mature erythrocytes. Red blood cell production is regulated by the hormone erythropoietin, which is produced by the kidney in response to a decrease in oxygen levels.
The life span of a red blood cell is approximately 120 days. Red cell destruction normally occurs in the spleen, liver, bone marrow, and lymph nodes. In the process of destruction, the heme portion of the hemoglobin molecule is converted to bilirubin. Bilirubin, which is insoluble in plasma, attaches to plasma proteins for transport in the blood. It is removed from the blood by the liver and conjugated to a water-soluble form so that it can be excreted in the bile.

The red blood cell, which lacks mitochondria, relies on glucose and the glycolytic pathway for its metabolic needs. The end product of the glycolytic pathway, 2,3-DPG, increases the release of oxygen to the tissues during conditions of hypoxia by reducing hemoglobin’s affinity for oxygen.

In the laboratory, automated blood cell counters rapidly provide accurate measurements of red blood cell count and cell indices. A stained blood smear provides information about the size, color, and shape of red cells, and the presence of immature or abnormal cells. If blood smear results are abnormal, examination of the bone marrow may be indicated.

**ANEMIA**

After completing this section of the chapter, you should be able to meet the following objectives:

- Describe the manifestations of anemia and their mechanisms.
- Explain the difference between intravascular and extravascular hemolysis.
- Compare the hemoglobinopathies associated with sickle cell disease and thalassemia.
- Explain the cause of sickling in sickle cell disease.
- Cite common causes of iron-deficiency anemia in infancy, adolescence, and adulthood.
- Describe the relation between vitamin B₁₂ deficiency and megaloblastic anemia.
- List three causes of aplastic anemia.
- Compare characteristics of the red blood cells in acute blood loss, hereditary spherocytosis, sickle cell disease, iron-deficiency anemia, and aplastic anemia.

Anemia is defined as an abnormally low number of circulating red blood cells or level of hemoglobin, or both, resulting in diminished oxygen-carrying capacity. Anemia usually results from excessive loss (bleeding) or destruction (hemolysis) of red blood cells or from deficient red blood cell production because of a lack of nutritional elements or bone marrow failure.

Anemia is not a disease, but an indication of some disease process or alteration in body function. The effects of anemia can be grouped into three categories: (1) manifestations of impaired oxygen transport and the resulting compensatory mechanisms, (2) reduction in red cell indices and hemoglobin levels, and (3) signs and symptoms associated with the pathologic process that is causing the anemia. The manifestations of anemia depend on its severity, the rapidity of its development, and the person’s age and health status.

In anemia, the oxygen-carrying capacity of hemoglobin is reduced, causing tissue hypoxia. Tissue hypoxia can give rise to fatigue, weakness, dyspnea, and sometimes angina. Hypoxia of brain tissue results in headache, faintness, and dim vision. The redistribution of the blood from cutaneous tissues or a lack of hemoglobin causes pallor of the skin, mucous membranes, conjunctiva, and nail beds. Tachycardia and palpitations may occur as the body tries to compensate with an increase in cardiac output. A flow-type systolic heart murmur may result from changes in blood viscosity. Ventricular hypertrophy and high-output heart failure may develop in persons with severe anemia, particularly those with preexisting heart disease. Erythropoiesis is accelerated and may be recognized by diffuse bone pain and sternal tenderness. In addition to the common anemic manifestations, hemolytic anemias are accompanied by jaundice caused by increased blood levels of bilirubin. In aplastic anemia, petechiae and purpura (i.e., minute hemorrhagic spots and purplish areas on the skin caused by small vessel bleeding) are the result of reduced platelet function.

Laboratory tests are useful in determining the severity and cause of the anemia. The red cell count and hemoglobin levels provide information about the severity of the anemia, whereas red cell characteristics such as size (normocytic, microcytic, macrocytic), color (normochromic, hypochromic), and shape often provide information about the cause of anemia (Fig. 14-8).

### Blood Loss Anemia

The clinical and red cell manifestations associated with blood loss anemia depend on the rate of hemorrhage and whether the
bleeding loss is internal or external. With rapid blood loss, circulatory shock and circulatory collapse may occur. With more slowly developing anemia, the amount of red cell mass lost may reach 50% without the occurrence of signs and symptoms. The effects of acute blood loss are mainly due to loss of intravascular volume, which can lead to cardiovascular collapse and shock (see Chapter 26). A fall in the red blood cell count, hematocrit, and hemoglobin is caused by hemodilution resulting from movement of fluid into the vascular compartment. Initially, the red cells are normal in size and color (normocytic, normochromic). The hypoxia that results from blood loss stimulates proliferation of committed erythroid stem cells in the bone marrow. It takes about 5 days for the progeny of stem cells to differentiate fully, an event that is marked by increased reticulocytes in the blood. If the bleeding is controlled and sufficient iron stores are available, the red cell concentration returns to normal within 3 to 4 weeks. External bleeding leads to iron loss and possible iron deficiency, which can hamper restoration of red cell counts.

Chronic blood loss does not affect blood volume but instead leads to iron-deficiency anemia when iron stores are depleted. It is commonly caused by gastrointestinal bleeding and menstrual disorders. Because of compensatory mechanisms, patients are commonly asymptomatic until the hemoglobin level is less than 8 g/dL. The red cells that are produced have too little hemoglobin, giving rise to microcytic hypochromic anemia (see Fig. 14-8).

**FIGURE 14-8** - Red cell characteristics seen in different types of anemia: (A) microcytic and hypochromic red cells, characteristic of iron-deficiency anemia; (B) macrocytic and misshaped red blood cells, characteristic of megaloblastic anemia; (C) abnormally shaped red blood cells seen in sickle cell disease; and (D) normocytic and normochromic red blood cells, as a comparison.

### Hemolytic Anemias

Hemolytic anemia is characterized by the premature destruction of red cells, the retention in the body of iron and the other products of hemoglobin destruction, and an increase in erythropoiesis. Almost all types of hemolytic anemia are distinguished by normocytic and normochromic red cells. Because of the red blood cell's shortened life span, the bone marrow usually is hyperactive, resulting in an increased number of reticulocytes in the circulating blood. As with other types of anemias, the person experiences easy fatigability, dyspnea, and other signs and symptoms of impaired oxygen transport.

In hemolytic anemia, red cell breakdown can occur within or outside the vascular compartment. Intravascular hemolysis is less common and occurs as a result of complement fixation in transfusion reactions, mechanical injury, or toxic factors. It is characterized by hemoglobinemia, hemoglobinuria, jaundice, and hemosiderinuria. Extravascular hemolysis occurs when red cells become less deformable, making it difficult for them to traverse the splenic sinusoids. The abnormal red cells are sequestered and phagocytized by macrophages in the spleen. The manifestations of extravascular hemolysis include anemia and jaundice.

Another classification of hemolytic anemia is based on whether the cause is intrinsic or extrinsic. Intrinsic causes include defects of the red cell membrane, the various hemoglobinopathies, and inherited enzyme defects. Two main types of hemoglobinopathies can cause red cell hemolysis: the abnormal substitution of an amino acid in the hemoglobin molecule, as in sickle cell disease, and the defective synthesis of one of the polypeptide chains that form the globin portion of hemoglobin, as in the thalassemias. Extrinsic or acquired forms of hemolytic anemia are caused by agents external to the red blood cell, such as drugs, bacterial and other toxins, antibodies, and physical trauma. Although all these factors can cause premature and accelerated destruction of red cells, they cannot all be treated in the same way. Some respond to splenectomy, others to treatment with corticosteroid hormones, and still others do not resolve until the primary disorder is corrected.

### Inherited Disorders of the Red Cell Membrane

Hereditary spherocytosis, transmitted as an autosomal dominant trait in 75% of the cases, is the most common inherited disorder of the red cell membrane. The disorder is caused by abnormalities of the spectrin and ankyrin membrane proteins that lead to a gradual loss of the membrane surface. The loss of membrane relative to cytoplasm causes the cell to become a tight sphere instead of a concave disk. Although the spherical cell retains its ability to transport oxygen, it is poorly deformable and susceptible to destruction as it passes through the venous sinuses of the splenic circulation. Clinical signs are variable but typically include mild hemolytic anemia, jaundice, splenomegaly, and bilirubin gallstones. A life-threatening aplastic crisis may occur when a sudden disruption of red cell production (often from a viral infection) causes a rapid drop in hematocrit and the hemoglobin level. The disorder usually is treated with splenectomy to reduce red cell destruction, and blood transfusions may be required in a crisis.

### Sickle Cell Disease

Sickle cell disease is an inherited disorder in which an abnormal hemoglobin (hemoglobin S [HbS]) leads to chronic hemolytic
anemia, pain, and organ failure. The HbS gene is transmitted by recessive inheritance and can manifest as sickle cell trait (i.e., heterozygote with one HbS gene) or sickle cell disease (i.e., homozygote with two HbS genes). Sickle cell disease affects approximately 50,000 (0.1% to 0.2%) black Americans and about 10% of black Americans carry the trait. In parts of Africa, where malaria is endemic, the gene frequency approaches 30%, attributed to the slight protective effect it confers against Plasmodium falciparum malaria.

The abnormal structure of HbS results from a point mutation in the β chain of the hemoglobin molecule, with an abnormal substitution of a single amino acid, valine, for glutamic acid (Fig. 14-9). In the heterozygote, only approximately 40% of the hemoglobin is HbS, but in the homozygote, 80% to 95% of the hemoglobin is HbS. Variations in proportions exist, and the concentration of HbS correlates with the risk of sickling. In the homozygote with sickle cell disease, the HbS becomes sickled when deoxygenated or at a low oxygen tension. The deoxygenated hemoglobin aggregates and polymerizes in the cytoplasm, creating a semisolid gel that changes the shape and deformability of the cell. The sickled cell may return to normal shape with oxygenation in the lungs. However, after repeated episodes of deoxygenation, the cells remain permanently sickled. The person with sickle cell trait who has less HbS has little tendency to sickle and is virtually asymptomatic. Fetal hemoglobin (HbF) inhibits the polymerization of HbS; therefore, most infants with sickle cell disease do not begin to experience the effects of the sickling until after 8 to 10 weeks of age, when the HbF has been replaced by HbS.

There are two major consequences of red blood cell sickling: chronic hemolytic anemia and blood vessel occlusion. Premature destruction of the cells due to the rigid, non-deformable membrane occurs in the spleen, causing hemolysis and anemia from a decrease in red cell numbers. Recent evidence suggests that vessel occlusion is a complex process involving an interaction among the sickled cells, endothelial cells, leukocytes, platelets, other plasma proteins. The process is initiated by the adherence of sickled cells to the vessel endothelium through adhesion molecules, causing endothelial activation with liberation of inflammatory mediators and substances that increase platelet activation and promote blood coagulation. The process also leads to the release of vasoc constrictor substances, whereas the liberation of nitric oxide, an important vasodilator, is impaired.

Factors associated with sickling and vessel occlusion include cold, stress, physical exertion, infection, and illnesses that cause hypoxia, dehydration, or acidosis. The rate of HbS polymerization is affected by the concentration of hemoglobin in the cell. Dehydration increases the hemoglobin concentration and contributes to the polymerization and resulting sickling. Acidosis reduces the affinity of hemoglobin for oxygen, resulting in more deoxygenated hemoglobin and increased sickling. Even such trivial incidents as reduced oxygen tension induced by sleep may contribute to the sickling process.

Clinical Course. Persons who are homozygous for the HbS gene experience severe hemolytic anemia, chronic hyperbilirubinemia, and vaso-occlusive crises. Hemolysis produces an anemia with hematocrit values ranging from 18% to 30%. The hyperbilirubinemia that results from the breakdown products of hemoglobin often leads to jaundice and the production of pigment stones in the gallbladder.

Blood vessel occlusion causes most of the severe complications. An acute pain episode results from vessel occlusion and hypoxia and can occur suddenly in almost any part of the body. Common sites obstructed by sickled cells include the abdomen, chest, bones, and joints. Many areas may be affected simultaneously. Infarctions caused by sluggish blood flow may cause chronic damage to the liver, spleen, heart, kidneys, retina, and other organs (Fig. 14-10). Acute chest syndrome is an atypical pneumonia resulting from pulmonary infarction. It is the second leading cause of hospitalization in persons with sickle cell disease and is characterized by pulmonary infiltrates, shortness of breath, fever, chest pain, and cough. The syndrome can cause chronic respiratory insufficiency and is a leading cause of death in sickle cell disease. Children may experience growth retardation and susceptibility to osteomyelitis. Painful bone crises may be caused by marrow infarcts of the bones of the
Kidney infarcts: Approximately 25% of persons with sickle cell disease have hands and feet, resulting in swelling of those extremities. Approximately 25% of persons with sickle cell disease have neurologic complications related to vessel occlusion. Stroke occurs in children 1 to 15 years of age and may recur in two thirds of those afflicted. Transient ischemic attack or cerebral hemorrhage may precede the stroke.

The spleen is especially susceptible to damage by HbS. Because of the spleen’s sluggish blood flow and low oxygen tension, hemoglobin in red cells traversing the spleen becomes deoxygenated, causing ischemia. Splenic injury begins in early childhood, characterized by intense congestion, and is usually asymptomatic. The congestion causes functional asplenia and predisposes the person to life-threatening infections by encapsulated organisms such as Streptococcus pneumoniae, Haemophilus influenzae type b, and Klebsiella species. Neonates and small children have not had time to create antibodies to these organisms and rely on the spleen for their removal. In the absence of specific antibody to the polysaccharide capsular antigens of these organisms, splenic activity is essential for removing these organisms when they enter the blood.

**Diagnosis and Screening.** Neonatal diagnosis of sickle cell disease is made on the basis of clinical findings and hemoglobin solubility results, which are confirmed by hemoglobin electrophoresis. Prenatal diagnosis is done by the analysis of fetal DNA obtained by amniocentesis.

In the United States, screening programs have been implemented to detect newborns with sickle cell disease and other hemoglobinopathies. Cord blood or heel-stick samples are subjected to electrophoresis to separate the HbF from the small amount of HbA and HbS. Other hemoglobins may be detected and quantified by further laboratory evaluation. Many states mandate screening of all newborns, regardless of ethnic origin. Ideally, the effective screening program also includes expert genetic counseling and education about pregnancy options.

**Management.** Currently, there is no known cure for sickle cell disease; hence, treatment strategies focus on prevention of sickling episodes, symptom management, and treatment of complications. The person is advised to avoid situations that precipitate sickling episodes, such as infections, cold exposure, severe physical exertion, acidosis, and dehydration. Infections are aggressively treated, and blood transfusions may be warranted in a crisis or given chronically in severe disease.

Most children with sickle cell disease are at risk for fulminant septicemia from encapsulated organisms during the first 3 years of life. Prophylactic penicillin should be begun as early as 2 months of age and continued until at least 5 years of age.

Maintaining full immunization, including H. influenzae vaccine and hepatitis B vaccine, is recommended. The National Institutes of Health Committee on Management of Sickle Cell Disease also recommends administration of the 7-valent pneumococcal vaccine beginning at 2 to 6 months of age. The 7-valent vaccine should be followed by immunization with the 23-valent pneumococcal vaccine at 24 months of age or later.

Hydroxyurea is a cytotoxic drug used to prevent complications of sickle cell disease. The drug allows synthesis of more HbF and less HbS, thereby decreasing sickling. Hydroxyurea reduces by 50% the pain episodes and pulmonary events in about 60% of the persons treated. The others do not respond. Long-term effects regarding organ damage, growth and development, and risk of malignancies are unknown. Other therapies under investigation include drugs that affect globin gene expression; prevent polymerization, membrane damage, and cell dehydration; and inhibit sickle cell adhesion to endothelial cells and promote anticoagulation. Nitric oxide appears to be a promising new drug. It regulates blood vessel tone, platelet activity, and endothelial cell adhesion—all factors that contribute to vessel occlusion. It has been shown that when nitric oxide concentrations, which are low in sickle cell disease, are increased through the use of supplemental oral arginine (a precursor of nitric oxide), pulmonary complications are reduced.
Bone marrow or stem cell transplantation has the potential for cure in symptomatic children but carries the risk of graft-versus-host disease. The bone marrow transplantation survival rate for human leukocyte antigen (HLA)-identical sibling donor transplants is about 93%. For those without family donors, transplants of unrelated donor stem cells, cord blood, and mixed donor–host transplants offer possibilities of cure with about a 70% success rate. Progress in gene therapy to treat sickle cell disease has been slow but promising, and may be a future option.

The Thalassemias

The thalassemias are a group of inherited disorders of hemoglobin synthesis leading to decreased synthesis of either the α- or β-globin chains of HbA. β-Thalassemias are caused by deficient synthesis of the β chain and α-thalassemias by deficient synthesis of the α chain. The defect is inherited as a mendelian trait, and a person may be heterozygous for the trait and have a mild form of the disease or be homozygous and have the severe form of the disease. Like sickle cell disease, the thalassemias occur with high degree of frequency in certain populations. The β-thalassemias, sometimes called Cooley anemia or Mediterranean anemia, are most common in the Mediterranean populations of southern Italy and Greece, and the α-thalassemias are most common among Asians. Both α- and β-thalassemias are common in Africans and African Americans.

Two factors contribute to the anemia that occurs in thalassemia: low intracellular hemoglobin (hypochromia) due to the decreased synthesis of the affected chain, coupled with continued production and accumulation of the unaffected globin chain. The reduced hemoglobin synthesis results in a hypochromic, microcytic anemia, whereas the accumulation of the unaffected chain interferes with normal red cell maturation and contributes to membrane changes that lead to hemolysis and anemia.

The β-Thalassemias. The β-thalassemias result from 1 of nearly 200 point mutations in the β-globin gene causing a defect in β-chain synthesis. In β-thalassemias, the excess α chains are denatured to form precipitates (i.e., Heinz bodies) in the bone marrow red cell precursors. The Heinz bodies impair DNA synthesis and cause damage to the red cell membrane. Severely affected red cell precursors are destroyed in the bone marrow, and those that escape intramedullary death are at increased risk of destruction in the spleen. In addition to the anemia, persons with moderate to severe forms of the disease suffer from coagulation abnormalities. Thrombotic events (stroke and pulmonary embolism) appear to be related to altered platelet function, endothelial activation, and an imbalance of procoagulants and anticoagulants.

The clinical manifestations of the β-thalassemias are based on the severity of the anemia. The presence of one normal gene in heterozygous persons (thalassemia minor) usually results in sufficient normal hemoglobin synthesis to prevent severe anemia. Persons who are homozygous for the trait (thalassemia major) have severe, transfusion-dependent anemia that is evident at 6 to 9 months of age when the hemoglobin switches from HbF to HbA. If transfusion therapy is not started early in life, severe growth retardation occurs in children with the disorder.

In severe β-thalassemia, marked anemia produced by ineffective hematopoiesis and hemolysis lead to increased erythropoietin secretion and hyperplasia in the bone marrow and sites of extramedullary hematopoiesis. The expanding mass of erythroid marrow invades the bony cortex, impairs bone growth, and produces other bone abnormalities. There is thinning of the cortical bone, with new bone formation evident on the maxilla and frontal bones of the face (i.e., chipmunk facies). The long bones, ribs, and vertebrae may become vulnerable to fracture because of osteoporosis or osteopenia, which contributes to increased morbidity in older patients. Enlargement of the spleen (splenomegaly) and liver (hepatomegaly) result from extramedullary hematopoiesis and increased red cell destruction.

Iron overload is a major complication of β-thalassemia. Excess iron stores, which accumulate from increased dietary absorption and repeated transfusions, are deposited in the myocardium, liver, and endocrine organs and induce organ damage. Cardiac, hepatic, and endocrine diseases are common causes of morbidity and mortality from iron overload. Disorders of the pituitary, thyroid, and adrenal glands and the pancreas result in significant morbidity and require hormone replacement therapy.12

Regular blood transfusions to maintain hemoglobin levels at 9 to 10 g/dL improve growth and development and prevent most of the complications, and iron chelation therapy can reduce the iron overload and extend life expectancy.13 Stem cell transplantation is a potential cure for low-risk patients, particularly in younger persons with no complications of the disease or its treatment, and has excellent results.12 In the future, stem cell gene replacement may provide a cure for many with the disease.

The α-Thalassemias. The α-thalassemias are caused by a gene deletion that results in defective α-chain synthesis. Synthesis of the α-globin chains of hemoglobin is controlled by two pairs or four genes; hence, α-thalassemia shows great variation in severity related to the number of gene deletions. Silent carriers who have a deletion of a single α-globin gene are asymptomatic, and those with deletion of two genes have the α-thalassemia trait and exhibit mild hemolytic anemia. Deletion of three of the four α-chain genes leads to unstable aggregates of α chains called hemoglobin H (HbH). This disorder is the most important clinical form and is common in Asians. The β chains are more soluble than the α chains, and their accumulation is less toxic to the red cells, so that senescent rather than precursor red cells are affected. Most persons with HbH have chronic moderate hemolytic anemia and may require transfusions in time of fever or illness or with certain medications.13 The most severe form of α-thalassemia occurs in infants in whom all four α-globin genes are deleted. Such a defect results in a hemoglobin molecule (Hb Bart) that is formed exclusively from the chains of HbF. Hb Bart, which has an extremely high oxygen affinity, cannot release oxygen in the tissues. This disorder usually results in death in utero or shortly after birth. The few survivors are transfusion dependent and have other malformations.13
**Inherited Enzyme Defects**

The most common inherited enzyme defect that results in hemolytic anemia is a deficiency of G6PD. The gene that determines this enzyme is located on the X chromosome, and the defect is expressed only in males and homozygous females. There are more than 350 genetic variants of this disorder found in all populations, but particularly in African and Mediterranean groups. The African variant has been found in 10% to 15% of African Americans. The disorder makes red cells more vulnerable to oxidants and causes direct oxidation of hemoglobin to methemoglobin, which cannot transport oxygen, and denaturing of the hemoglobin molecule to form Heinz bodies, which are precipitated in the red blood cell. Hemolysis usually occurs as the damaged red blood cells move through the narrow vessels of the spleen, causing hemoglobinemia, hemoglobinuria, and jaundice. The hemolysis is short-lived, occurring 2 to 3 days after the trigger event. In blacks, the defect is mildly expressed and is not associated with chronic hemolytic anemia unless triggered by oxidant drugs, acidosis, or infection.

The antimalarial drug primaquine, the sulfonamides, nitrofurantoin, aspirin, phenacetin, some chemotherapeutics, and other drugs cause hemolysis. Free radicals generated by phagocytes during infections also are possible triggers. A more severe deficiency of G6PD is found in people of Mediterranean descent (e.g., Sardinians, Sephardic Jews, Arabs). In some of these persons, chronic hemolysis occurs in the absence of exposure to oxidants. The disorder can be diagnosed through the use of a G6PD assay or screening test.

**Acquired Hemolytic Anemias**

Several acquired factors exogenous to the red blood cell produce hemolysis by direct membrane destruction or by antibody-mediated lysis. Various drugs, chemicals, toxins, venoms, and infections such as malaria destroy red cell membranes. Hemolysis can also be caused by mechanical factors such as prosthetic heart valves, vasculitis, and severe burns. Obstructions in the microcirculation, as in disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, and renal disease, may traumatize the red cells by producing turbulence and changing pressure gradients.

Many hemolytic anemias are immune mediated, caused by antibodies that destroy the red cell. Autoantibodies may be produced in response to drugs and disease. Allantibodies come from an exogenous source and are responsible for transfusion reactions and hemolytic disease of the newborn.

The autoantibodies that cause red cell destruction are of two types: warm-reacting antibodies of the immunoglobulin G (IgG) type, which are maximally active at 37°C, and cold-reacting antibodies of the IgM type, which are optimally active at or near 4°C. The warm-reacting antibodies account for 80% of cases of hemolytic anemia. They cause no morphologic or metabolic alteration in the red cell. Instead, they react with antigens on the red cell membrane, causing destructive changes that lead to spherocytosis, with subsequent phagocytic destruction in the spleen or reticuloendothelial system. They lack specificity for the ABO antigens but may react with the Rh antigens. The hemolytic reactions associated with the warm-reacting antibodies occur with an incidence of approximately 10 per 1 million and affect women more frequently than men. The reactions have a rapid onset and may be severe and life-threatening. Fatigue is a common complaint and jaundice and moderate splenomegaly are present. Angina or congestive heart failure may also occur. There are varied causes for this anemia; approximately 50% are idiopathic, and 50% are drug induced (e.g., penicillin) or related to cancers of the lymphoproliferative system (e.g., chronic lymphocytic leukemia, lymphoma), collagen diseases (e.g., systemic lupus erythematosus), viral infections, and inflammatory disorders (e.g., ulcerative colitis). The antihypertensive drug alpha-methyldopa and the antiarrhythmic drug quinidine account for a small number of cases. The drug-induced hemolysis is commonly benign.

The cold-reacting antibodies activate complement. Chronic hemolytic anemia caused by cold-reacting antibodies occurs with lymphoproliferative disorders and as an idiopathic disorder of unknown cause. The hemolytic process occurs in distal body parts, where the temperature may fall below 30°C. Vascular obstruction by red cells results in pallor, cyanosis of the body parts exposed to cold temperatures, and Raynaud phenomenon (see Chapter 22). Hemolytic anemia caused by cold-reacting antibodies develops in only a few persons and is rarely severe. The Coombs test, or antiglobulin test, is used to diagnosis immune hemolytic anemias. It detects the presence of antibody or complement on the surface of the red cell. The direct antiglobulin test (DAT) detects the antibody on red blood cells. In this test, red cells that have been washed free of serum are mixed with anti-human globulin reagent. The red cells agglutinate if the reagent binds to and bridges the antibody or complement on adjacent red cells. The DAT result is positive in cases of autoimmune hemolytic anemia, erythroblastosis fetalis (Rh disease of the newborn), transfusion reactions, and drug-induced hemolysis. The indirect antiglobulin test detects antibody in the serum, and the result is positive for specific antibodies. It is used for antibody detection and crossmatching before transfusion.

**Anemias of Deficient Red Cell Production**

Anemia may result from the decreased production of erythrocytes by the bone marrow. A deficiency of nutrients for hemoglobin synthesis (iron) or DNA synthesis (cobalamin or folic acid) may reduce red cell production by the bone marrow. A deficiency of red cells also results when the marrow itself fails or is replaced by nonfunctional tissue.

**Iron-Deficiency Anemia**

Iron deficiency is a common worldwide cause of anemia affecting persons of all ages. The anemia results from dietary deficiency, loss of iron through bleeding, or increased demands. Because iron is a component of heme, a deficiency leads to
decreased hemoglobin synthesis and consequent impairment of oxygen delivery.

Body iron is used repeatedly. When red cells become senescent and are broken down, their iron is released and reused in the production of new red cells. Despite this efficiency, small amounts of iron are lost in the feces and need to be replaced by dietary uptake. Iron balance is maintained by the absorption of 0.5 to 1.5 mg daily to replace the 1 mg lost in the feces. The average Western diet supplies about 20 mg. The absorbed iron is more than sufficient to supply the needs of most individuals, but may be barely adequate in toddlers, adolescents, and women of child-bearing age. Dietary deficiency of iron is not common in developed countries except in certain populations. Most iron is derived from meat, and when meat is not available, as for deprived populations, or is not a dietary constituent, as for vegetarians, iron deficiency may occur.

The usual reason for iron deficiency in adults in the Western world is chronic blood loss because iron cannot be recycled to the pool. In men and postmenopausal women, blood loss may occur from gastrointestinal bleeding because of peptic ulcer, intestinal polyps, hemorrhoids, or cancer. Excessive aspirin intake may cause undetected gastrointestinal bleeding. In women, menstruation may account for an average of 1.5 mg of iron lost per day, causing a deficiency. Although cessation of menstruation removes a major source of iron loss in the pregnant woman, iron requirements increase at this time, and deficiency is common. The expansion of the mother's blood volume requires approximately 500 mg of additional iron, and the growing fetus requires approximately 360 mg during pregnancy. In the postnatal period, lactation requires approximately 1 mg of iron daily.

A child's growth places extra demands on the body. Blood volume increases, with a greater need for iron. Iron requirements are proportionally higher in infancy (3 to 24 months) than at any other age, although they are also increased in childhood and adolescence. In infancy, the two main causes of iron deficiency anemia are low iron levels at birth because of maternal deficiency and a diet consisting mainly of cow's milk, which is low in absorbable iron. Adolescents are also susceptible to iron deficiency because of high requirements due to growth spurts, dietary deficiencies, and menstrual loss.

Iron-deficiency anemia is characterized by low hemoglobin and hematocrit, decreased iron stores, and low serum iron and ferritin. The red cells are decreased in number and are microcytic and hypochromic (see Fig. 14-8). Poikilocytosis (irregular shape) and anisocytosis (irregular size) are also present. Laboratory values indicate reduced MCHC and MCV. Membrane changes may predispose to hemolysis, causing further loss of red cells.

The manifestations of iron-deficiency anemia are related to impaired oxygen transport and lack of hemoglobin. Depending on the severity of the anemia, fatigue, palpatations, dyspnea, angina, and tachycardia may occur. Epithelial atrophy is common and results in waxy pallor, brittle hair and nails, sometimes a spoon-shaped deformity of the fingernails, smooth tongue, sores in the corners of the mouth, and sometimes dysphagia and decreased acid secretion. A poorly understood symptom occasionally seen is pica, the bizarre, compulsive eating of ice, dirt, or other abnormal substances. Iron deficiency in infants may also result in long-term manifestations such as poor cognitive, motor, and emotional function that may be related to effects on brain development or neurotransmitter function.

Prevention of iron deficiency is a primary concern in infants and children. Avoidance of cow's milk, iron supplementation at 4 to 6 months of age in breast-fed infants, and use of iron-fortified formulas and cereals are recommended for infants younger than 1 year of age. In the second year, a diet rich in iron-containing foods and use of iron-fortified vitamins will help prevent iron deficiency. The treatment of iron-deficiency anemia in children and adults is directed toward controlling chronic blood loss, increasing dietary intake of iron, and administering supplemental iron. Ferrous sulfate, which is the usual oral replacement therapy, replenishes iron stores in several months. Parenteral iron (iron dextran or sodium ferric gluconate) therapy may be used when oral forms are not tolerated or are ineffective. Because of the possibility of severe hypersensitivity reactions, an initial test dose should be done before administration of the first therapeutic dose of the drug. It is recommended that the test dose be administered in an environment equipped for treatment of severe allergic or anaphylactic reactions. Parenteral iron can be given intravenously or as a deep intramuscular injection by the Z-track method, in which the skin is pulled to one side before inserting the needle to prevent leakage into the tissues, with subsequent skin discoloration. In the future, gastric delivery systems may provide good therapy without side effects.

**Megaloblastic Anemias**

Megaloblastic anemias are caused by impaired DNA synthesis that results in enlarged red cells (MCV >100 fl) due to impaired maturation and division. Vitamin B12 and folic acid deficiencies are the most common conditions associated with megaloblastic anemias. Because megaloblastic anemias develop slowly, there are often few symptoms until the anemia is far advanced.

**Vitamin B12-Deficiency Anemia.** Vitamin B12, also known as cobalamin, serves as a cofactor for two important reactions in humans. It is essential for DNA synthesis and nuclear maturation, which in turn leads to normal red cell maturation and division. Vitamin B12 is also involved in a reaction that prevents abnormal fatty acids from being incorporated into neuronal lipids. This abnormality may predispose to myelin breakdown and produce some of the neurologic complications of vitamin B12 deficiency.

Vitamin B12 is found in all foods of animal origin. Dietary deficiency is rare and usually found only in strict vegetarians who avoid all dairy products as well as meat and fish. Normal body stores of 1000 to 5000 micrograms (μg) provide the daily requirement of 1 μg for a number of years. Therefore, deficiency of vitamin B12 develops slowly. Vitamin B12 is absorbed by
There it is bound to its carrier protein, transcobalamin II, which is secreted by the gastric parietal cells (Fig. 14-11). The vitamin B$_{12}$-intrinsic factor complex protects vitamin B$_{12}$ from digestion by intestinal enzymes. The complex travels to the ileum, where it binds to membrane receptors on the epithelial cells. Vitamin B$_{12}$ is then separated from intrinsic factor and transported across the membrane into the circulation. There it is bound to its carrier protein, transcobalamin II, which transports vitamin B$_{12}$ to its storage and tissue sites. Any defects in this pathway may cause a deficiency.

**Pernicious anemia** is a specific form of megaloblastic anemia caused by atrophic gastritis (see Chapter 37) and an attendant failure to produce intrinsic factor that leads to failure to absorb vitamin B$_{12}$. Pernicious anemia is believed to result from immunologically mediated, possibly autoimmune, destruction of the gastric mucosa. The resultant chronic atrophic gastritis is marked by loss of parietal cells and production of antibodies that interfere with binding of vitamin B$_{12}$ to intrinsic factor. Other causes of vitamin B$_{12}$-deficiency anemia include gastrectomy, ileal resection, inflammation or neoplasms in the terminal ileum, and malabsorption syndromes in which vitamin B$_{12}$ and other vitamin B compounds are poorly absorbed.

The hallmark of vitamin B$_{12}$ deficiency is megaloblastic anemia. When vitamin B$_{12}$ is deficient, the red cells that are produced are abnormally large because of excess cytoplasmic growth and structural proteins (see Fig. 14-8). The cells have immature nuclei and show evidence of cellular destruction. They have flimsy membranes and are oval rather than biconcave. These oddly shaped cells have a short life span that can be measured in weeks rather than months. The loss of red cells results in a moderate to severe anemia and mild jaundice. The MCV is elevated, and the MCHC is normal.

Neurologic changes that accompany the disorder are caused by deranged methylation of myelin protein. Demyelination of the dorsal and lateral columns of the spinal cord causes symmetric paresthesias of the feet and fingers, loss of vibratory and position sense, and eventual spastic ataxia. In more advanced cases, cerebral function may be altered. In some cases, dementia and other neuropsychiatric changes may precede hematologic changes.

Diagnosis of vitamin B$_{12}$ deficiency is made by finding an abnormally low vitamin B$_{12}$ serum level. The Schilling test, which measures the 24-hour urinary excretion of radiolabeled vitamin B$_{12}$ administered orally, has been commonly used in the past to document decreased absorption of vitamin B$_{12}$. Currently, the diagnosis of pernicious anemia is usually made by the detection of parietal cell and intrinsic factor antibodies. Lifelong treatment consisting of intramuscular injections or high oral doses of vitamin B$_{12}$ reverses the anemia and improves the neurologic changes.

**Folic Acid–Deficiency Anemia.** Folic acid is also required for DNA synthesis and red cell maturation, and its deficiency produces the same type of megaloblastic red cell changes that occur in vitamin B$_{12}$-deficiency anemia (i.e., increased MCV and normal MCHC). Symptoms are also similar, but without the neurologic manifestations.

Folic acid is readily absorbed from the intestine. It is found in vegetables (particularly the green leafy types), fruits, cereals, and meats. Much of the vitamin, however, is lost in cooking. The most common causes of folic acid deficiency are malnutrition or dietary lack, especially in the elderly or in association with alcoholism. Total body stores of folic acid amount to 2000 to 5000 µg, and 50 µg is required in the daily diet. A dietary deficiency may result in anemia in a few months. Malabsorption of folic acid may be due to syndromes such as sprue or other intestinal disorders. Some drugs used to treat seizure disorders (e.g., primidone, phenytoin, phenobarbital) and triamterene, a diuretic, predispose to a deficiency by interfering with folic acid absorption. In neoplastic disease, tumor cells compete for folate, and deficiency is common. Methotrexate, a folic acid analog used in the treatment of cancer, impairs the action of folic acid by blocking its conversion to the active form.

Because pregnancy increases the need for folic acid 5- to 10-fold, a deficiency commonly occurs. Poor dietary habits, anorexia, and nausea are other reasons for folic acid deficiency during pregnancy. Studies also show an association between folate deficiency and neural tube defects. The U.S. Public Health Service recommends that all women of childbearing age should take 400 µg of folic acid daily. It is estimated that 50% of neural tube defects could thus be prevented. The Institute of Medicine Panel on Folate and Other B Vitamins has revised the recommended daily allowances for pregnant women to 600 µg/day. To ensure adequate folate consumption, the U.S. Food and Drug Administration mandated the addition of folate to cereal grain products, effective January 1, 1998.

**Aplastic Anemia**

Aplastic anemia describes a disorder of pluripotential bone marrow stem cells that results in a reduction of all three hematopoietic processes. The hallmark of aplastic anemia is a decrease in one or more of the major blood cells (red blood cells, white blood cells, and platelets). The bone marrow is usually replaced by fat tissue. The exact cause of aplastic anemia is unknown, but it can be due to a variety of factors, including...
poietic cell lines—red blood cells, white blood cells, and platelets. Pure red cell aplasia, in which only the red cells are affected, rarely occurs. Anemia results from the failure of the marrow to replace senescent red cells that are destroyed and leave the circulation, although the cells that remain are of normal size and color. At the same time, because the leukocytes, particularly the neutrophils, and the thrombocytes have a short life span, a deficiency of these cells usually is apparent before the anemia becomes severe.

The onset of aplastic anemia may be insidious, or it may strike with suddenness and great severity. It can occur at any age. The initial presenting symptoms include weakness, fatigue, and pallor caused by anemia. Petechiae (i.e., small, punctate skin hemorrhages) and ecchymoses (i.e., bruises) often occur on the skin, and bleeding from the nose, gums, vagina, or gastrointestinal tract may occur because of decreased platelet levels. The decrease in the number of neutrophils increases susceptibility to infection.

Among the causes of aplastic anemia are exposure to high doses of radiation, chemicals, and toxins that suppress hematopoiesis directly or through immune mechanisms. Chemotherapy and irradiation commonly result in bone marrow depression, which causes anemia, thrombocytopenia, and neutropenia. Identified toxic agents include benzene, the antibiotic chloramphenicol, and the alkylating agents and antimetabolites used in the treatment of cancer (see Chapter 8). Aplastic anemia caused by exposure to chemical agents may be an idiosyncratic reaction because it affects only certain susceptible persons. It typically occurs weeks after a drug is initiated. Such reactions often are severe and sometimes irreversible and fatal. Aplastic anemia can develop in the course of many infections and has been reported most often as a complication of viral hepatitis, mononucleosis, and other viral illnesses, including acquired immunodeficiency syndrome (AIDS). In two thirds of cases, the cause is unknown, and these are called idiopathic aplastic anemia. The mechanisms underlying the pathogenesis of aplastic anemia are unknown. It is suggested that exposure to the chemicals, infectious agents, and other insults generates a cellular immune response resulting in production of cytokines by activated T cells. These cytokines (e.g., interferon, tumor necrosis factor [TNF]) then suppress normal stem cell growth and development.

Therapy for aplastic anemia in the young and severely affected includes stem cell replacement by bone marrow or peripheral blood transplantation. Histocompatible donors supply the stem cells to replace the patient’s destroyed marrow cells. Graft-versus-host disease, rejection, and infection are major risks of the procedure, yet 75% or more survive. For those who are not transplantation candidates, immunosuppressive therapy with lymphocyte immune globulin (i.e., antithymocyte globulin) prevents suppression of proliferating stem cells, producing remission in up to 50% of patients. Persons with aplastic anemia should avoid the offending agents and be treated with antibiotics for infection. Red cell transfusions to correct the anemia and platelets and corticosteroid therapy to minimize bleeding may also be required.

Chronic Disease Anemias

Anemia often occurs as a complication of chronic infections, inflammation, and cancer. The most common causes of chronic disease anemias are acute and chronic infections, including AIDS and osteomyelitis; cancers; autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease; and chronic kidney disease. It is theorized that the short red cell life span, deficient red cell production, a blunted response to erythropoietin, and low serum iron are caused by actions of cytokines and cells of the reticuloendothelial system (RES). Microorganisms, tumor cells, and autoimmune dysregulation lead to T-cell activation and production of cytokines (e.g., interleukin-1, interferon, and TNF) that suppress the erythropoietin response, inhibit erythroid precursors, and cause changes in iron homeostasis. In addition, macrophages take up iron and store it, thus reducing its availability for erythropoiesis. The mild anemia is normocytic and normochromic with low reticulocyte counts.

Chronic renal failure almost always results in anemia, primarily because of a deficiency of erythropoietin. Unidentified uremic toxins and retained nitrogen also interfere with the actions of erythropoietin and with red cell production and survival. Hemolysis and blood loss associated with hemodialysis and bleeding tendencies also contribute to the anemia of renal failure. Therapy for these anemias includes treatment for the underlying disease, short-term erythropoietin therapy, iron supplementation, and blood transfusions. Future treatments may include iron-chelating drugs, agents that lower iron retention in RES cells, and cytokines to stimulate erythropoietin production.

“Anemia of critical illness” is common in the intensive care unit, with more than 90% of patients having subnormal hemoglobin levels by the third day. In critically ill persons, low erythropoietin concentrations and anemia also appear to be caused by inflammatory cytokines. In this population, it is suggested that red blood cell transfusions be restricted to reduce the risk of transmission of newer infectious agents and immune modulation (e.g., immunosuppression) predisposing to infections, cancer recurrence, and autoimmune disease.

In Summary, anemia is a condition of an abnormally low number of circulating red blood cells or hemoglobin level, or both. It is not a disease, but a manifestation of a disease process or alteration in body function. The manifestations of anemia are those associated with impaired oxygen transport; alterations in red blood cell number, hemoglobin content, and cell structure, as well as the signs and symptoms of the underlying process causing the anemia.

Anemia can result from excessive blood loss, red cell destruction due to hemolysis, or deficient hemoglobin or red cell production. Blood loss anemia can be acute or chronic. With bleeding, iron and other components of the erythrocyte are lost from the body. Hemolytic anemia is characterized by
the premature destruction of red cells, with retention in the body of iron and the other products of red cell destruction. Hemolytic anemia can be caused by defects in the red cell membrane, hemoglobinopathies (sickle cell disease or thalassemia), or inherited enzyme defects (G6PD deficiency). Acquired forms of hemolytic anemia are caused by agents extrinsic to the red blood cell, such as drugs, bacterial and other toxins, antibodies, and physical trauma. Iron-deficiency anemia, which is characterized by decreased hemoglobin synthesis, can result from dietary deficiency, loss of iron through bleeding, or increased demands for red cell production. Vitamin B₁₂ and folic acid deficiencies impair red cell production by interfering with DNA synthesis. Aplastic anemia is caused by bone marrow suppression and usually results in a reduction of white blood cells and platelets, as well as red blood cells. Chronic diseases such as inflammatory disorders (rheumatoid arthritis), cancers, and renal failure cause anemia through the production of inflammatory cytokines that interfere with erythropoietin production or response.

**TRANSFUSION THERAPY**

*After completing this section of the chapter, you should be able to meet the following objectives:*

- Differentiate red cell antigens from antibodies in persons with type A, B, AB, or O blood.
- Explain the determination of the Rh factor.
- List the signs and symptoms of a blood transfusion reaction.

Anemias of various causes are treated with transfusions of whole blood or red blood cells only when oxygen delivery to the tissues is compromised, as evidenced by measures of oxygen transport and use, hemoglobin, and hematocrit. Current recommendations suggest transfusion for patients with hemoglobin levels less than 7 g/dL, depending on age, illness, risk factors, and surgical procedures. Acute massive blood loss usually is replaced with whole-blood transfusion. Most anemias, however, are treated with transfusions of red cell concentrates, which supply only the blood component that is deficient. Since the 1960s, devices that mechanically separate a unit of blood into its constituents provide red cell components, platelets, fresh-frozen plasma, cryoprecipitate, and clotting factor concentrates. In this way, a unit of blood can be used efficiently for several recipients to correct specific deficiencies.

Several red cell components that are used for transfusion are prepared and stored under specific conditions and have unique uses, as described in Table 14-2. These red cell components are derived principally from voluntary blood donors. In the future, red cell substitutes, such as hemoglobin solutions, may be used, particularly in the trauma setting. The potential advantages are better storage, longer shelf life, and no risk of transfusion reaction.

The use of autologous donation and transfusion has been advocated since the early 1980s. Autologous transfusion refers to the procedure of receiving one’s own blood—usually to replenish a surgical loss—thereby eliminating the risk of blood-borne disease or transfusion reaction. In 1992, a reported 8.5% of transfusions were autologous. Autologous blood can be provided by several means: predeposit, hemodilution, and intraoperative salvage. A patient who is anticipating elective orthopedic, vascular, or open heart surgery may predeposit blood (i.e., have the blood collected up to 6 weeks in advance and stored) for later transfusion during the surgery. Hemodilution involves phlebotomy before surgery with transfusion of the patient’s blood at the completion of surgery. The procedure requires the use of fluid infusions to maintain blood volume and is commonly used in open heart surgery. Intraoperative blood salvage is the collection of blood shed from the operative site for transfusion into the patient. Semiautomated devices are used to collect, anticoagulate, wash, and resuspend red cells for reinfusion during many procedures, including vascular, cardiac, and orthopedic surgery. Potential risks of autologous transfusion may include bacterial and other contamination, volume overload, and administrative errors.

Before a red cell or whole-blood transfusion from a volunteer donor source can occur, a series of procedures is required to ensure a successful transfusion. Donor samples are first tested for blood-borne diseases, such as hepatitis B and C viruses, HIV types 1 and 2, human T-cell lymphotropic viruses (HTLV-I and -II), and syphilis. Donor and recipient samples are typed to determine ABO and Rh groups and screened for unexpected red cell antibodies. The cross-match is performed by incubating the donor cells with the recipient's serum and observing for agglutination. If none appears, the donor and recipient blood types are compatible.

**ABO Blood Groups**

ABO compatibility is essential for effective transfusion therapy and requires knowledge of ABO antigens and antibodies. There are four major ABO blood groups determined by the presence or absence of two red cell antigens (A and B). Persons who have neither A nor B antigens are classified as having type O blood; those with A antigens are classified as having type A blood; those with B antigens, as having type B blood; and those with A and B antigens, as having type AB blood (Table 14-3). The ABO blood groups are genetically determined. The type O gene is apparently functionless in production of a red cell antigen. Each of the other genes is expressed by the presence of a strong antigen on the surface of the red cell. Six genotypes, or gene combinations, result in four phenotypes, or blood type expressions. In the United States the frequencies of ABO blood groups among whites are approximately 46% for type O, 41% for type A, 9% for type B, and 4% for type AB. Although the distribution varies