44 The Biochemistry of the Erythrocyte and other Blood Cells

*The cells of the blood are classified as **erythrocytes, leukocytes,** or **thrombocytes.** The erythrocytes (**red cells**) carry oxygen to the tissues and are the most numerous cells in the blood. The leukocytes (**white cells**) are involved in defense against infection, and the thrombocytes (**platelets**) function in blood clotting. All of the cells in the blood can be generated from hematopoietic stem cells in the bone marrow on demand. For example, in response to infection, leukocytes secrete **cytokines** called **interleukins** that stimulate the production of additional leukocytes to fight the infection. Decreased supply of oxygen to the tissues signals the kidney to release **erythropoietin**, a hormone that stimulates the production of red cells.*

*The red cell has limited metabolic function, owing to its lack of internal organelles. **Glycolysis** is the main energy-generating pathway, with lactate production regenerating NAD^+ for glycolysis to continue. The NADH produced in glycolysis is also used to reduce the ferric form of hemoglobin, **methemoglobin**, to the normal ferrous state. Glycolysis also leads to a side pathway in which **2,3 bisphosphoglycerate** is produced, which is a major allosteric effector for oxygen binding to hemoglobin. The **hexose monophosphate shunt** pathway generates **NADPH** to protect red cell membrane lipids and proteins from oxidation, through regeneration of reduced glutathione. **Heme synthesis** occurs in the precursors of red cells and is a complex pathway that originates from succinyl-CoA and glycine. Mutations in any of the steps of heme synthesis lead to a group of diseases known collectively as **porphyrias**.*

*The red cell membrane must be highly deformable to allow it to travel throughout the capillary system in the body. This is because of a complex **cytoskeletal** structure that consists of the major proteins **spectrin**, **ankyrin**, and **band 3 protein**. Mutations in these proteins lead to improper formation of the membrane cytoskeleton, ultimately resulting in malformed red cells, **spherocytes**, in the circulation. Spherocytes have a shortened life span, leading to loss of blood cells.*

*When the body does not have sufficient red cells, the patient is said to be **anemic**. Anemia can result from many causes. **Nutritional deficiencies** of **iron**, **folate**, or **vitamin B12** prevent the formation of adequate numbers of red cells. **Mutations** in the genes that encode red cell metabolic **enzymes**, **membrane structural proteins**, and **globins** cause **hereditary anemias**. The appearance of red cells on a blood smear frequently provides clues to the cause of an anemia. Because the mutations that give rise to hereditary anemias also provide some protection against malaria, hereditary anemias are some of the most common genetic diseases known.*

*The human alters globin gene expression during development, a process known as **hemoglobin switching**. The switch between expression of one gene to another is regulated by transcription factor binding to the promoter regions of these genes. Current research is attempting to reactivate fetal hemoglobin genes to combat sickle-cell disease and thalassemia.*

THE WAITING ROOM

Anne Niemick, who has β^+ thalassemia, complains of pain in her lower spine (see Chapters 14 and 15). A quantitative computed tomogram (CT) of the vertebral bodies of the lumbar spine shows evidence of an area of early spinal cord compression in the upper lumbar region. She is suffering from severe anemia, resulting in stimulation of production of red blood cell precursors (the erythroid mass) from the stem cells in her bone marrow. This expansion of marrow volume causes compression of tissues in this area, which, in turn, causes pain. Local irradiation is considered, as is a program of regular blood transfusions to maintain the oxygen-carrying capacity of circulating red blood cells. The results of special studies related to the genetic defect underlying her thalassemia are pending, although preliminary studies have shown that she has elevated levels of fetal hemoglobin, which, in part, moderates the manifestations of her disease. **Anne Niemick's** parents have returned to the clinic to discuss the results of these tests.

Spiro Site is a 21-year-old college student who complains of feeling tired all the time. Two years previously he had had gallstones removed, which consisted mostly of bilirubin. His spleen is palpable, and jaundice is evidenced by yellowing of the whites of his eyes. His hemoglobin was low (8 g/dL; reference value 13.5–17.5 gm/dL). A blood smear showed dark, rounded, abnormally small red cells called spherocytes as well as an increase in the number of circulating immature red blood cells known as reticulocytes.

I. CELLS OF THE BLOOD

The blood, together with the bone marrow, makes up the organ system that makes a significant contribution to achieving **homeostasis,** the maintenance of the normal composition of the body's internal environment. Blood can be considered a liquid tissue consisting of water, proteins, and specialized cells. The most abundant cell in the blood is the **erythrocyte** or red blood cell, which transports oxygen to the tissues and contributes to buffering of the blood through the binding of protons by hemoglobin (see section IV of this chapter, and the material in Chapter 4, section IV.D.2., and Chapter 7, section VII). Red blood cells lose all internal organelles during the process of differentiation. The white blood cells (**leukocytes**) are nucleated cells present in blood that function in the defense against infection. The platelets (**thrombocytes**), which contain cytoplasmic organelles but no nucleus, are involved in the control of bleeding by contributing to normal thrombus (clot) formation within the lumen of the blood vessel. The average concentration of these cells in the blood of normal individuals is presented in Table 44.1.

Table 44.1. Normal Values of Blood Cell Concentrations in Adults

Cell Type	Mean (cells/mm^3)
Erythrocytes	5.2×10^6 (men); 4.6×10^6 women
Neutrophils	4,300
Lymphocytes	2,700
Monocytes	500
Eosinophils	230
Basophils	40

A. Classification and Functions of Leukocytes and Thrombocytes

The leukocytes can be classified either as polymorphonuclear leukocytes (granulocytes) or mononuclear leukocytes, depending on the morphology of the nucleus in these cells. The mononuclear leukocyte has a rounded nucleus, whereas the polymorphonuclear leukocytes have a multilobed nucleus.

1. THE GRANULOCYTES

The granulocytes, so named because of the presence of secretory granules visible on staining, are the **neutrophils**, **eosinophils,** and **basophils**. When these cells are activated in response to chemical stimuli, the vesicle membranes fuse with the cell plasma membrane, resulting in the release of the granule contents (**degranulation**). The granules contain many cell-signaling molecules that mediate inflammatory processes. The granulocytes, in addition to displaying segmented nuclei (are polymorphonuclear), can be distinguished from each other by their staining properties (caused by different granular contents) in standard hematologic blood smears; neutrophils stain pink, eosinophils stain red, and basophils stain blue.

Neutrophils are phagocytic cells that rapidly migrate to areas of infection or tissue damage. As part of the response to acute infection, neutrophils engulf foreign bodies, and destroy them, in part, by initiating the respiratory burst (see Chapter 24). The respiratory burst creates oxygen radicals that rapidly destroy the foreign material found at the site of infection.

A primary function of eosinophils is to destroy parasites such as worms. The eosinophilic granules are lysosomes containing hydrolytic enzymes and cationic proteins, which are toxic to parasitic worms. Eosinophils have also been implicated in asthma and allergic responses, although their exact role in the development of these disorders is still unknown, and this is an active area of research.

Basophils, the least abundant of the leukocytes, participate in hypersensitivity reactions, such as allergic responses. Histamine, produced by the decarboxylation of histidine, is stored in the secretory granules of basophils. Release of histamine during basophil activation stimulates smooth muscle cell contraction and increases vascular permeability. The granules also contain enzymes such as proteases, β-glucuronidase, and lysophospholipase. These enzymes degrade microbial structures and assist in the remodeling of damaged tissue.

2. MONONUCLEAR LEUKOCYTES

The mononuclear leukocytes consist of various classes of lymphocytes and the monocytes. Lymphocytes are small, round cells originally identified in lymph fluid. These cells have a high ratio of nuclear volume to cytoplasmic volume and are the primary antigen (foreign body)-recognizing cells. There are three major types of lymphocytes: T cells, B cells, and NK cells. The precursors of T cells (thymus-derived lymphocytes) are produced in the bone marrow and then migrate to the thymus, where they mature before being released to the circulation. Several subclasses of T cells exist. These subclasses are identified by different surface membrane proteins, the presence of which correlate with the function of the subclass. Lymphocytes that mature in the bone marrow are the B cells, which secrete antibodies in response to antigen binding. The third class of lymphocytes are the natural killer cells (NK cells), which target virally infected and malignant cells for destruction.

Circulatory monocytes are the precursors of tissue macrophages. Macrophages (large eater) are phagocytic cells that enter inflammatory sites and consume microorganisms and necrotic host cell debris left behind by granulocyte attack of the foreign material. Macrophages in the spleen play an important role in maintaining

Table 44.2. Normal Hemoglobin Levels in Blood (g/dL)

Adult	
Males	13.5–17.5
Females	11.5–15.5
Children	
Newborns	15.0–21.0
3–12 mo.	9.5–12.5
1 yr to puberty	11.0–13.5

Other measurements used to classify the type of anemia present include the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC). The MCV is the average volume of the red blood cell, expressed in femto (10^{-15}) liters. Normal MCV range from 80 to 100 fL. The MCHC is the average concentration of hemoglobin in each individual erythrocyte, expressed in g/L. The normal range is 32 to 37; a value of less than 32 would indicate hypochromic cells. Thus, microcytic, hypochromic red blood cells have an MCV of less than 80 and an MCHC of less than 32. Macrocytic, normochromic cells have an MCV of greater than 100, with an MCHC between 32 and 37.

The trace amounts of 2,3 BPG found in cells other than erythrocytes is required for the phosphoglycerate mutase reaction of glycolysis, in which 3-phosphoglycerate is isomerized to 2-phosphoglycerate. As the 2,3 BPG is regenerated during each reaction cycle, it is only required in catalytic amounts.

the oxygen-delivering capabilities of the blood by removing damaged red blood cells that have a reduced oxygen-carrying capacity.

3. THE THROMBOCYTES

Platelets are heavily granulated disc-like cells that aid in intravascular clotting. Like the erythrocyte, platelets lack a nucleus. Their function is discussed in the following chapter. Platelets arise by budding of the cytoplasm of megakaryocytes, multinucleated cells that reside in the bone marrow.

B. Anemia

The major function of erythrocytes is to deliver oxygen to the tissues. To do this, a sufficient concentration of hemoglobin in the red blood cells is necessary for efficient oxygen delivery to occur. When the hemoglobin concentration falls below normal values (Table 44.2), the patient is classified as anemic. Anemias can be categorized based on red cell size and hemoglobin concentration. Red cells can be of normal size (normocytic), small (microcytic), or large (macrocytic). Cells containing a normal hemoglobin concentration are termed normochromic; those with decreased concentration are hypochromic. This classification system provides important diagnostic tools (Table 44.3) that enable one to properly classify, diagnose, and treat the anemia.

II. ERYTHROCYTE METABOLISM

A. The Mature Erythrocyte

To best understand how the erythrocyte can carry out its major function, a discussion of erythrocyte metabolism is required. Mature erythrocytes contain no intracellular organelles, so the metabolic enzymes of the red blood cell are limited to those found in the cytoplasm. In addition to hemoglobin, the cytosol of the red blood cell contains enzymes necessary for the prevention and repair of damage done by reactive oxygen species (see Chapter 24) and the generation of energy (Fig. 44.1). Erythrocytes can only generate adenosine triphosphate (ATP) by glycolysis (see Chapter 22). The ATP is used for ion transport across the cell membrane (primarily Na^+, K^+, and Ca^{+2}), the phosphorylation of membrane proteins, and the priming reactions of glycolysis. Erythrocyte glycolysis also uses the Rapaport-Luebering shunt to generate 2,3-bisphosphoglycerate (2,3-BPG). Red cells contain 4 to 5 mM 2,3-BPG, compared with trace amounts in other cells. As discussed in more detail in Section IV, 2,3-BPG is a modulator of oxygen binding to hemoglobin that stabilizes the deoxy form of hemoglobin, thereby facilitating the release of oxygen to the tissues.

To bind oxygen, the iron of hemoglobin must be in the ferrous ($+2$) state. Reactive oxygen species can oxidize the iron to the ferric ($+3$) state, producing

Table 44.3. Classification of the Anemias on the Basis of Red Cell Morphology

| Red Cell Morphology	Functional Deficit	Possible Causes
Microcytic, hypochromic	Impaired hemoglobin synthesis	Iron deficiency, thalassemia mutation, lead poisoning
Macrocytic, normochromic	Impaired DNA synthesis	B12 or folic acid deficiency, erythroleukemia
Normocytic, normochromic	Red cell loss	Acute bleeding, sickle cell disease, red cell metabolic defects, red cell membrane defects
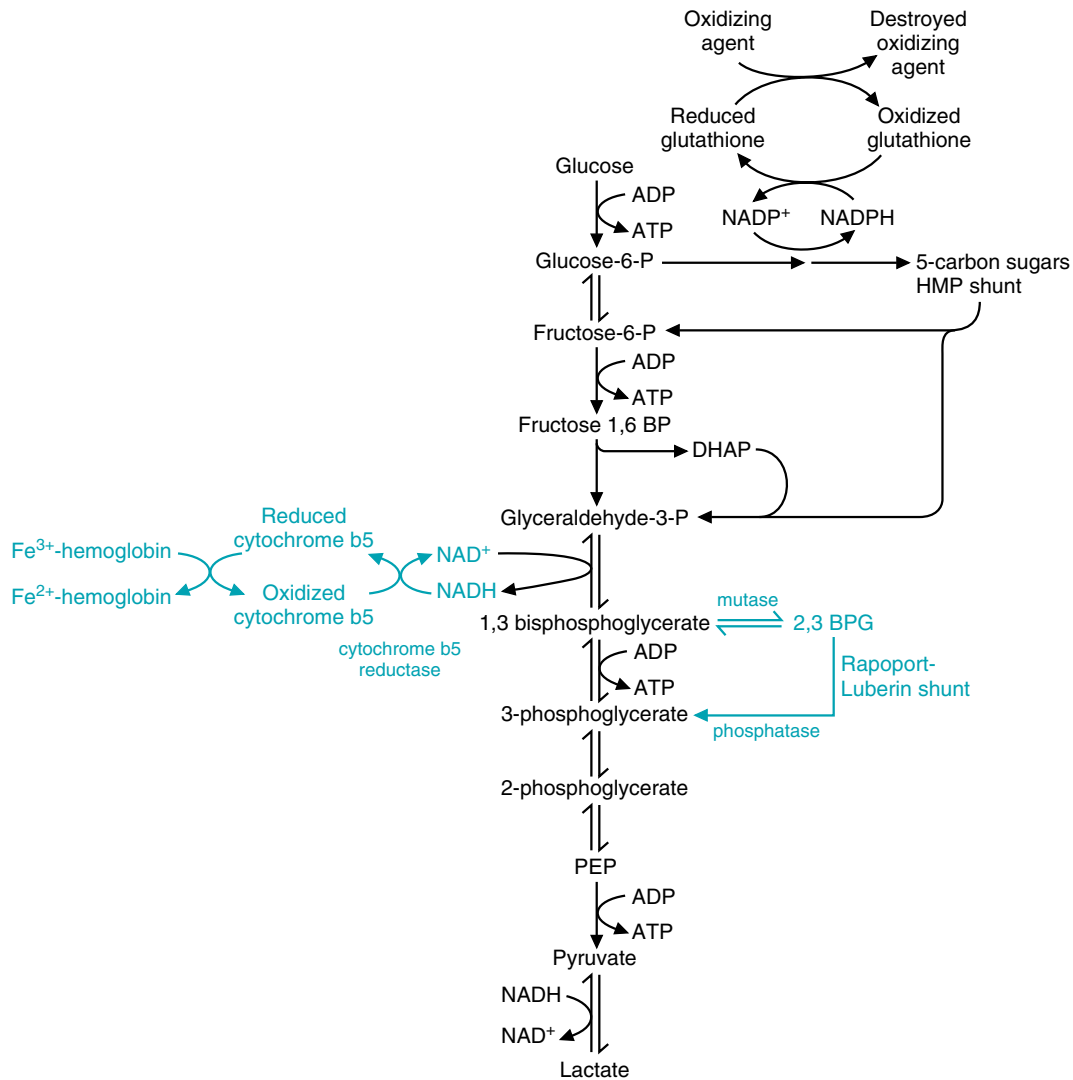
Fig. 44.1. Overview of erythrocyte metabolism. Glycolysis is the major pathway, with branches for the hexose monophosphate shunt (for protection against oxidizing agents) and the Rapoport-Luebering shunt (which generates 2,3 bisphosphoglycerate, which moderates oxygen binding to hemoglobin). The NADH generated from glycolysis can be used to reduce methemoglobin (Fe^{+3}) to normal hemoglobin (Fe^{+2}), or to convert pyruvate to lactate, such that NAD^+ can be regenerated and used for glycolysis. Pathways unique to the erythrocyte are indicated in blue.

methemoglobin. Some of the NADH produced by glycolysis is used to regenerate hemoglobin from methemoglobin by the NADH-cytochrome b_5 methemoglobin reductase system. Cytochrome b_5 reduces the Fe^{+3} of methemoglobin. The oxidized cytochrome b_5 is then reduced by a flavin-containing enzyme, cytochrome b_5 reductase (also called methemoglobin reductase), using NADH as the reducing agent.

An inherited deficiency in pyruvate kinase leads to hemolytic anemia (an anemia caused by the destruction of red blood cells; hemoglobin values typically drop to 4 to 10 g/dL in this condition). Because the amount of ATP formed from glycolysis is decreased by 50%, red blood cell ion transporters cannot function effectively. The red blood cells tend to gain Ca^{2+} and lose K^+ and water. The water loss increases the intracellular hemoglobin concentration. With the increase in intracellular hemoglobin concentration, the internal viscosity of the cell is increased to the point that the cell becomes rigid and, therefore, more susceptible to damage by shear forces in the circulation. Once damaged, the red blood cells are removed from circulation, leading to the anemia. However, the effects of the anemia are frequently moderated by the twofold to threefold elevation in 2,3-BPG concentration that results from the blockage of the conversion of phosphoenol pyruvate to pyruvate. Because 2,3-BPG binding to hemoglobin decreases the affinity of hemoglobin of oxygen, the red blood cells that remain in circulation are highly efficient in releasing their bound oxygen to the tissues.

Congenital methemoglobinemia, the presence of excess methemoglobin, is found in people with an enzymatic deficiency in cytochrome b_5 reductase or in people who have inherited hemoglobin M. In hemoglobin M, a single amino acid substitution in the heme-binding pocket stabilizes the ferric (Fe^{+3}) oxygen. Individuals with congenital methemoglobinemia appear cyanotic but have few clinical problems. Methemoglobinemia can be acquired by ingestion of certain oxidants such as nitrites, quinones, aniline, and sulfonamides. Acquired methemoglobinemia can be treated by the administration of reducing agents, such as ascorbic acid or methylene blue.

G6PD deficiency is the most common enzyme deficiency known in humans, probably, in part, because individuals with G6PD deficiency are resistant to malaria. The resistance to malaria counterbalances the deleterious effects of the deficiency. G6PD-deficient red cells have a shorter life span and are more likely to lyse under conditions of oxidative stress. When soldiers during the Korean War were given the antimalarial drug primaquine prophylactically, approximately 10% of the soldiers of African ancestry developed a spontaneous anemia. Because the gene for G6PD is found on the X chromosome, these men had only one copy of a variant G6PD gene

All known G6PD variant genes contain small in-frame deletions or missense mutations. The corresponding proteins, therefore, have decreased stability or lowered activity, leading to a reduced half-life or lifespan for the red cell. No mutations have been found that result in complete absence of G6PD. Based on studies with knockout mice, those mutations would be expected to result in embryonic lethality.

Heme, which is red, is responsible for the color of red blood cells and of muscles that contain a large number of mitochondria.

Chlorophyll, the major porphyrin in plants, is similar to heme, except that it is coordinated with magnesium rather than iron, and it contains different substituents on the rings, including a long-chain alcohol (phytol). As a result of these structural differences, chlorophyll is green.

Approximately 5 to 10% of the glucose metabolized by red blood cells is used to generate NADPH by way of the hexose monophosphate shunt. The NADPH is used to maintain glutathione in the reduced state. The glutathione cycle is the red blood cell's chief defense against damage to proteins and lipids by reactive oxygen species (see Chapter 24).

The enzyme that catalyzes the first step of the hexose monophosphate shunt is glucose-6-phosphate dehydrogenase (G6PD). The lifetime of the red blood cell correlates with G6PD activity. Lacking ribosomes, the red blood cell cannot synthesize new G6PD protein. Consequently, as the G6PD activity decreases, oxidative damage accumulates, leading to lysis of the erythrocyte. When red blood cell lysis (hemolysis) substantially exceeds the normal rate of red blood cell production, the number of erythrocytes in the blood drops below normal values, leading to a **hemolytic anemia.**

B. The Erythrocyte Precursor Cells and Heme Synthesis

1.   HEME STRUCTURE

Heme consists of a porphyrin ring coordinated with an atom of iron (Fig. 44.2). Four pyrrole rings are joined by methionyl bridges (—CH—) to form the porphyrin ring (see Fig. 7.12). Eight side chains serve as substituents on the porphyrin ring, two on each pyrrole. These side chains may be acetyl (A), propionyl (P), methyl (M), or vinyl (V) groups. In heme, the order of these groups is M V M V M P P M. This order, in which the position of the methyl group is reversed on the fourth ring, is characteristic of the porphyrins of the type III series, the most abundant in nature.

Heme is the most common porphyrin found in the body. It is complexed with proteins to form hemoglobin, myoglobin, and the cytochromes (see Chapters 7 and 21), including cytochrome P450 (see Chapter 24).

2.   SYNTHESIS OF HEME

Heme is synthesized from glycine and succinyl CoA (Fig. 44.3), which condense in the initial reaction to form δ-aminolevulinic acid (δ-ALA) (Fig 44.4). The enzyme that catalyzes this reaction, δ-ALA synthase, requires the participation of pyridoxal phosphate, as the reaction is an amino acid decarboxylation reaction (glycine is decarboxylated; see Chapter 39).

The next reaction of heme synthesis is catalyzed by δ-ALA dehydratase, in which two molecules of δ-ALA condense to form the pyrrole, porphobilinogen (Fig. 44.5). Four of these pyrrole rings condense to form a linear chain and then a series of porphyrinogens. The side chains of these porphyrinogens initially contain
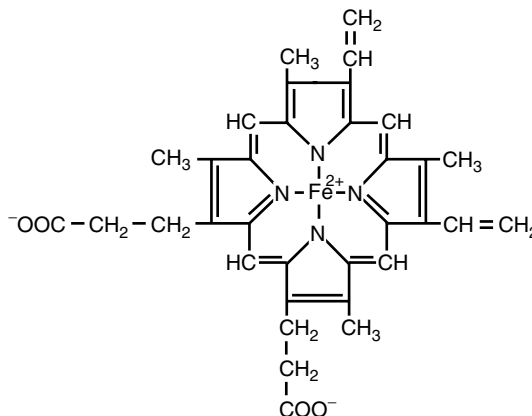

Fig. 44.2. Structure of heme. The side chains can be abbreviated as MVMVMPPM. M = methyl (CH_3); V = vinyl (—CH=CH_2); P = propionyl (—CH_2—CH_2—COO^-).
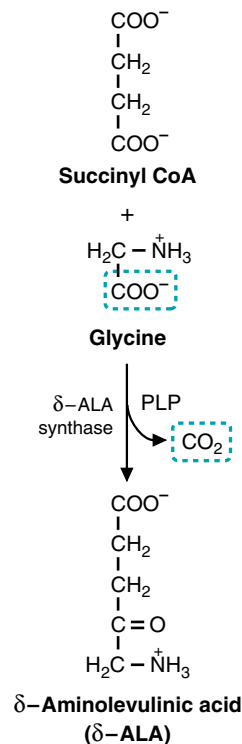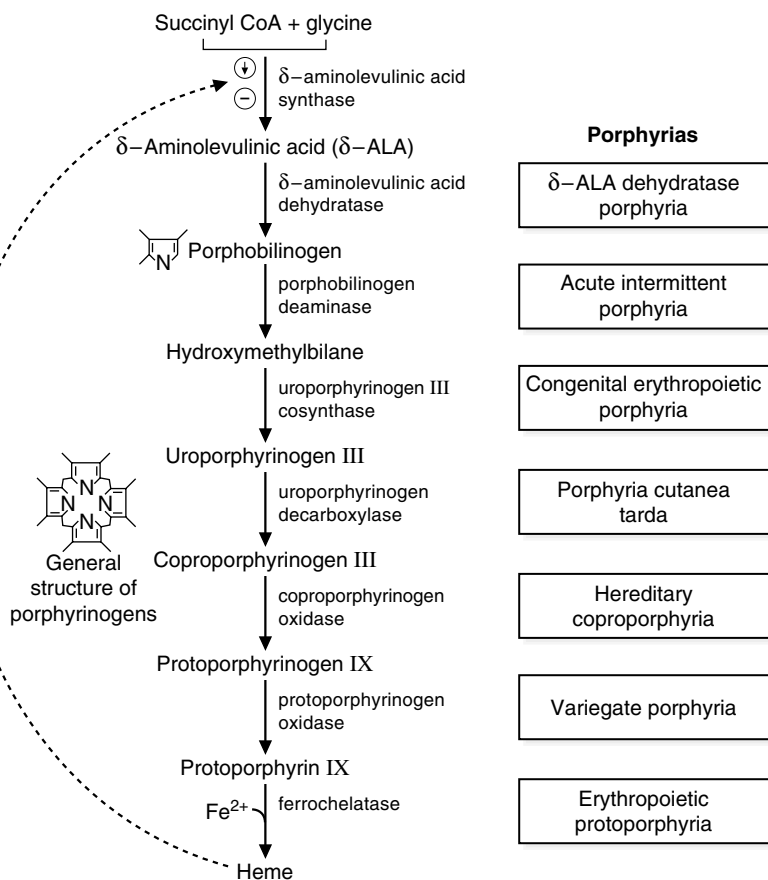
Fig. 44.3. Synthesis of heme. To produce one molecule of heme, 8 molecules each of glycine and succinyl CoA are required. A series of porphyrinogens are generated in sequence. Finally, iron is added to produce heme. Heme regulates its own production by repressing the synthesis of δ-aminolevulinic acid (δ-ALA) synthase (circled ↓) and by directly inhibiting the activity of this enzyme (circled −). Deficiencies of enzymes in the pathway result in a series of diseases known as porphyrias (listed on the right, beside the deficient enzyme).

Fig. 44.4. Synthesis of δ-aminolevulinic acid (δ-ALA). PLP = pyridoxal phosphate.

acetyl (A) and propionyl (P) groups. The acetyl groups are decarboxylated to form methyl groups. Then the first two propionyl side chains are decarboxylated and oxidized to vinyl groups, forming a protoporphyrinogen. The methylene bridges are subsequently oxidized to form protoporphyrin IX (see Fig. 44.3).

In the final step of the pathway, iron (as Fe^{2+}) is incorporated into protoporphyrin IX in a reaction catalyzed by ferrochelatase (also known as heme synthase).

3.   SOURCE OF IRON

Iron, which is obtained from the diet, has a Recommended Dietary Allowance (RDA) of 10 mg for men and postmenopausal women, and 15 mg for premenopausal women. The average American diet contains 10 to 50 mg of iron. However, only 10 to 15% is normally absorbed, and iron deficiencies are fairly common.

Q: Pyridoxine (vitamin B6) deficiencies are often associated with a microcytic, hypochromic anemia. Why would a B6 deficiency result in small (microcytic), pale (hypochromic) red blood cells?

δ-ALA dehydratase, which contains zinc, and ferrochelatase are inactivated by lead. Thus, in lead poisoning, δ-ALA and protoporphyrin IX accumulate, and the production of heme is decreased. Anemia results from a lack of hemoglobin, and energy production decreases because of the lack of cytochromes for the electron transport chain.
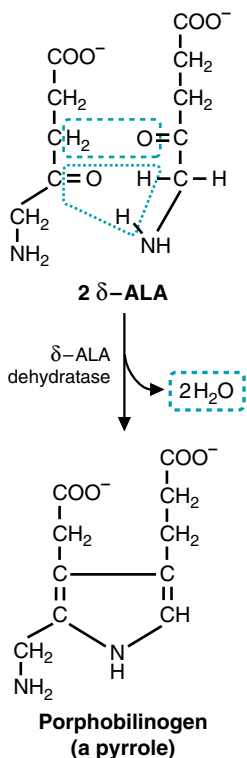
Porphyrias are a group of rare inherited disorders resulting from deficiencies of enzymes in the pathway for heme biosynthesis (see Fig. 44.3). Intermediates of the pathway accumulate and may have toxic effects on the nervous system that cause neuropsychiatric symptoms. When porphyrinogens accumulate, they may be converted by light to porphyrins, which react with molecular oxygen to form oxygen radicals. These radicals may cause severe damage to the skin. Thus, individuals with excessive production of porphyrins are photosensitive. The scarring and increased growth of facial hair seen in some porphyrias may have contributed to the development of the werewolf legends.

Fig. 44.5. Two molecules of δ-ALA condense to form porphobilinogen.

In a B6 deficiency, the rate of heme production is slow because the first reaction in heme synthesis requires pyridoxal phosphate (see Fig. 44.4). Thus, less heme is synthesized, causing red blood cells to be small and pale. Iron stores are usually elevated.

The iron lost by adult males (approximately 1 mg/day) by desquamation of the skin and in bile, feces, urine, and sweat is replaced by iron absorbed from the diet. Men are not as likely to suffer from iron deficiencies as premenopausal adult women, who also lose iron during menstruation and who must supply iron to meet the needs of the growing fetus during a pregnancy. If a man eating a Western diet has iron-deficiency anemia, his physician should suspect bleeding from the gastrointestinal tract due to ulcers or colon cancer.

Although spinach has been touted as a wonderful source of iron (mostly by the cartoon character Popeye), this iron is not readily absorbed because spinach has a high content of phytate (inositol with a phosphate group attached to each of its 6 hydroxyl groups).

The iron in meats is in the form of heme, which is readily absorbed. The non-heme iron in plants is not as readily absorbed, in part because plants often contain oxalates, phytates, tannins, and other phenolic compounds that chelate or form insoluble precipitates with iron, preventing its absorption. Conversely, vitamin C (ascorbic acid) increases the uptake of non-heme iron from the digestive tract. The uptake of iron is also increased in times of need by mechanisms that are not yet understood. Iron is absorbed in the ferrous (Fe^{2+}) state (Fig. 44.6), but is oxidized to the ferric state by a ferroxidase known as ceruloplasmin (a copper-containing enzyme) for transport through the body.

Because free iron is toxic, it is usually found in the body bound to proteins (see Fig. 44.6). Iron is carried in the blood (as Fe^{3+}) by the protein apotransferrin, with which it forms a complex known as transferrin. Transferrin is usually only one-third saturated with iron. The total iron-binding capacity of blood, mainly due to its content of transferrin, is approximately 300 μg/dL.

Storage of iron occurs in most cells but especially those of the liver, spleen, and bone marrow. In these cells, the storage protein, apoferritin, forms a complex with iron (Fe^{3+}) known as ferritin. Normally, little ferritin is present in the blood. This amount increases, however, as iron stores increase. Therefore, the amount of ferritin in the blood is the most sensitive indicator of the amount of iron in the body's stores.

Iron can be drawn from ferritin stores, transported in the blood as transferrin, and taken up via receptor-mediated endocytosis by cells that require iron (e.g., by reticulocytes that are synthesizing hemoglobin). When excess iron is absorbed from the diet, it is stored as hemosiderin, a form of ferritin complexed with additional iron that cannot be readily mobilized.

4.   REGULATION OF HEME SYNTHESIS

Heme regulates its own synthesis by mechanisms that affect the first enzyme in the pathway, δ-ALA synthase (see Fig. 44.3). Heme represses the synthesis of this enzyme, and also directly inhibits the activity of the enzyme (an allosteric modifier). Thus, heme is synthesized when heme levels fall. As heme levels rise, the rate of heme synthesis decreases.

Heme also regulates the synthesis of hemoglobin by stimulating synthesis of the protein globin. Heme maintains the ribosomal initiation complex for globin synthesis in an active state (see Chapter 15).

5.   DEGRADATION OF HEME

Heme is degraded to form bilirubin, which is conjugated with glucuronic acid and excreted in the bile (Fig. 44.7). Although heme from cytochromes and myoglobin also undergoes conversion to bilirubin, the major source of this bile pigment is hemoglobin. After red blood cells reach the end of their lifespan (approximately 120 days), they are phagocytosed by cells of the reticuloendothelial system. Globin is cleaved to its constituent amino acids, and iron is returned to the body's iron stores. Heme is oxidized and cleaved to produce carbon monoxide and biliverdin (Fig. 44.8). Biliverdin is reduced to bilirubin, which is transported to the liver complexed with serum albumin.

In the liver, bilirubin is converted to a more water-soluble compound by reacting with UDP-glucuronate to form bilirubin monoglucuronide, which is converted to the diglucuronide (see Fig. 30.5). This conjugated form of bilirubin is excreted into the bile.

Drugs, such as phenobarbital, induce enzymes of the drug metabolizing systems of the endoplasmic reticulum that contain cytochrome P450. Because heme is used for synthesis of cytochrome P450, free heme levels will fall and δ-ALA synthase will be induced to increase the rate of heme synthesis.
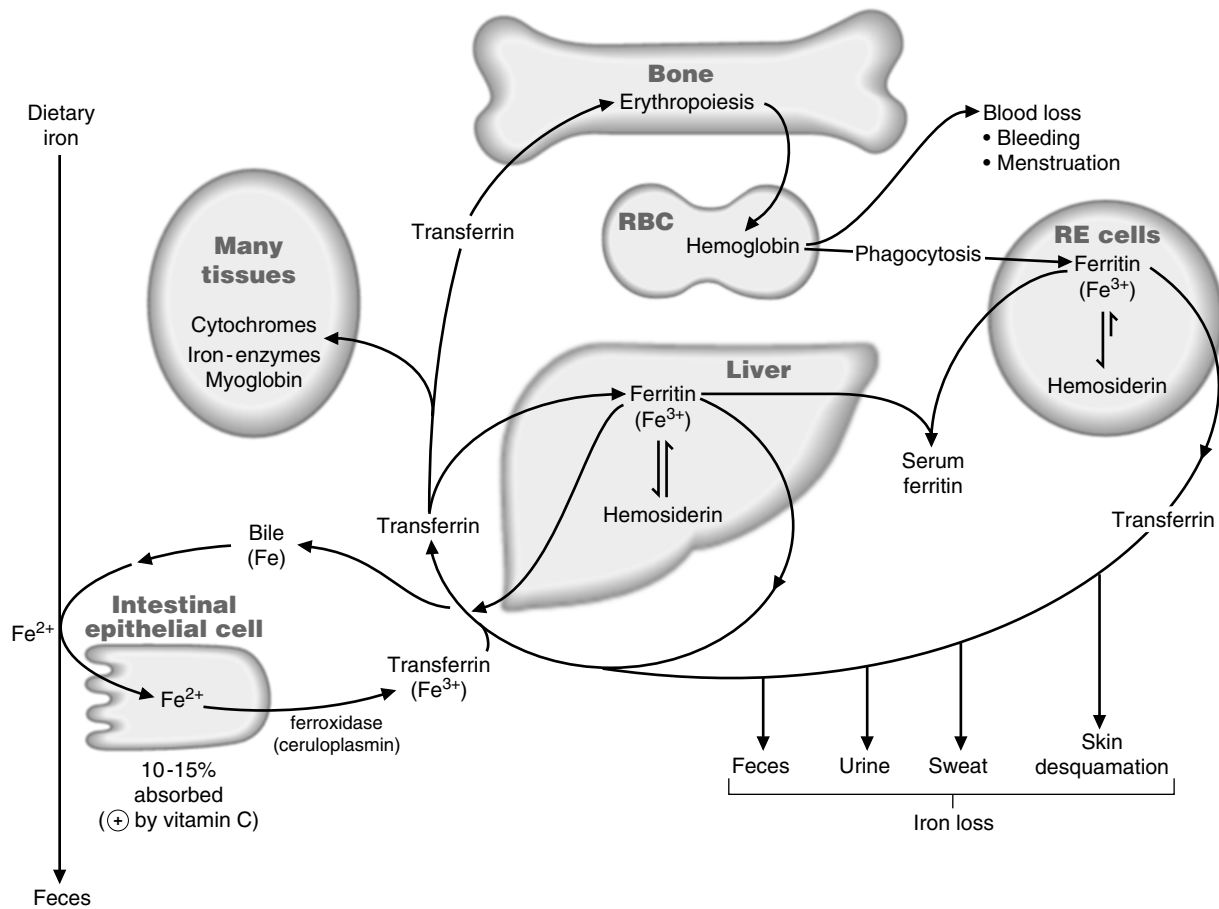
Fig. 44.6. Iron metabolism. Iron is absorbed from the diet, transported in the blood in transferrin, stored in ferritin, and used for the synthesis of cytochromes, iron-containing enzymes, hemoglobin, and myoglobin. It is lost from the body with bleeding and sloughed-off cells, sweat, urine, and feces. Hemosiderin is the protein in which excess iron is stored. Small amounts of ferritin enter the blood and can be used to measure the adequacy of iron stores. RE = reticuloendothelial.

In the intestine, bacteria deconjugate bilirubin diglucuronide and convert the bilirubin to urobilinogens (see Fig. 44.7). Some urobilinogen is absorbed into the blood and excreted in the urine. However, most of the urobilinogen is oxidized to urobilins, such as stercobilin, and excreted in the feces. These pigments give feces their brown color.

Q: In an iron deficiency, what characteristics would blood exhibit?

III. THE RED BLOOD CELL MEMBRANE

Under the microscope, the red blood cell appears to be a red disc with a pale central area (biconcave disc) (Fig.44.9). The biconcave disc shape (as opposed to a spherical shape) serves to facilitate gas exchange across the cell membrane. The membrane proteins that maintain the shape of the red blood cell also allow the red blood cell to traverse the capillaries with very small luminal diameters to deliver oxygen to the tissues. The interior diameters of many capillaries are smaller than the approximately 7.5-μm diameter of the red cell. Furthermore, in passing through the kidney, red blood cells traverse hypertonic areas that are up to six times the normal isotonicity, and back again, causing the red cell to shrink and expand during its travels. The spleen is the organ responsible for determining the viability of the red blood cells. Erythrocytes pass through the spleen 120 times per day. The elliptical passageways through the spleen are approximately 3 μm in diameter, and
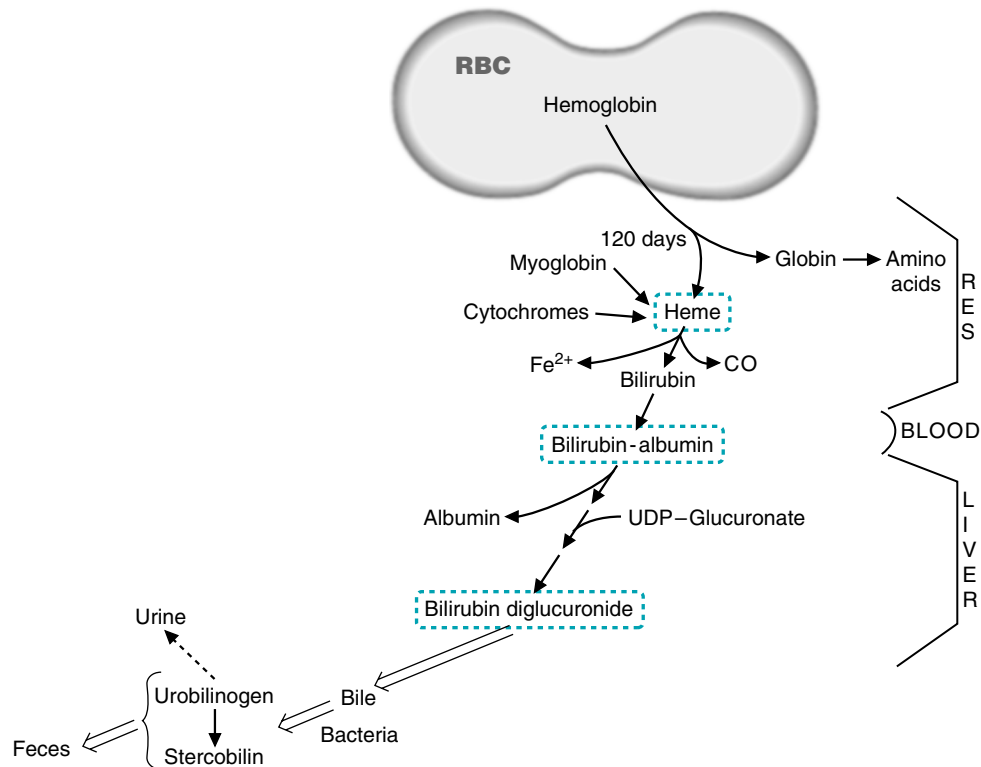
Fig. 44.7. Overview of heme degradation. Heme is degraded to bilirubin, carried in the blood by albumin, conjugated to form the diglucuronide in the liver, and excreted in the bile. The iron is returned to the body's iron stores. RES = reticuloendothelial system: RBC = red blood cells.

A: Iron deficiency would result in a microcytic, hypochromic anemia. Red blood cells would be small and pale. In contrast to a vitamin B6 deficiency, which also results in a microcytic, hypochromic anemia, iron stores are low in an iron-deficiency anemia.

The unusual names for some erythrocyte membrane proteins, such as band 4.1, arose through analysis of red blood cell membranes by polyacrylamide gel electrophoresis. The stained bands observed in the gel were numbered according to molecular weight (band 1, band 2, and so on), and as functions were assigned to the proteins, more common names were assigned to the proteins (for example, spectrin is actually band 1).

normal red cells traverse them in approximately 30 seconds. Thus, to survive in the circulation, the red cell must be highly deformable. Damaged red cells that are no longer deformable become trapped in the passages in the spleen, where they are destroyed by macrophages. The reason for the erythrocyte's deformability lies in its shape and in the organization of the proteins that make up the red blood cell membrane.

The surface area of the red cell is approximately 140 μm^2, which is greater than the surface of a sphere needed to enclose the contents of the red cell (98 μm^2). The presence of this extra membrane and the cytoskeleton that supports it allows the red cell to be stretched and deformed by mechanical stresses as the cell passes through narrow vascular beds. On the cytoplasmic side of the membrane, proteins form a two-dimensional lattice that gives the red cell its flexibility (Fig. 44.10). The major proteins are spectrin, actin, band 4.1, band 4.2, and ankyrin. Spectrin, the major protein, is a heterodimer composed of α and β subunits wound around each other. The dimers self-associate at the heads. At the opposite end of the spectrin dimers, actin and band 4.1 bind near to each other. Multiple spectrins can bind to each actin filament, resulting in a branched membrane cytoskeleton.

The spectrin cytoskeleton is connected to the membrane lipid bilayer by ankyrin, which interacts with β-spectrin and the integral membrane protein, band 3. Band 4.2 helps to stabilize this connection. Band 4.1 anchors the spectrin skeleton with the membrane by binding the integral membrane protein glycophorin C and the actin complex, which has bound multiple spectrin dimers.

When the red blood cell is subjected to mechanical stress, the spectrin network rearranges. Some spectrin molecules become uncoiled and extended; others
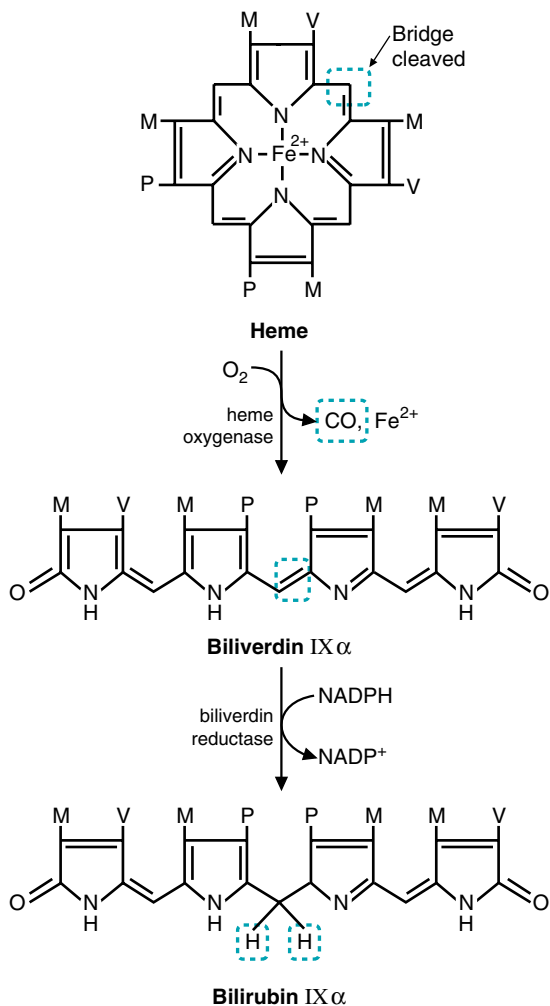
Heme

O₂

heme oxygenase → CO, Fe²⁺

Biliverdin IXα

biliverdin reductase

NADPH
NADP⁺

Bilirubin IXα

Fig. 44.8. Conversion of heme to bilirubin. A methylene bridge in heme is cleaved, releasing carbon monoxide (CO) and iron. Then, the center methylene bridge is reduced.

Fig. 44.9. The shape of the red blood cell. A. Wright-stained cells, displaying the pale staining in the center. Three leukocytes also are present in the preparation. The magnification is $350\times$. B. Scanning electron micrograph, showing the biconcave disc structure of the cells. The stacks of erythrocytes in this preparation (collected from a blood tube) is not unusual. The magnification is $28,000\times$. These photographs were obtained, with permission, from Ross et al, Histology, A Text and Atlas with Cell and Molecular Biology, 4th Ed. Philadelphia: Lippincott, 2003:216–217.

become compressed, thereby changing the shape of the cell, but not its surface area.

The mature erythrocyte cannot synthesize new membrane proteins or lipids. However, membrane lipids can be freely exchanged with circulating lipoprotein lipids. The glutathione system protects the proteins and lipids from oxidative damage.

IV. AGENTS THAT AFFECT OXYGEN BINDING

The major agents that affect oxygen binding to hemoglobin are shown in Figure 44.11.

A. 2,3-Bisphosphoglycerate

2,3-Bisphosphoglycerate (2,3-BPG) is formed in red blood cells from the glycolytic intermediate 1,3-bisphosphoglycerate, as indicated in Figure 44.1. 2,3-BPG binds to hemoglobin in the central cavity formed by the four subunits, increasing the energy required for the conformational changes that facilitate the binding of oxygen. Thus, 2,3-BPG lowers the affinity of hemoglobin for oxygen. Therefore, oxygen is less readily bound (i.e., more readily released in tissues) when hemoglobin contains 2,3-BPG.
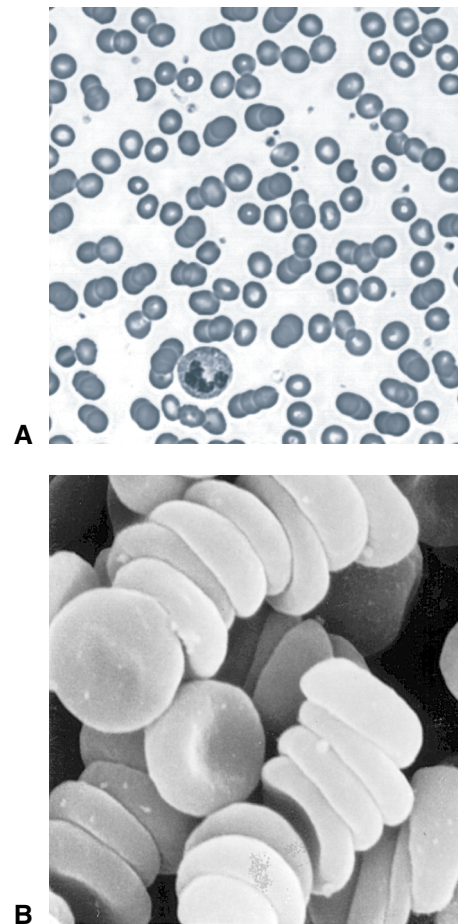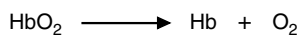
Defects in erythrocyte cytoskeletal proteins lead to hemolytic anemia. Shear stresses in the circulation result in the loss of pieces of the red cell membrane. As the membrane is lost, the red blood cell becomes more spherical and loses its deformability. As these cells become more spherical, they are more likely to lyse in response to mechanical stresses in the circulation, or to be trapped and destroyed in the spleen.

Fig. 44.10. A generalized view of the erythrocyte cytoskeleton. A. The major protein, spectrin, is linked to the plasma membrane either through interactions with ankyrin and band 3, or with actin, band 4.1, and glycophorin. Other proteins in this complex, but not shown, are tropomyosin and adducin. B. A view from inside the cell, looking up at the cytoskeleton. This view displays the cross-linking of the spectrin dimers to actin and band 3 anchor sites.

$$HbO_2 \longrightarrow Hb + O_2$$

① Hydrogen ions

② 2,3–Bisphosphoglycerate

③ Covalent binding of CO_2

Fig. 44.11. Agents that affect oxygen binding by hemoglobin. Binding of hydrogen ions, 2,3 bisphosphoglycerate, and carbon dioxide to hemoglobin decrease its affinity for oxygen.
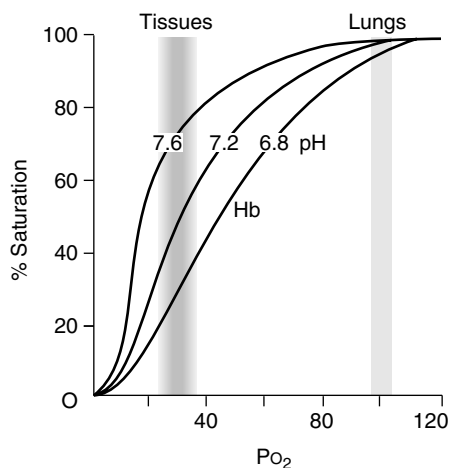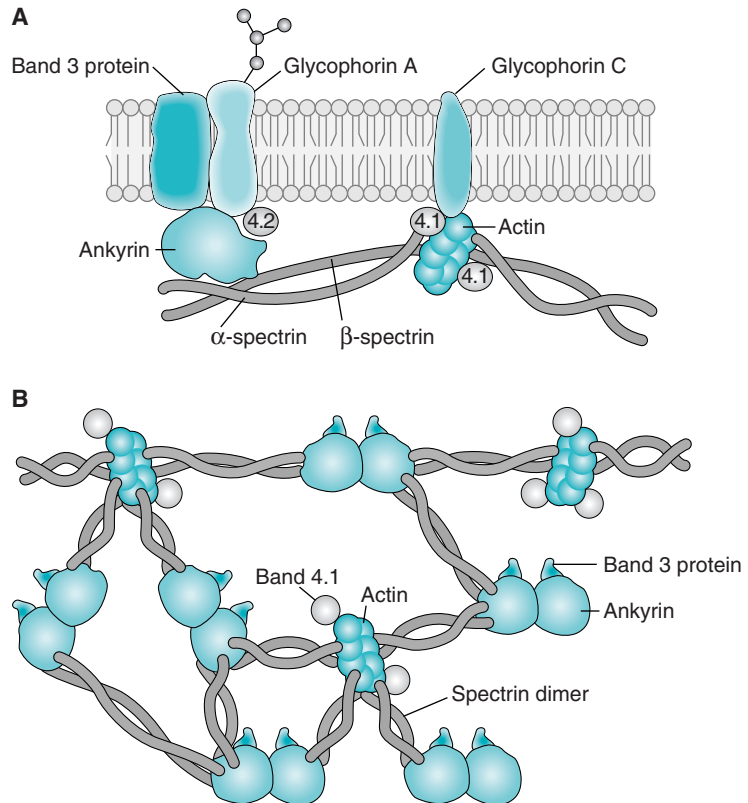
B. Proton Binding (Bohr effect)

The binding of protons by hemoglobin lowers its affinity for oxygen (Fig. 44.12), contributing to a phenomenon known as the Bohr effect (Fig. 44.13). The pH of the blood decreases as it enters the tissues (and the proton concentration rises) because the CO_2 produced by metabolism is converted to carbonic acid by the reaction catalyzed by carbonic anhydrase in red blood cells. Dissociation of carbonic acid produces protons that react with several amino acid residues in hemoglobin, causing conformational changes that promote the release of oxygen.

In the lungs, this process is reversed. Oxygen binds to hemoglobin, causing a release of protons, which combine with bicarbonate to form carbonic acid. This decrease of protons causes the pH of the blood to rise. Carbonic anhydrase cleaves the carbonic acid to H_2O and CO_2, and the CO_2 is exhaled. Thus, in tissues in which the pH of the blood is low because of the CO_2 produced by metabolism, oxygen is released from hemoglobin. In the lungs, where the pH of the blood is higher because CO_2 is being exhaled, oxygen binds to hemoglobin.

Fig. 44.12. Effect of pH on oxygen saturation curves. As the pH decreases, the affinity of hemoglobin for oxygen decreases, producing the Bohr effect.

C. Carbon Dioxide

Although most of the CO_2 produced by metabolism in the tissues is carried to the lungs as bicarbonate, some of the CO_2 is covalently bound to hemoglobin (Fig. 44.14). In the tissues, CO_2 forms carbamate adducts with the N-terminal amino groups of deoxyhemoglobin and stabilizes the deoxy conformation. In the
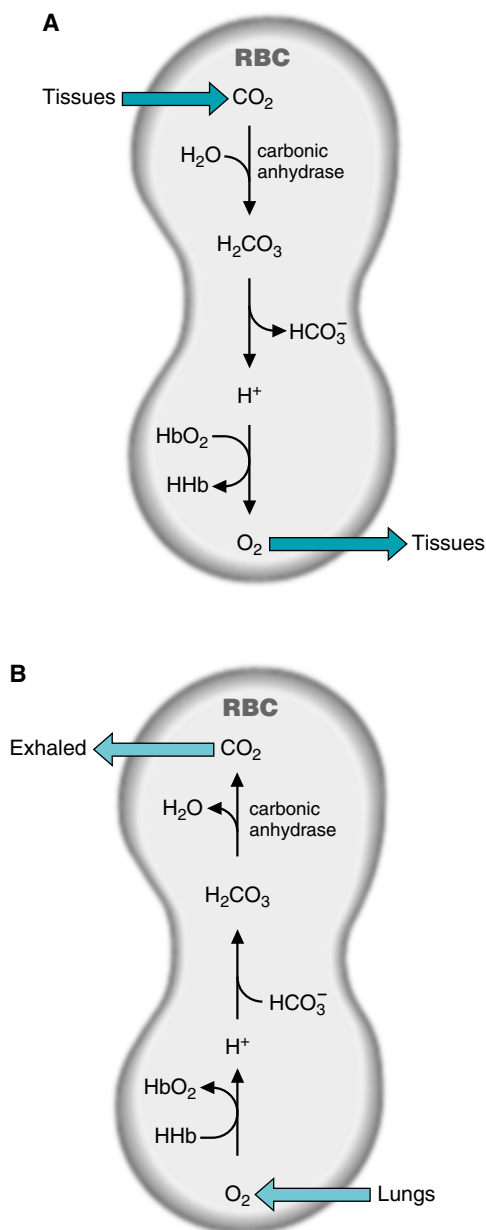
$$Hb - \overset{+}{N}H_3 \ + \ \boxed{CO_2}$$

Hemoglobin

$$Hb - \underset{\underset{H}{|}}{N} - \boxed{COO^-} \ + \ H^+$$

Carbamate of hemoglobin

Fig. 44.14. Binding of CO_2 to hemoglobin. CO_2 forms carbamates with the N-terminal amino groups of Hb chains. Approximately 15% of the CO_2 in blood is carried to the lungs bound to Hb. The reaction releases protons, which contribute to the Bohr effect. The overall effect is the stabilization of the deoxy form of hemoglobin.

Fig. 44.13. Effect of H^+ on oxygen binding by hemoglobin (Hb). A. In the tissues, CO_2 is released. In the red blood cell, this CO_2 forms carbonic acid, which releases protons. The protons bind to Hb, causing it to release oxygen to the tissues. B. In the lungs, the reactions are reversed. O_2 binds to protonated Hb, causing the release of protons. They bind to bicarbonate (HCO_3^-), forming carbonic acid, which is cleaved to water and CO_2, which is exhaled.

lungs, where the pO_2 is high, oxygen binds to hemoglobin and this bound CO_2 is released.

V. HEMATOPOIESIS

The various types of cells (lineages) that make up the blood are constantly being produced in the bone marrow. All cell lineages are descended from hematopoietic stem cells, cells that are renewable throughout the life of the host. The population of hematopoietic stem cells is quite small. Estimates vary between 1 to 10 per 10^5 bone marrow cells. In the presence of the appropriate signals, hematopoietic stem
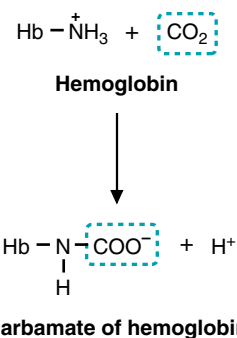
Populations of hematopoietic cells enriched with stem cells can be isolated by fluorescence activated cell sorting, based on the expression of specific cell surface markers. Increasing the population of stem cells in cells used for a bone marrow transplantation increases the chances of success of the transplantation.
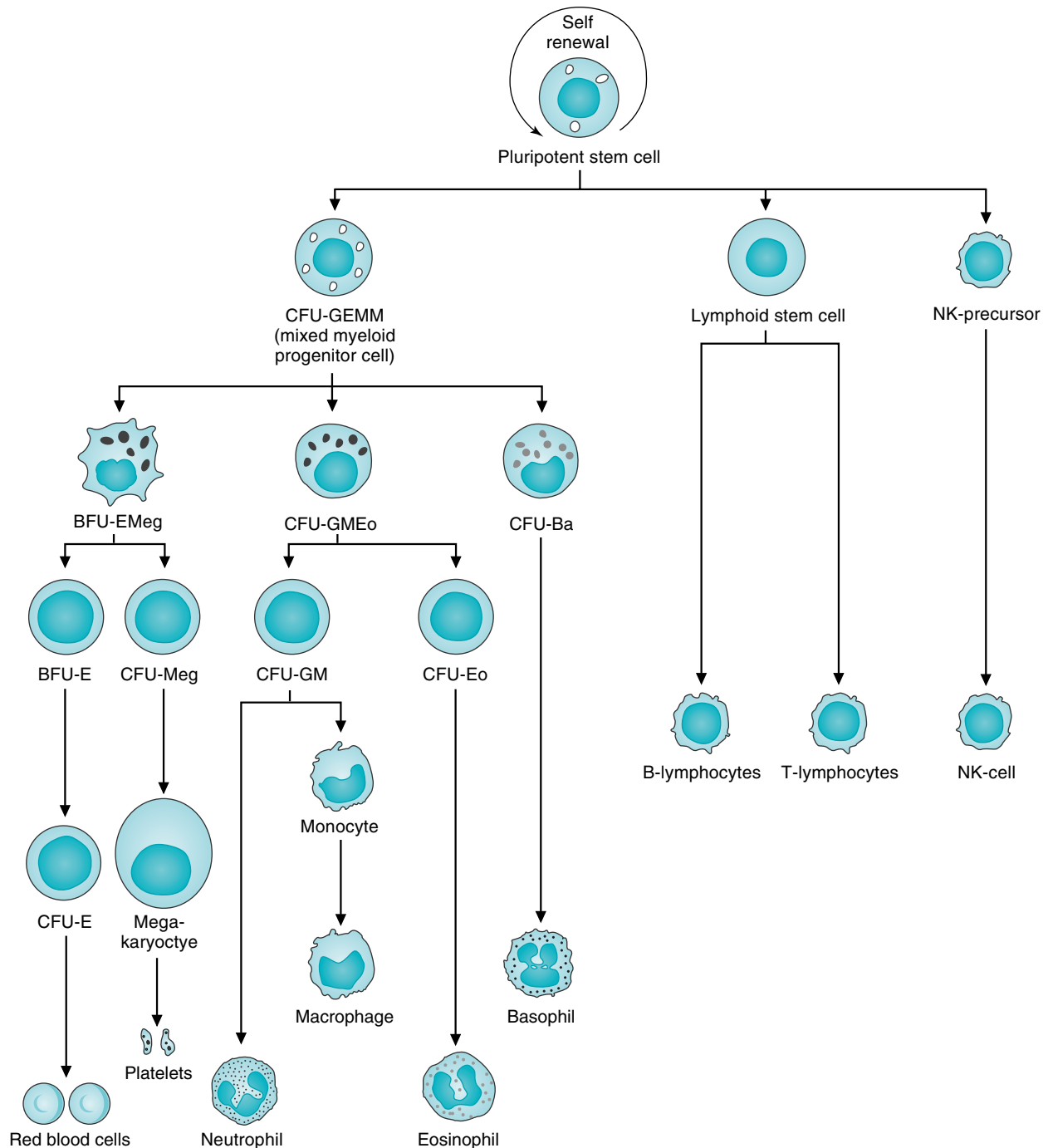
Fig. 44.15. The hematopoietic tree. All blood cells arise from the self-renewing pluripotent stem cell. Different cytokines are required at each step for these events to occur. CFU = colony-forming unit; BFU = burst-forming unit.

Leukemias, malignancies of the blood, arise when a differentiating hematopoietic cell does not complete its developmental program but remains in an immature, proliferative state. Leukemias have been found in every hematopoietic lineage.

cells proliferate, differentiate, and mature into any of the types of cells that make up the blood (Figure 44.15).

Hematopoietic differentiation is hierarchical. The number of fates a developing blood cell may adopt becomes progressively restricted. Hematopoietic progenitors are designated colony-forming unit–lineage, or colony-forming unit–erythroid (CFU-E). Progenitors that form very large colonies are termed burst-forming units.

A. Cytokines and Hematopoiesis

Developing progenitor cells in the marrow grow in proximity with marrow stromal cells. These include fibroblasts, endothelial cells, adipocytes, and macrophages. The stromal cells form an extracellular matrix and secrete growth factors that regulate hematopoietic development.

The hematopoietic growth factors have multiple effects. An individual growth factor may stimulate proliferation, differentiation, and maturation of the progenitor cells and also may prevent apoptosis. These factors also may activate various functions within the mature cell. Some hematopoietic growth factors act on multiple lineages, whereas others have more limited targets.

Most hematopoietic growth factors are recognized by receptors belonging to the cytokine receptor superfamily. Binding of ligand to receptor results in receptor aggregation, which induces phosphorylation of Janus kinases (JAKs). The JAKs are a family of cytoplasmic tyrosine kinases that are active when phosphorylated (see Chapter 11, section III.C., and Fig. 11.15). The activated JAKs then phosphorylate the cytokine receptor. Phosphorylation of the receptor creates docking regions where additional signal transduction molecules bind, including members of the signal transducer and activator of transcription (STAT) family of transcription factors. The JAKs phosphorylate the STATs, which dimerize and translocate to the nucleus, where they activate target genes. Additional signal transduction proteins bind to the phosphorylated cytokine receptor, leading to activation of the Ras/Raf/MAP kinase pathways. Other pathways are also activated, some of which lead to an inhibition of apoptosis (see Chapter 18).

The response to cytokine binding is usually transient because the cell contains multiple negative regulators of cytokine signaling. The family of **s**ilencer **o**f **c**ytokine **s**ignaling (SOCS) proteins are induced by cytokine binding. One member of the family binds to the phosphorylated receptor and prevents the docking of signal transduction proteins. Other SOCS proteins bind to JAKs and inhibit them. Whether SOCS inhibition of JAKs is a consequence of steric inhibition or whether SOCS recruit phosphatases that then dephosphorylate the JAKs (Figure 44.16) is uncertain.

SHP-1 is a tyrosine phosphatase found primarily in hematopoietic cells that is necessary for proper development of myeloid and lymphoid lineages. Its function is to dephosphorylate JAK2, thereby inactivating it.

STATs are also inactivated. The **p**rotein **i**nhibitors of **a**ctivated **S**TAT (PIAS) family of proteins bind to phosphorylated STATs and prevent their dimerization or promote the dissociation of STAT dimers. STATs also may be inactivated by dephosphorylation, although the specific phosphatases have not yet been identified, or by targeting activated STATs for proteolytic degradation.

B. Erythropoiesis

The production of red cells is regulated by the demands of oxygen delivery to the tissues. In response to reduced tissue oxygenation, the kidney releases the hormone erythropoietin, which stimulates the multiplication and maturation of erythroid progenitors. The progression along the erythroid pathway begins with the stem cell and passes through the mixed myeloid progenitor cell, (CFU-GEMM, colony-forming unit–granulocyte, erythroid, monocyte, megakaryocyte), burst-forming unit–erythroid (BFU-E), colony-forming unit–erythroid (CFU-E), and to the first recognizable red cell precursor, the normoblast. Each normoblast undergoes four more cycles of cell division. During these four cycles, the nucleus becomes smaller and more condensed. After the last division, the nucleus is extruded. The red cell at this state is called a reticulocyte. Reticulocytes still retain ribosomes and mRNA and are capable of synthesizing hemoglobin. They are released from the bone marrow and circulate for 1 to 2 days. Reticulocytes mature in the spleen, where the ribosomes and mRNA are lost (Fig. 44.17).

Bone marrow cells can be cultured in semisolid media with the addition of the appropriate growth factors. After 14 to 18 days in culture, colonies of blood cells can be seen. The type (lineage) of these cells can be determined based on morphological or staining properties. Most colonies will be of single lineage, indicating descent from a hematopoietic progenitor that was committed to a lineage. Occasionally a multilineage colony will be obtained, indicating that it was derived from a more primitive hematopoietic progenitor.

In X-linked severe combined immunodeficiency disease (SCID), the most common form of SCID, circulating T lymphocytes are not formed, and B lymphocytes are not active. The affected gene encodes the gamma chain of the interleukin 2 receptor. Mutant receptors are unable to activate JAK3, and the cells are unresponsive to the cytokines that stimulate growth and differentiation. Recall also that adenosine deaminase deficiency (see Chapter 41), which is not X-linked, also leads to a form of SCID, but for different reasons.

Families have been identified whose members have a mutant erythropoietin (epo) receptor that is unable to bind SHP-1. Erythropoietin is the hematopoietic cytokine that stimulates production of red blood cells. Individuals with the mutant epo receptor have a higher than normal percentage of red blood cells in the circulation, because the mutant epo receptor cannot be deactivated by SHP-1. Erythropoietin causes sustained activation of JAK2 and STAT 5 in this case.

Perturbed JAK/STAT signaling is associated with development of lymphoid and myeloid leukemias, severe congenital neutropenia, a condition in which levels of circulating neutrophils are severely reduced, and Fanconi anemia, which is characterized by bone marrow failure and increased susceptibility to malignancy.
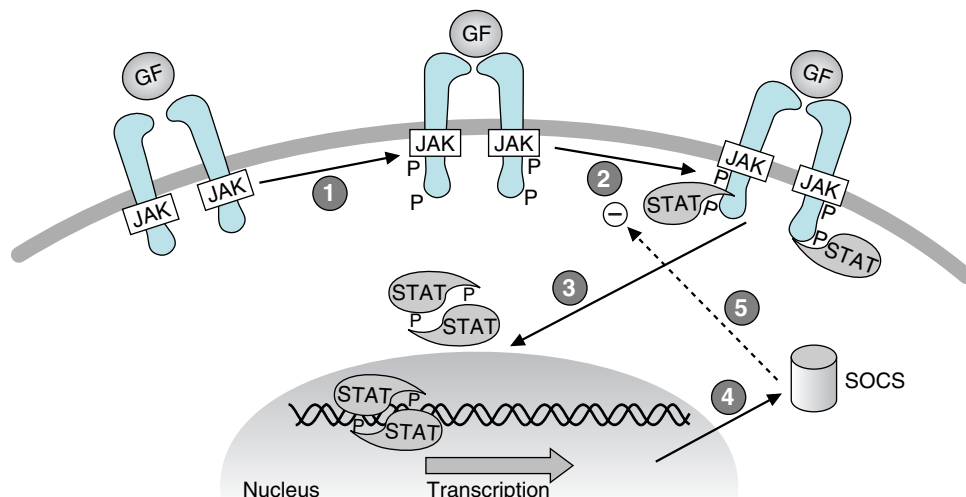
Fig. 44.16. Cytokine signaling through the JAK/STAT pathway. 1. Cytokine binding to receptors initiates dimerization and activation of the JAK kinase, which phosphorylates the receptor on tyrosine residues. 2. STAT proteins bind to the activated receptors and are themselves phosphorylated. 3. Phosphorylated STAT proteins dimerize, travel to the nucleus, and initiate gene transcription. 4. One of the proteins whose synthesis is stimulated by STATs is SOCS (**s**uppressor **o**f **c**ytokine **s**ignaling), which inhibits further activation of STAT proteins (circle 5) by a variety of mechanisms.

C. Nutritional Anemias

Each person produces approximately 10^{12} red blood cells per day. Because so many cells must be produced, nutritional deficiencies in iron, vitamin B12, and folate prevent adequate red blood cell formation. The physical appearance of the cells in the case of a nutritional anemia frequently provides a clue as to the nature of the deficiency.

In the case of iron deficiency, the cells are smaller and paler than normal. The lack of iron results in decreased heme synthesis, which in turn affects globin synthesis. Maturing red cells following their normal developmental program divide until their hemoglobin has reached the appropriate concentration. Iron- (and hemoglobin-) deficient developing red blood cells continue dividing past their normal stopping point, resulting in small (microcytic) red cells. The cells are also pale because of the lack of hemoglobin, as compared with normal cells (thus, a pale, microcytic anemia results).
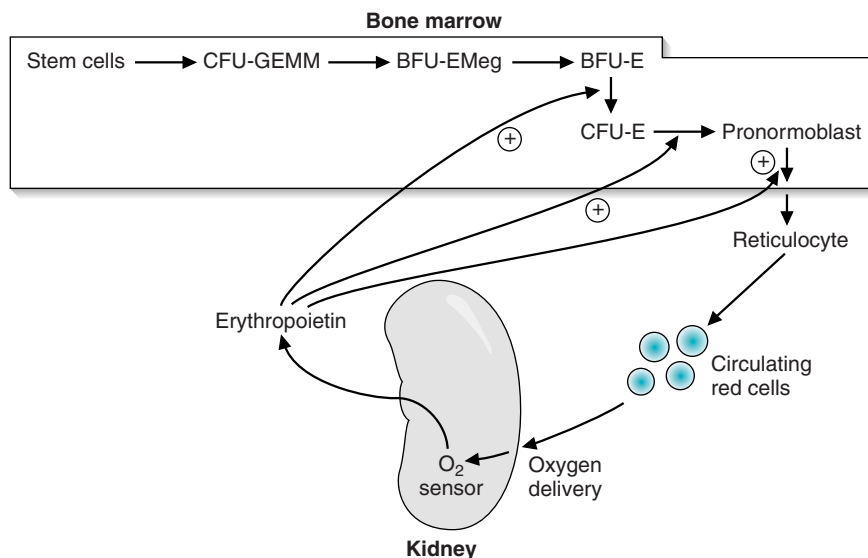

Fig. 44.17. Erythropoietin stimulation of erythrocyte maturation. The abbreviations are described in the text.

Deficiencies of folate or vitamin B12 can cause megaloblastic anemia, in which the cells are larger than normal. Folate and B12 are required for DNA synthesis (see Chapters 40 and 41). When these vitamins are deficient, DNA replication and nuclear division do not keep pace with the maturation of the cytoplasm. Consequently, the nucleus is extruded before the requisite number of cell divisions has taken place, and the cell volume is greater than it should be, and fewer blood cells are produced.

VI. HEMOGLOBINOPATHIES, HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN, AND HEMOGLOBIN SWITCHING

A. Hemoglobinopathies: Disorders in the Structure or Amount of the Globin Chains

More than 700 different mutant hemoglobins have been discovered. Most arise from a single base substitution, resulting in a single amino acid replacement. Many have been discovered during population screenings and are not clinically significant. However, in patients with hemoglobin S (HbS, sickle cell anemia), the most common hemoglobin mutation, the amino acid substitution has a devastating effect in the homozygote (see **Will Sichel** in Chapter 6). Another common hemoglobin variant, HbC, results from a glu to lys replacement in the same position as the HbS mutation. This mutation has two effects. It promotes water loss from the cell by activating the K^+ transporter by an unknown mechanism, resulting in a higher than normal concentration of hemoglobin within the cell. The amino acid replacement also substantially lowers the hemoglobin solubility in the homozygote, resulting in a tendency of the mutant hemoglobin to precipitate within the red cell, although, unlike sickle cells, the cell does not become deformed. Homozygotes for the HbC mutation have a mild hemolytic anemia. Heterozygous individuals are clinically unaffected.

B. Thalassemias

For optimum function, the hemoglobin α and β-globin chains must have the proper structure and be synthesized in a 1:1 ratio. A large excess of one subunit over the other results in the class of diseases called thalassemias. These anemias are clinically very heterogeneous, as they can arise by multiple mechanisms. Like sickle cell anemia, the thalassemia mutations provide resistance to malaria in the heterozygous state.

Hemoglobin single amino acid replacement mutations that give rise to a globin subunit of decreased stability is one mechanism by which thalassemia arises. More common, however, are mutations that result in decreased synthesis of one subunit. Alpha thalassemias usually arise from complete gene deletions. Two copies of the α-globin gene are found on each chromosome 16, for a total of 4 α-globin genes per precursor cell. If one copy of the gene is deleted, the size and hemoglobin concentration of the individual red blood cells is minimally reduced. If two copies are deleted, the red blood cells are of decreased size (microcytic) and reduced hemoglobin concentration (hypochromic). However, the individual is usually not anemic. The loss of three α-globin genes causes a moderately severe microcytic hypochromic anemia (hemoglobin 7–10 g/dL) with splenomegaly (enlarged spleen). The absence of four α-globin genes (hydrops fetalis) is usually fatal in utero.

There are two ways in which an individual could have two α-globin genes deleted. In one case, one copy of chromosome 16 could have both α-globin genes deleted, whereas the other copy had two functional α genes. In the second case, both chromosomes could have lost one of their two copies of the α-globin gene. The former possibility is more common among Asians; the latter among Africans.

A registry of hemoglobin mutations is found at the International Hemoglobin Information Center http://e20.manu.edu.mk/rcgeb/ihic/.

A complication of sickle cell disease is an increased formation of gallstones. A sickle cell crisis accompanied by the intravascular destruction of red blood cells (hemolysis) experienced by patients with sickle cell disease, such as **Will Sichel**, increases the amount of unconjugated bilirubin that is transported to the liver. If the concentration of this unconjugated bilirubin exceeds the capacity of the hepatocytes to conjugate it to the more soluble diglucuronide through interaction with hepatic UDP-glucuronate, both the total and the unconjugated bilirubin levels would rise in the blood. More unconjugated bilirubin would be secreted by the liver into the bile. The increase in unconjugated bilirubin (which is not very water-soluble) results in its precipitation within the gallbladder lumen, leading to the formation of pigmented (calcium bilirubinate) gallstones.

HbC is found in high frequency in West Africa, in regions with a high frequency of HbS. Consequently, compound heterozygotes for HbS and HbC are not uncommon both in some African regions and among African-Americans. HbS/HbC individuals have significantly more hematopathology than individuals with sickle cell trait (HbA/HbS). Polymerization of deoxygenated HbS is dependent on the HbS concentration within the cell. The presence of HbC in the compound heterozygote increases the HbS concentration by stimulating K^+ and water efflux from the cell. Because the HbC globin is produced more slowly than HbA or HbS, the proportion of HbS tends to be higher in HbS/HbC cells than in the cells of individuals with sickle cell trait (HbS/HbA). The way in which multiple mutations ameliorate or exacerbate hematologic diseases has provided insights into the molecular mechanisms of hemoglobin function and developmental regulation.

The difference in amino acid composition between the β-chains of HbA and γ-chains of HbF results in structural changes that cause HbF to have a lower affinity for 2,3-BPG than adult hemoglobin (HbA) and, thus, a greater affinity for oxygen. Therefore, the oxygen released from the mother's hemoglobin (HbA) is readily bound by HbF in the fetus. Thus, the transfer of oxygen from the mother to the fetus is facilitated by the structural difference between the hemoglobin molecule of the mother and that of the fetus.

Individuals with sickle cell anemia or beta thalassemia (usually) have intact γ-globin loci. If a way could be found to reactivate the γ-globin loci (the drug hydroxyurea is a potential candidate for this), it would be an attractive therapeutic option for the treatment of these diseases.

An additional source of variation in the levels of fetal hemoglobin is the FCP (**F-c**ell **p**roducing) locus on the short arm of the X chromosome in a region thought not to be susceptible to X inactivation. Both normal individuals and individuals with hemoglobinopathies vary in the amount of hemoglobin F they produce. In studies of normal individuals, a high level of hemoglobin F appears to be inherited as an X-linked dominant trait. The FCP locus is responsible for a substantial amount of the variation in Hemoglobin F seen among sickle cell patients. The protein encoded at the FCP locus has not been identified; current speculations are that it is a transcription factor involved in the regulation of the globin locus.

As discussed in Chapter 14, beta thalassemia is a very heterogeneous genetic disease. Insufficient β-globin synthesis can result from deletions, promoter mutations, and splice junction mutations. Heterozygotes for β^+ (some globin chain synthesis) or β null (β^0, no globin chain synthesis) are generally asymptomatic, though they typically have microcytic, hypochromic red blood cells and may have a mild anemia. β^+/β^+ homozygotes have an anemia of variable severity, β^+/β^0 compound heterozygotes tend to be more severely affected, and β^0/β^0 homozygotes have severe disease. In general, diseases of β chain deficiency are more severe than diseases of α chain deficiency. Excess β chains form a homotetramer, hemoglobin H (HbH), which is useless for delivering oxygen to the tissues because of its high oxygen affinity. As red blood cells age, HbH will precipitate in the cells, forming inclusion bodies. Red blood cells with inclusion bodies have a shortened life span, because they are more likely to be trapped and destroyed in the spleen. Excess α chains are unable to form a stable tetramer. However, excess α chains precipitate in erythrocytes at every developmental stage. The α chain precipitation in erythroid precursors results in their widespread destruction, a process called ineffective erythropoiesis. The precipitated α chains also damage red blood cell membranes through the heme-facilitated lipid oxidation by reactive oxygen species. Both lipids and proteins, particularly band 4.1, are damaged.

C. Hereditary Persistence of Fetal Hemoglobin

Fetal hemoglobin (HbF), the predominant hemoglobin of the fetal period, consists of two alpha chains and two gamma chains, whereas adult Hb consists of two alpha and two beta chains. The process that regulates the conversion of HbF to HbA is called hemoglobin switching. Hb switching is not 100%; most individuals continue to produce a small amount of HbF throughout life. However, some people, who are clinically normal, produce abnormally high levels (up to 100%) of fetal hemoglobin (Hemoglobin F) in place of HbA. Patients with hemoglobinopathies such as β-thalassemia or sickle cell anemia frequently have less severe illnesses if their levels of fetal hemoglobin are elevated. One goal of much research on hemoglobin switching is to discover a way to reactivate transcription of the γ-globin genes to compensate for defective β-globin synthesis. Individuals who express fetal hemoglobin past birth have hereditary persistence of fetal hemoglobin (HPFH).

1.   NON-DELETION FORMS OF HPFH

The non-deletion forms of HPFH are those that derive from point mutations in the Aγ and Gγ promoters. When these mutations are found with sickle cell or beta thalassemia mutations, they have an ameliorating effect on the disease, because of the increased production of gamma chains.

2.   DELETION FORMS OF HPFH

In deletion HPFH, both the entire delta and beta genes have been deleted from one copy of chromosome 11 and only HbF can be produced. In some individuals the fetal globins remain activated after birth, and enough HbF is produced that the individuals are clinically normal. Other individuals with similar deletions that remove the entire delta and beta genes do not produce enough fetal hemoglobin to compensate for the deletion and are considered to have $\delta^0\beta^0$ thalassemia. The difference between these two outcomes is believed to be the site at which the deletions end within the β-globin gene cluster. In deletion HPFH, powerful enhancer sequences 3′ of the β-globin gene are resituated because of the deletion such that they activate the gamma promoters. In individuals with $\delta^0\beta^0$ thalassemia, the enhancer sequences have not been relocated such that they can interact with the gamma promoters.

D. Hemoglobin Switching: A Developmental Process Controlled by Transcription Factors

In humans, embryonic megaloblasts (the embryonic red blood cell is large and is termed a "blast" because it retains its nucleus) are first produced in the yolk sac approximately 15 days after fertilization. After 6 weeks, the site of erythropoiesis shifts to the liver. The liver and to a lesser extent the spleen are the major sites of fetal erythropoiesis. In the last few weeks before birth, the bone marrow begins producing red blood cells. By 8 to 10 weeks after birth, the bone marrow is the sole site of erythrocyte production. The composition of the hemoglobin also changes with development, because both the α-globin locus and the β-globin locus have multiple genes that are differentially expressed during development (Figure 44.18).

E. Structure and Transcriptional Regulation of the Alpha and Beta Globin Gene Loci

The α-globin locus on chromosome 16 contains the embryonic ζ (zeta) gene and two copies of the alpha gene, α_2 and α_1. The β-globin locus on chromosome 11 contains the embryonic ϵ gene, two copies of the fetal β-globin gene G_γ and A_γ

A Chromosome 16

HS40 ζ α_2 α_1

Chromosome 11

LCR ϵ G_γ A_γ δ β

Embryo: $\zeta_2\epsilon_2$ = Gower 1

$\zeta_2\gamma_2$ = Portland

$\alpha_2\epsilon_2$ = Gower 2

Fetus: $\alpha_2\gamma_2$ = HbF

Adult: $\alpha_2\gamma_2$ = HbF
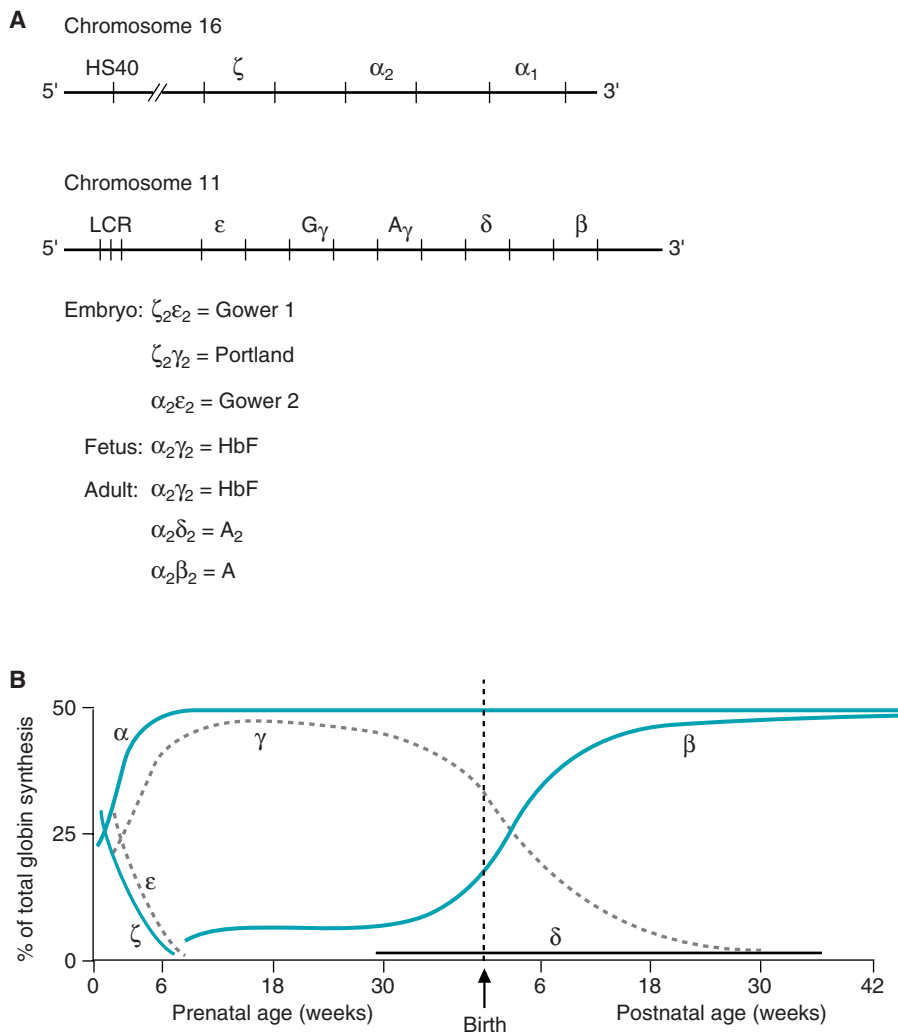
$\alpha_2\delta_2$ = A$_2$

$\alpha_2\beta_2$ = A

B

Fig 44.18. Globin gene clusters and expression during development. A. The globin gene clusters with the α genes on chromosome 16 and the β genes on chromosome 11. LCR = locus control region. B. The switching of globin chain synthesis during development.

(which differ by one amino acid), and two adult genes, δ and β. The order of the genes along the chromosome parallels the order of expression of the genes during development (see Fig. 44.18). The embryonic hemoglobins are $\zeta_2\epsilon_2$ (Gower 1), $\zeta_2\gamma_2$ (Portland), and $\alpha_2\gamma_2$ (Gower 2). Fetal hemoglobin is predominantly $\alpha_2G_{\gamma2}$. The major adult species is $\alpha_2\beta_2$ (hemoglobin A); the minor adult species is $\alpha_2\delta_2$ (hemoglobin A_2). The fetal hemoglobin found in adult cells is $\alpha_2A_{\gamma2}$. The timing of hemoglobin switching is controlled by a developmental clock not significantly altered by environmental conditions and is related to changes in expression of specific transcription factors. Premature newborns convert from HbF to HbA on schedule with their gestational ages.

CLINICAL COMMENTS

Spiro Site's red blood cells are deficient in spectrin. This deficiency impairs the ability of his erythrocytes to maintain the redundant surface area necessary to maintain deformability. Mechanical stresses in the circulation cause progressive loss of pieces of membrane. As membrane components are lost, **Spiro Site's** red blood cells become spherical and unable to deform. His spleen is enlarged because of the large number of red blood cells that have become trapped within it. His erythrocytes are lysed by mechanical stresses in the circulation and by macrophages in the spleen. Consequently, this hemolytic process results in an anemia. His gallstones were the result of the large amounts of bilirubin that were produced and stored in the gallbladder as a result of the hemolysis. The abnormally rounded red cells seen on a blood smear are characteristic of hereditary spherocytosis.

Mutations in the genes for ankyrin, β-spectrin, or band 3 account for three quarters of the cases of hereditary spherocytosis, whereas mutations in the genes for α-spectrin or band 4.2 account for the remainder. The result of defective synthesis of any of the membrane cytoskeletal proteins results in improper formation of the membrane cytoskeleton. Excess membrane proteins are catabolized, resulting in a net deficiency of spectrin. **Spiro Site** underwent a splenectomy. Because the spleen was the major site of destruction of his red blood cells, his anemia significantly improved after surgery. He was discharged with the recommendation to take a folate supplement daily. It was explained to Mr. Site that because the spleen plays a major role in protection against certain bacterial agents, he would require immunizations against pneumococcus, meningococcus, and Haemophilus influenzae type b.

Anne Niemick was found to be a compound heterozygote for mutations in the β-globin gene. On one gene, a mutation in position 6 of intron 1 converted a T to a C. The presence of this mutation, for unknown reasons, raises HbF production. The other β-globin gene had a mutation in position 110 of exon 1 (a C to T mutation). Both β-globin chains have reduced activity, but combined with the increased expression of HbF, results in a β^+ thalassemia.

BIOCHEMICAL COMMENTS

How is hemoglobin switching controlled? Although there are still many unanswered questions, some of the molecular mechanisms have been identified. The α-globin locus covers ~100 kb. The major regulatory element, HS 40, is a nuclease-sensitive region of DNA that lies 5′ of the ζ gene (see Fig. 44.18). HS 40 acts as an erythroid-specific enhancer that interacts with the upstream regulatory regions of the ζ and α genes, and stimulates their transcription. The region immediately 5′ of the ζ gene contains the regulatory sequences responsible for silencing ζ gene transcription. However, the exact sequences and transcription factors responsible for this silencing have not yet been identified. Even after silencing, low levels of
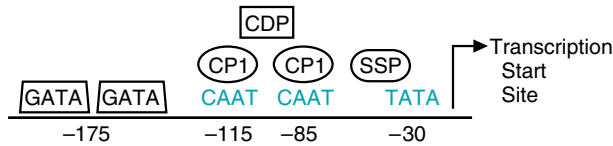
Fig. 44.19. The γ-globin gene promoter indicating some of the transcription factor binding sites associated with hereditary persistence of fetal hemoglobin.

ζ gene transcripts are still produced after the embryonic period; however, they are not translated. This is because both the ζ globin and α-globin transcripts have regions that bind to a messenger ribonucleoprotein (mRNP) stability-determining complex. Binding to this complex prevents the mRNA from being degraded. The α-globin messenger RNA has a much higher affinity for the mRNP than the ζ-globin message, which leads to the ζ-globin message being rapidly degraded.

The β-globin locus covers ~100 kb. From 5 to 25 kb upstream of the ε gene is the locus control region (LCR), containing five DNAse hypersensitive sites. The LCR is necessary for the function of the β-globin locus. It maintains the chromatin of the entire locus in an active configuration and acts as an enhancer and entry point for the factors that transcribe the genes of the β-globin locus. One model of the control of hemoglobin switching postulates that proteins bound at the promoters of the ε–, γ–, and β-globin genes compete to interact with the enhancers of the LCR.

Each gene in the β-globin locus has individual regulatory elements—a promoter, silencers, or enhancers that control its developmental regulation. The promoters controlling the γ and β-globin genes have been intensively studied because of their clinical relevance.

The ε-globin gene, like the ζ globin gene, has silencers in the 5′ regulatory region. Binding of proteins to these regions turns off the ε gene.

The proximal region of the γ-globin gene promoter has multiple transcription factor binding sites (Fig. 44.19). Many HPFH mutations map to these transcription factor–binding sites, either by destroying a site or by creating a new one, but the exact mechanisms are still not understood. Two sites that appear to be significant in the control of hemoglobin switching are the stage selector protein binding (SSP) site and the CAAT box region. When the SSP complex is bound to the promoter, the γ-globin gene has a competitive advantage over the β-globin promoter for interaction with the LCR. A second transcription factor, Sp1, also binds at the SSP-binding site, where it may act as a repressor, and competition between these two protein complexes for the SSP-binding site helps to determine the activity of the γ-globin gene. A similar mechanism appears to be operating at the CAAT box. CP1, thought to be a transcription activator, binds at the CAAT box. CAAT displacement protein (CDP) is a repressor that binds at the CAAT site and displaces CP1. Part of the mechanism of hemoglobin switching appears to be the binding of repressors at the ε-globin and γ-globin upstream regulatory regions.

The β-globin gene also has binding sites for multiple transcription factors in its regulatory regions. Mutations that affect binding of transcription factors can produce thalassemia by reducing the activity of the β-globin promoter. There is also an enhancer 3′ of the poly A signal that seems to be required for stage-specific activation of the β-globin promoter.

Transgenic mice are an invaluable tool for studying the roles of transcription factors in developmental processes in general and hemoglobin switching in particular. Transgenic mice were created with mutations in the silencer regions of the ε-globin genes. These mice continued to express the ε-globin gene into adulthood.

Suggested References

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✏ REVIEW QUESTIONS—CHAPTER 44

1. A compensatory mechanism to allow adequate oxygen delivery to the tissues at high altitudes, where oxygen concentrations are low, would be which of the following?

   (A) An increase in 2,3-bisphosphoglycerate synthesis by the red cell
   (B) A decrease in 2,3-bisphosphoglycerate synthesis by the red cell
   (C) An increase in hemoglobin synthesis by the red cell
   (D) A decrease in hemoglobin synthesis by the red cell
   (E) Decreasing the blood pH

2. A 2-year-old boy of normal weight and height is brought to a clinic because of excessive fatigue. Blood work indicates an anemia, with microcytic hypochromic red cells. The boy lives in a 100-year-old apartment building and has been caught ingesting paint chips. His parents indicate that the child eats a healthy diet and takes a Flintstones vitamin supplement every day. His anemia is most likely attributable to a deficiency in which of the following?

   (A) Iron
   (B) B12
   (C) Folate
   (D) Heme
   (E) B6

3. Drugs are being developed that will induce the transcription of globin genes, which are normally silent in patients affected with sickle cell disease. A good target gene for such therapy in this disease would be which of the following?

   (A) The $\alpha 1$ gene
   (B) The $\alpha 2$ gene
   (C) The γ gene
   (D) The β gene
   (E) The ζ gene

4. A mature blood cell that lacks a nucleus is which of the following?

   (A) Lymphocyte
   (B) Basophil
   (C) Eosinophil
   (D) Platelet
   (E) Neutrophil

5. A family has two children, one with a mild case of thalassemia, and a second with a severe case of thalassemia, requiring frequent blood transfusions as part of the treatment plan. One parent is of Mediterranean descent, the other is of Asian descent. Neither parent exhibits clinical signs of thalassemia. Both children express 20% of the expected level of β-globin; the more severely affected child expresses normal levels of α-globin, whereas the less severely affected child only expresses 50% of the normal levels of α-globin. Why is the child who has a deficiency in α-globin expression less severely affected?

   (A) Thalassemia is caused by a mutation in the α gene, and the more severely affected child expresses more of it.
   (B) The less severely affected child must be synthesizing the ζ gene to make up for the deficiency in a α chain synthesis.
   (C) The more severely affected child also has HPFH.
   (D) The more severely affected child produces more inactive globin tetramers than the less severely affected child.
   (E) Thalassemia is caused by an iron deficiency, and when the child is synthesizing normal levels of α-globin there is insufficient iron to populate all of the heme molecules synthesized.