42: The Biochemistry of Erythrocytes and Other Blood Cells

Introduction

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 nicotinamide adenine dinucleotide (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** (**2,3-BPG**) is produced, which is a major allosteric effector for oxygen binding to hemoglobin (see Chapter 7). 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 coenzyme A (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.

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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 B_{12}** 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.

In human, globin gene expression is altered 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

Lisa N., 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 (RBC) precursors (the erythroid mass) from the stem cells in her bone marrow. This expansion of marrow volume causes osteoporosis, leading to compression fractures in the lumbar spine area, which, in turn, cause pain. In addition to treatment of the osteoporosis, local irradiation to reduce the marrow volume in the lumbar spine is considered, as is a program of regular blood transfusions to maintain the oxygen-carrying capacity of circulating RBCs. 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. **Lisa N.'s** parents have returned to the clinic to discuss the results of these tests.

Edward R. 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 (icterus) is evidenced by yellowing of the whites of his eyes. His hemoglobin is low (8 g/dL; reference value, 13.5 to 17.5 g/dL). A blood smear showed dark, rounded, abnormally small red cells called spherocytes as well as an increase in the number of circulating immature RBCs known as reticulocytes.

Cells of the Blood

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The blood, together with the bone marrow, composes 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 cells in the blood are the erythrocytes or RBCs, which transport oxygen to the tissues and contribute to buffering of the blood through the binding of protons by hemoglobin (see the material in **Chapter 4**, Section IV.B, and **Chapter 7**, Section VII). RBCs 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 42.1**.

TABLE 42.1

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

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 leukocyte has a multilobed nucleus.

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 migrate rapidly 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 25**). 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 protect against parasites, such as worms, and to remove fibrin during inflammation. The eosinophilic granules are lysosomes containing hydrolytic enzymes and cationic proteins, which are toxic to parasitic worms. Increased eosinophils are also present in asthma and allergic responses, autoimmune diseases, and some cancers. Elucidating the function of eosinophils is currently 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.

Mononuclear Leukocytes

The mononuclear leukocytes consist of various classes of lymphocytes and the monocytes. Lymphocytes are small, round cells that were 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 natural killer (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 is the NK-cells, which target virally infected and malignant cells for destruction.

Circulatory monocytes are the precursors of tissue macrophages. Macrophages ("large eaters") 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 the oxygen-delivering capabilities of the blood by removing damaged RBCs that have a reduced oxygen-carrying capacity.

The Thrombocytes

Platelets are heavily granulated disclike 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.

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Anemia

The major function of erythrocytes is to deliver oxygen to the tissues. To do this, a sufficient concentration of hemoglobin in the RBCs is necessary for efficient oxygen delivery to occur. When the hemoglobin concentration falls below normal values (**Table 42.2**), 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 42.3**) that enable one to properly classify, diagnose, and treat the anemia.

TABLE 42.2

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 y to puberty	11.0–13.5

TABLE 42.3

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, mutation leading to thalassemia, lead poisoning
Macrocytic, normochromic	Impaired DNA synthesis	Vitamin B_{12} or folic acid deficiency, erythroleukemia
Normocytic, normochromic	Red cell loss	Acute bleeding, sickle cell disease, red cell metabolic defects, red cell membrane defects

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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 RBC, expressed in femtoliters (10^{-15} L). Normal MCV values range from 80 to 100 fL. The MCHC is the average concentration of hemoglobin in each individual erythrocyte, expressed in grams per liter. The normal range is 32 to 37 g/L; a value of <32 g/L indicates hypochromic cells. Thus, microcytic, hypochromic RBCs have an MCV of <80 fL and an MCHC of <32 g/L. Macrocytic, normochromic cells have an MCV of >100 fL, with an MCHC between 32 and 37 g/L.

A complete blood count (CBC) is ordered when a physician suspects a problem in the cellular composition of a patient's blood. The cells within the collected blood are counted and typed using an automated analyzer, based on flow cytometry (counting cells one at a time as they flow through a detector). As each cell flows through the machine, a laser shines light at the cell, which leads to predictable light-scattering and absorbance depending on the cell type. Based on the light-scattering and absorption pattern, the machine keeps track of the results of each cell that flows through the machine, leading to a very accurate count of each cell type present in the sample. The data from this analysis will include the total number of red cells per liter, the amount of hemoglobin in the red cells (in grams per liter), the hematocrit (the fraction of whole blood that consists of RBCs), the MCV, the total number of white blood cells, as well as a count of the different types of white blood cells (neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

Erythrocyte Metabolism

The Mature Erythrocyte

To 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 RBC are limited to those found in the cytoplasm. In addition to hemoglobin, the cytosol of the RBC contains enzymes necessary for the prevention and repair of damage done by reactive oxygen species (ROS; see **Chapter 25**) and the generation of energy (**Fig. 42.1**). 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 Rapoport-Luebering shunt to generate 2,3-BPG. Red cells contain 4 to 5 mM 2,3-BPG, compared with trace amounts in other cells. 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. Because the 2,3-BPG is regenerated during each reaction cycle, it is required in only catalytic amounts. As has been discussed in more detail in **Chapter 7**, 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.

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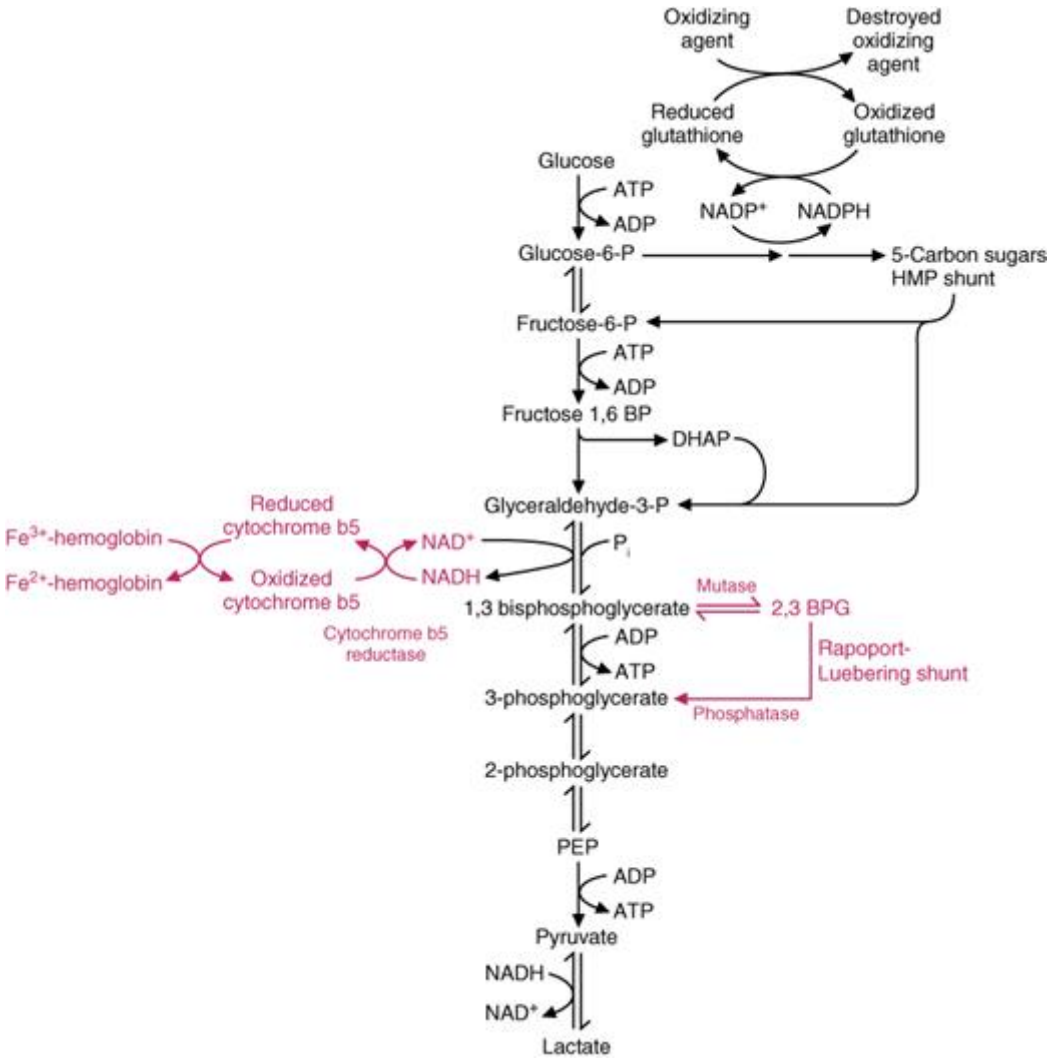
FIGURE 42.1

Overview of erythrocyte metabolism.

Glycolysis is the major pathway, with branches for the hexose monophosphate (HMP) shunt (for protection against oxidizing agents) and the Rapoport-Luebering shunt (which generates 2,3-bisphosphoglycerate [2,3-BPG], which moderates oxygen binding to hemoglobin). The reduced nicotinamide adenine dinucleotide (NADH) generated from glycolysis can be used to reduce methemoglobin (Fe^{3+}) to normal hemoglobin (Fe^{2+}), or to convert pyruvate to lactate, so that NAD^+ can be regenerated and used for glycolysis. Pathways that are unique to the erythrocyte are indicated in red. ADP, adenosine diphosphate; ATP, adenosine triphosphate; DHAP, dihydroxyacetone phosphate; Fructose 1,6-BP, fructose 1,6-bisphosphate; Fructose 6-P, fructose 6-phosphate; Glucose 6-P, glucose 6-phosphate; Glyceraldehyde 3-P, glyceraldehyde 3-phosphate; P_i, inorganic phosphate; PEP, phosphoenolpyruvate.



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An inherited deficiency in erythrocyte pyruvate kinase leads to hemolytic anemia (an anemia caused by the destruction of RBCs; hemoglobin values typically drop to 4 to 10 g/dL in this condition, with normal values being 13.5 to 17.5 in males or 11.5 to 15.5 in females). Because the amount of ATP formed from glycolysis can be decreased by 50%, RBC ion transporters cannot function effectively. The RBCs 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 they are damaged, the RBCs 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 phosphoenolpyruvate to pyruvate. Because 2,3-BPG binding to hemoglobin decreases the affinity of hemoglobin for oxygen, the RBCs that remain in circulation are highly efficient in releasing their bound oxygen to the tissues.

To bind oxygen, the iron of hemoglobin must be in the ferrous (+2) state. ROS can oxidize the iron to the ferric (+3) state, producing methemoglobin. Some of the reduced nicotinamide adenine dinucleotide (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.

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.

Approximately 5% to 10% of the glucose metabolized by RBCs 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 RBC's chief defense against damage to proteins and lipids by ROS (see **Chapter 25**).

Glucose 6-PD deficiency is the most common enzyme deficiency in humans, probably, in part, because individuals with glucose 6-PD deficiency have resistance to malaria. The resistance to malaria counterbalances the deleterious effects of the deficiency. Glucose 6-PD–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 spontaneous anemia. Because the gene for glucose 6-PD is found on the X chromosome, these men had only one copy of a variant G6PD gene.

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

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 life span for the red cell. No mutations have been found that result in complete absence of glucose 6-PD. Based on studies with knockout mice, those mutations would be expected to result in embryonic lethality.

The Erythrocyte Precursor Cells and Heme Synthesis

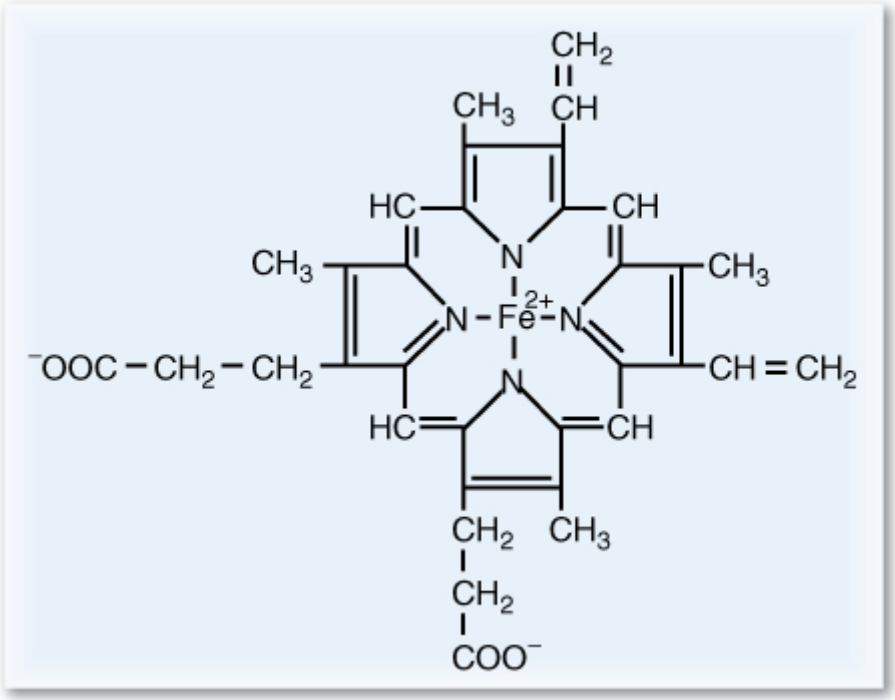
Heme Structure

Heme consists of a porphyrin ring coordinated with an atom of iron (**Fig. 42.2**). Four pyrrole rings are joined by methylene 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 acetate (A), propionate (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.

FIGURE 42.2

Structure of heme.

The side chains can be abbreviated as MVMVMPPM: M, methyl ($-CH_3$); P, propionate ($-CH_2-CH_2-COO^-$); V, vinyl ($-CH=CH_2$).



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 **24**), including cytochrome P450 (see **Chapter 25**).

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Synthesis of Heme

Heme is synthesized from glycine and succinyl-CoA (**Fig. 42.3**), which condense in the initial reaction to form δ-aminolevulinic acid (δ-ALA) (**Fig. 42.4**). The enzyme that catalyzes this reaction, δ-ALA synthase, requires the participation of pyridoxal phosphate because the reaction is an amino acid decarboxylation reaction (glycine is decarboxylated; see **Chapter 37**).

FIGURE 42.3

Synthesis of heme.

To produce one molecule of heme, eight molecules each of glycine and succinyl coenzyme A (succinyl-CoA) are required. A series of porphyrinogens is 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 ⊖ and by directly inhibiting the activity of this enzyme ⊖. Deficiencies of enzymes in the pathway result in a series of diseases known as porphyrias (listed on the right, beside the deficient enzyme).

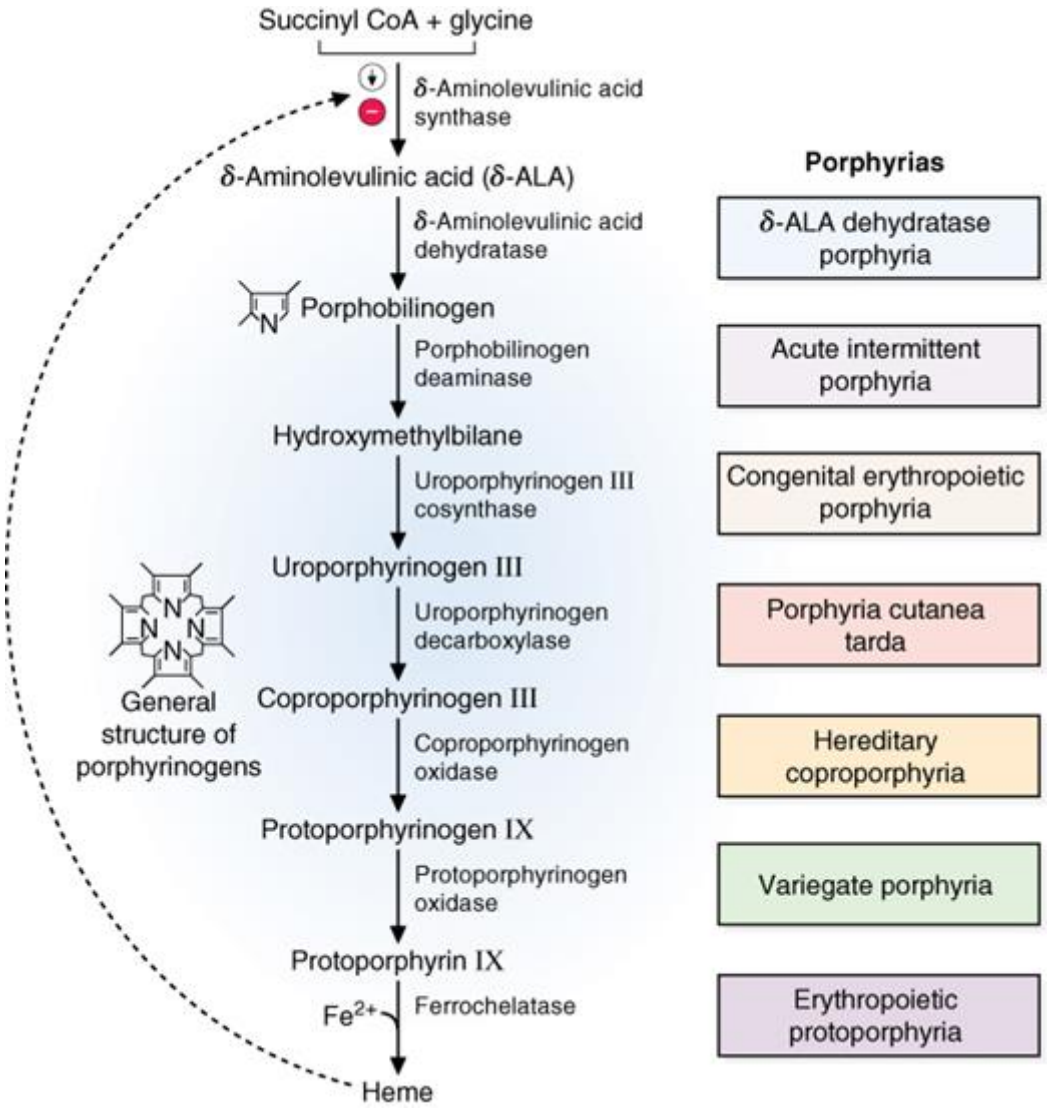

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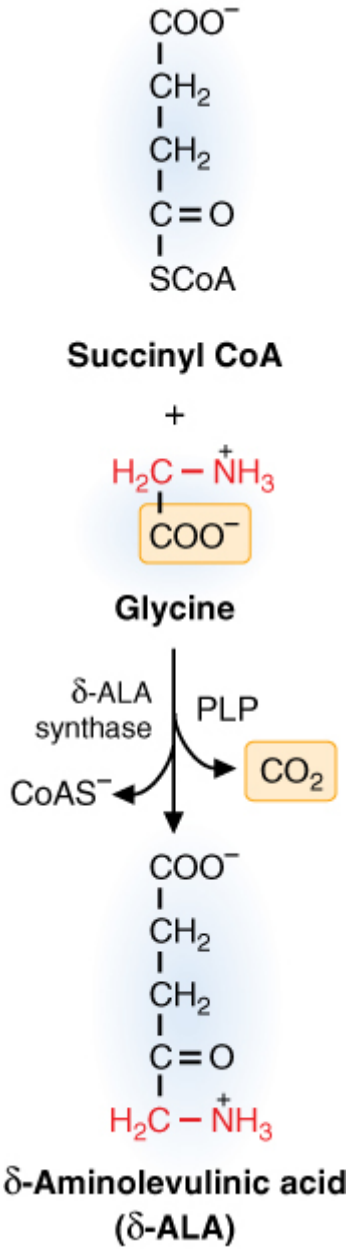
FIGURE 42.4

Synthesis of δ-aminolevulinic acid (δ-ALA).

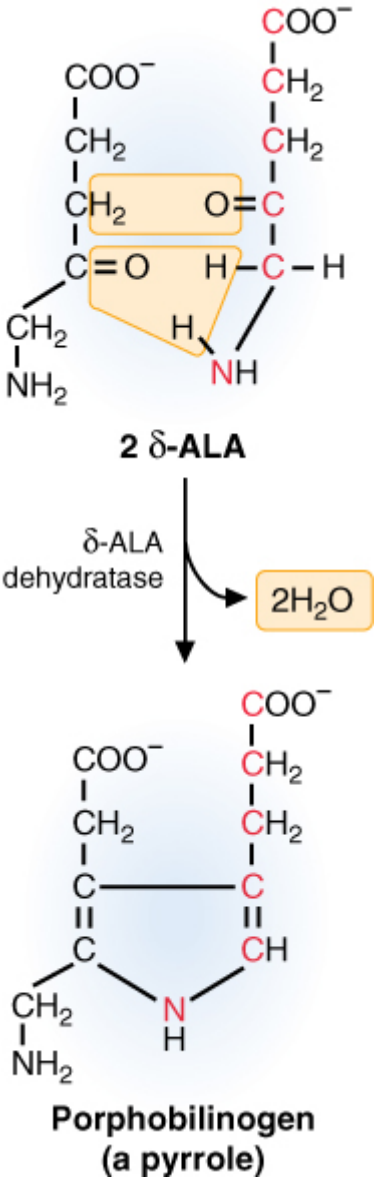
The atoms in red in δ-ALA are derived from glycine. CoA, coenzyme A; PLP, pyridoxal phosphate; succinyl CoA, succinyl coenzyme A.



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. 42.5**). 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 acetate (A) and propionate (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. 42.3**). Heme is red, and it is responsible for the color of RBCs and of muscles that contain a large number of mitochondria.

FIGURE 42.5

Two molecules of δ-aminolevulinic acid (δ-ALA) condense to form porphobilinogen.



Pyridoxine (vitamin B_6) deficiencies are often associated with a microcytic, hypochromic anemia. Why would a vitamin B_6 deficiency result in small (microcytic), pale (hypochromic) RBCs?

In a vitamin B_6 deficiency, the rate of heme production is slow because the first reaction in heme synthesis requires pyridoxal phosphate (see Fig. 42.4). Thus, less heme is synthesized, causing RBCs to be small and pale. Iron stores are usually elevated.

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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).

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.

Source of Iron

Iron, which is obtained from the diet, has a US Recommended Dietary Allowance (RDA) of 10 mg for men and postmenopausal women and 15 mg for premenopausal women. The average daily US diet contains 10 to 50 mg of iron. However, only 10% to 15% is normally absorbed, and iron deficiencies are fairly common. The iron in meats is in the form of heme, which is readily absorbed. The nonheme 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 nonheme 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. 42.6**) but is oxidized to the ferric state by a ferroxidase known as ceruloplasmin (a copper-containing enzyme) for transport through the body.

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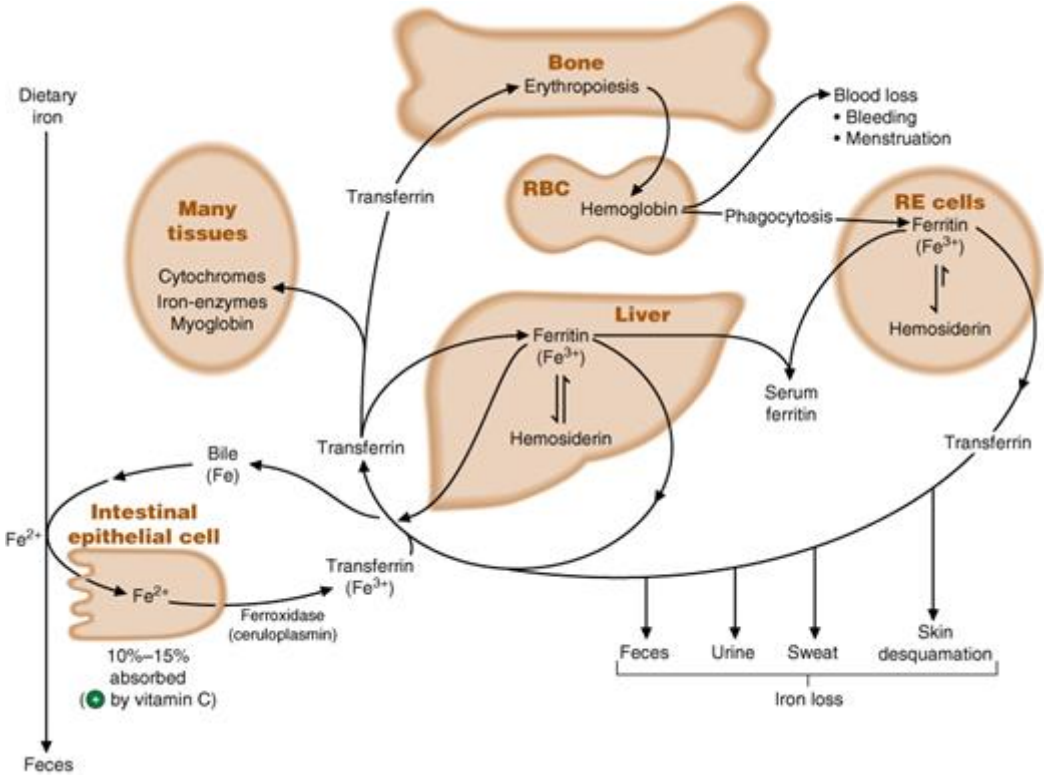
FIGURE 42.6

Iron metabolism.

Iron is absorbed from the diet, transported in the blood by 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. RBC, red blood cells; RE, reticuloendothelial.



Porphyrias are a group of rare inherited disorders resulting from deficiencies of enzymes in the pathway for heme biosynthesis (see Fig. 42.3). 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 rise of the werewolf legends.

Because free iron is toxic, it is usually found in the body bound to proteins (see **Fig. 42.6**). 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, owing mainly to its content of transferrin, is approximately 300 µg/dL. Transferrin, with bound iron, binds to the transferrin receptor on the cell surface, and the complex is internalized into the cell. The internalized membrane develops into an endosome, with a slightly acidic pH. The iron is reduced by a membrane-bound oxidoreductase, and the ferrous iron is transported out of the endosome into the cytoplasm via the divalent metal ion transporter 1 (DMT-1). Once in the cytoplasm, the iron is shunted to necessary enzymes, or it can be oxidized and bind to ferritin for long-term storage.

An inherited mutation in SLC11A2 (the gene encoding DMT-1) leads to an iron deficiency anemia, as indicated by a refractory hypochromic microcytic anemia. The iron is trapped in endosomal vesicles and cannot be released to bind to ferritin or used in other necessary biosynthetic reactions. This leads to reduced heme synthesis, reduced globin synthesis, and anemia.

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, ferritin is present in the blood in small amounts. The level 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.

The iron lost by adult men (~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 a growing fetus during pregnancy. If a man eating a Western diet has iron deficiency anemia, his physician should suspect bleeding from the gastrointestinal tract as a result of ulcers or colon cancer.

Regulation of Heme Synthesis

Heme regulates its own synthesis by mechanisms that affect the first enzyme in the pathway, δ-ALA synthase (see **Fig. 42.3**). 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**).

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 fall and δ-ALA synthase is induced to increase the rate of heme synthesis.

Degradation of Heme

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Heme is degraded to form bilirubin, which is conjugated with glucuronic acid and excreted in the bile (Fig. 42.7). Although heme from cytochromes and myoglobin also undergoes conversion to bilirubin, the major source of this bile pigment is hemoglobin. After RBCs reach the end of their life span (~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. 42.8). Biliverdin is reduced to bilirubin, which is transported to the liver complexed with serum albumin.

FIGURE 42.7

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. RBC, red blood cells; RES, reticuloendothelial system.

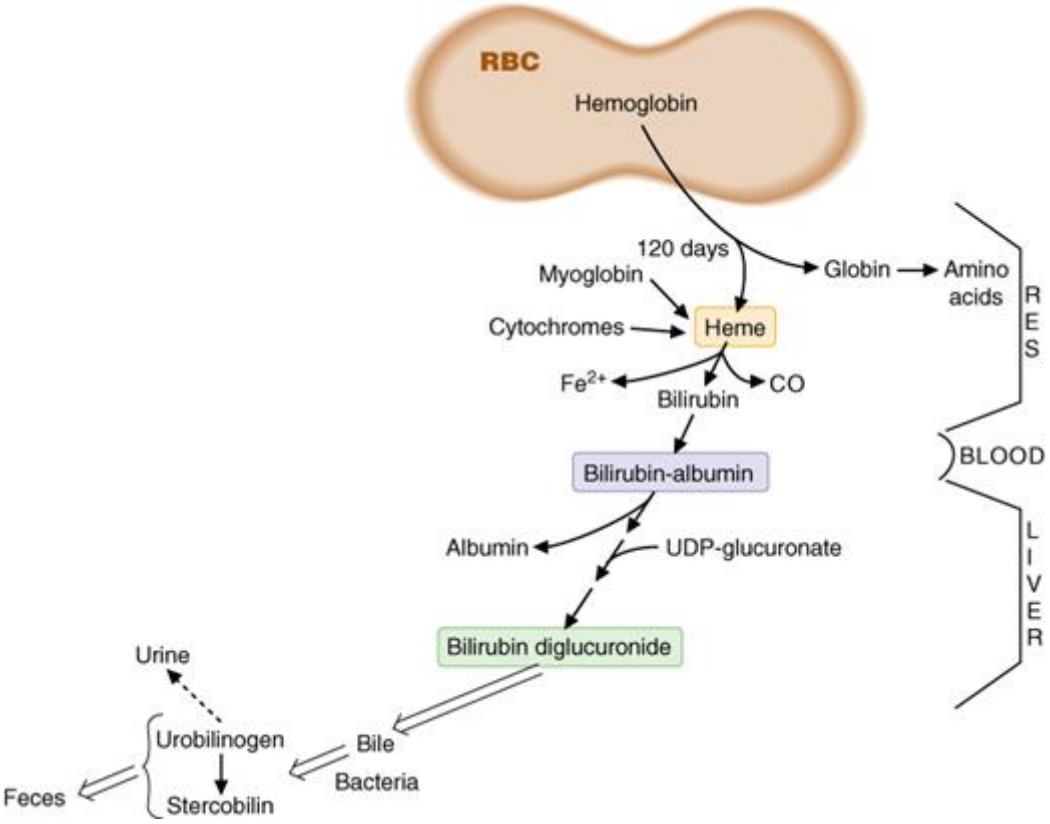

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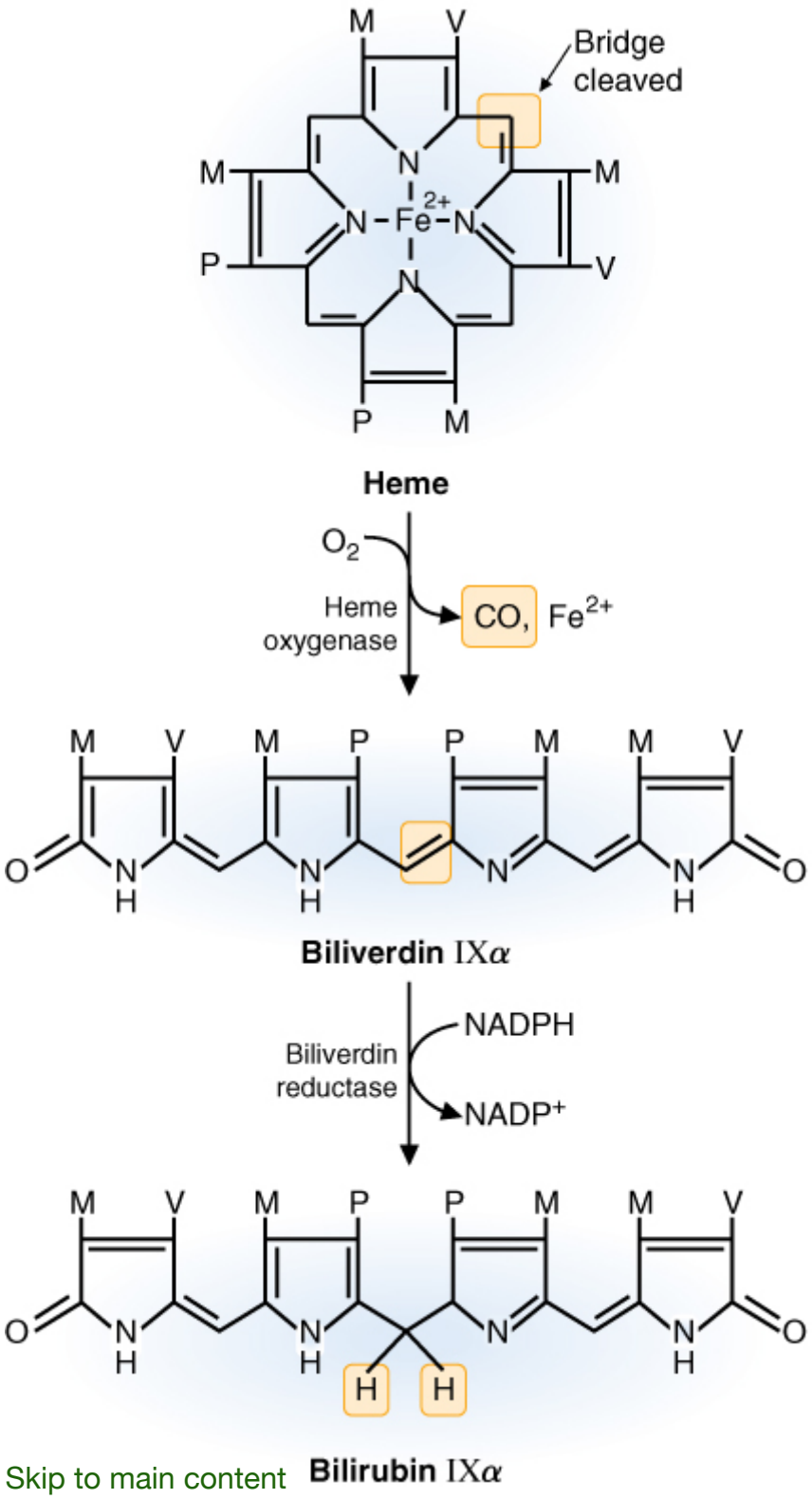
FIGURE 42.8

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. NADP, nicotinamide adenine dinucleotide phosphate.



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In the liver, bilirubin is converted to a more water-soluble compound by reacting with uridine diphosphate (UDP)-glucuronate to form bilirubin monoglucuronide, which is converted to the diglucuronide (see **Fig. 27.12**). This conjugated form of bilirubin is excreted into the bile.

In the intestine, bacteria deconjugate bilirubin diglucuronide and convert the bilirubin to urobilinogens (see **Fig. 42.7**). 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.

The Red Blood Cell Membrane

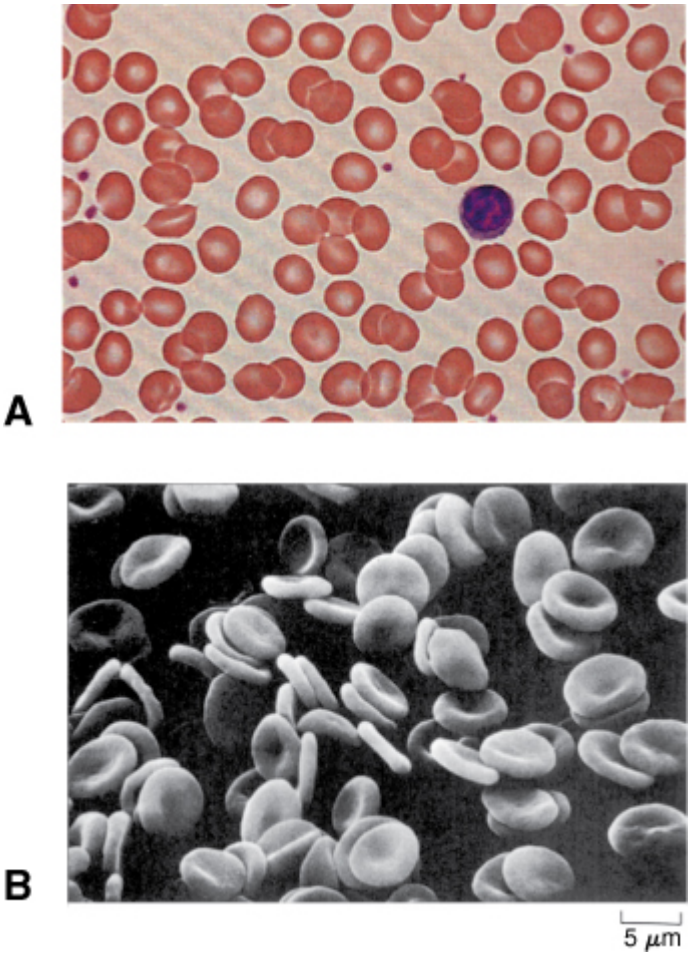
Under the microscope, the RBC appears to be a red disc with a pale central area (biconcave disc) (**Fig. 42.9**). The biconcave disc shape (as opposed to a spherical shape) serves to facilitate gas exchange across the cell membrane. The membrane proteins that maintain the shape of the RBC also allow the RBC 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, RBCs traverse hypertonic areas that are up to 6 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 RBCs. Erythrocytes pass through the spleen 120 times per day. The elliptical passageways through the spleen are approximately 3 μm in diameter, and 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 RBC membrane.

FIGURE 42.9

The shape of the RBC.

A. Wright-stained cells, displaying the pale staining in the center. **B.** Scanning electron micrograph, showing the biconcave disc structure of the cells. The stacks of erythrocytes in this preparation (collected from a blood tube) are not unusual.

(These photographs were obtained, with permission, from Cohen BJ, Wood DL. Memmler's the Human Body in Health and Disease. 9th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2000:230 [Panel A] ; and Alberts B, Johnson A, Lewis Jet al. Molecular Biology of the Cell. 4th ed. New York, NY: Garland Science, 2002:600 [Panel B].)



In an iron deficiency, what characteristics will be evident in the blood?

Iron deficiency will result in a microcytic, hypochromic anemia. RBCs will be small and pale. In contrast to a vitamin B_6 deficiency, which also results in a microcytic, hypochromic anemia, iron stores are low in an iron deficiency anemia.

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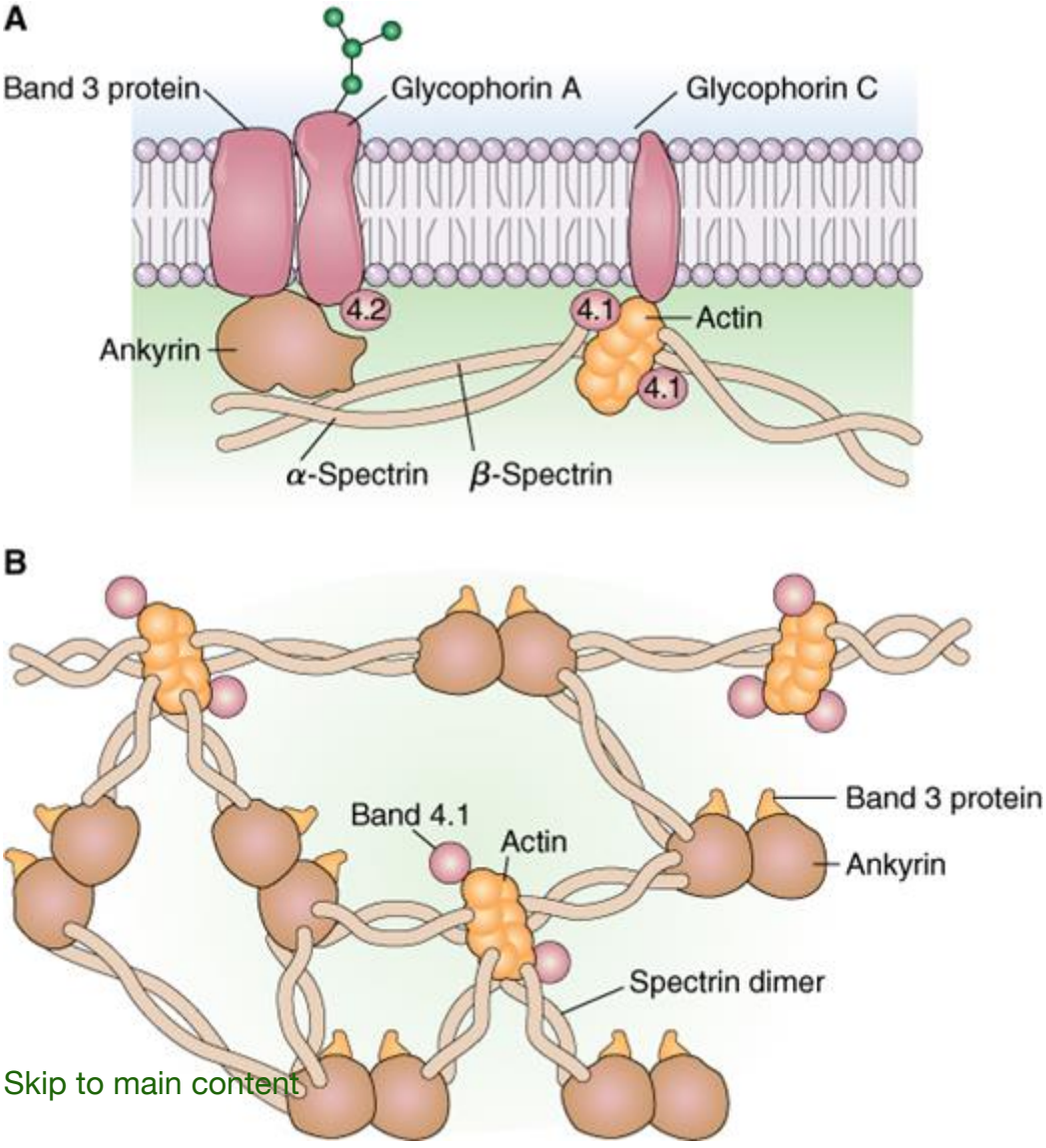
The surface area of the red cell is approximately 140 μm^2, which is greater than the surface of a sphere needed to enclose the contents of the red cell (98 μm^2). The presence of this extra membrane and the cytoskeleton that supports it allows the red cell to be stretched and deformed by mechanical 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. 42.10**). 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.

FIGURE 42.10

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, 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.



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.

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 RBC 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.

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

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

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 and 10 per 10^5 bone marrow cells. In the presence of the appropriate signals, hematopoietic stem cells proliferate, differentiate, and mature into any of the types of cells that make up the blood (**Fig. 42.11**).

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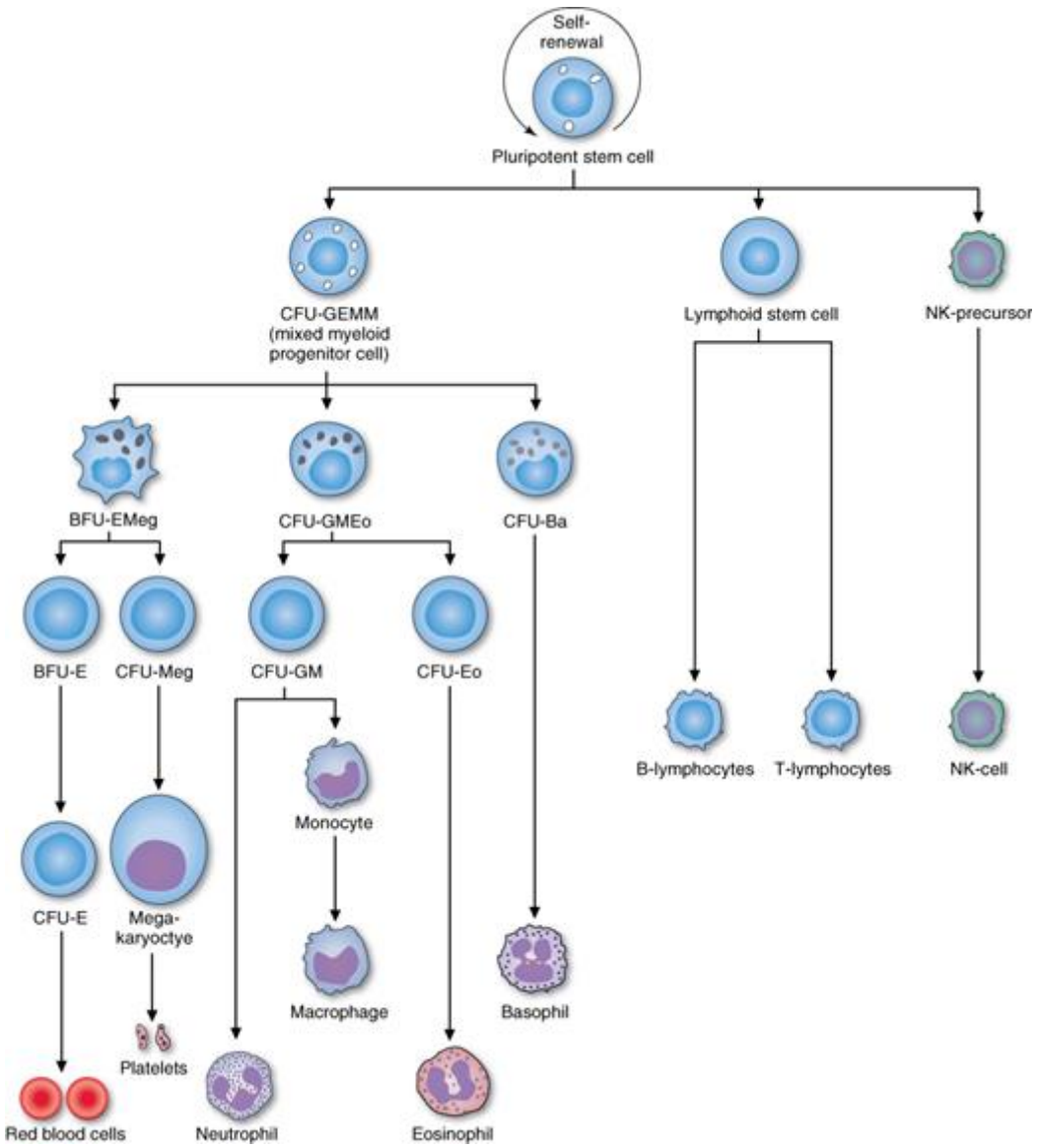
FIGURE 42.11

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. BFU, burst-forming unit; CFU, colony-forming unit.



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.

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.

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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.

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.

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/mitogen-activated protein (MAP) kinase pathways. Other pathways are also activated, some of which lead to an inhibition of apoptosis (see **Chapter 18**).

In X-linked severe combined immunodeficiency disease (SCID), the most common form of SCID, circulating mature T-lymphocytes are not formed, and therefore, B-lymphocytes are not active. The affected gene encodes the γ-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 39**), which is not X-linked, also leads to a form of SCID but for different reasons.

The response to cytokine binding is usually transient because the cell contains multiple negative regulators of cytokine signaling. The family of silencer of cytokine signaling (SOCS) proteins is 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 proteins recruit phosphatases that then dephosphorylate the JAKs (**Fig. 42.12**) is uncertain.

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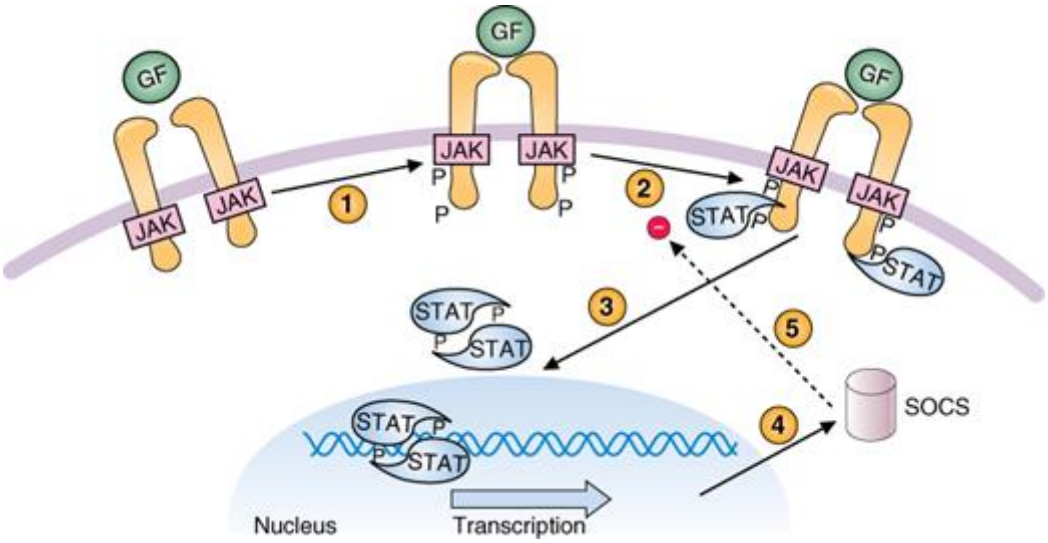
FIGURE 42.12

Cytokine signaling through the JAK (janus kinase)/STAT (signal transducer and activator of transcription) 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 family of proteins whose synthesis is stimulated by STATs is the SOCS (suppressor of cytokine signaling) family, which inhibits further activation of STAT proteins (5) by a variety of mechanisms. GF, growth factor.



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 protein inhibitors of activated STAT (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.

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 RBCs. Individuals with the mutant Epo receptor have a higher-than-normal percentage of RBCs in the circulation because the mutant Epo receptor cannot be deactivated by SHP-1. Erythropoietin causes sustained activation of JAK2 and STAT 5 in these cases.

Erythropoiesis

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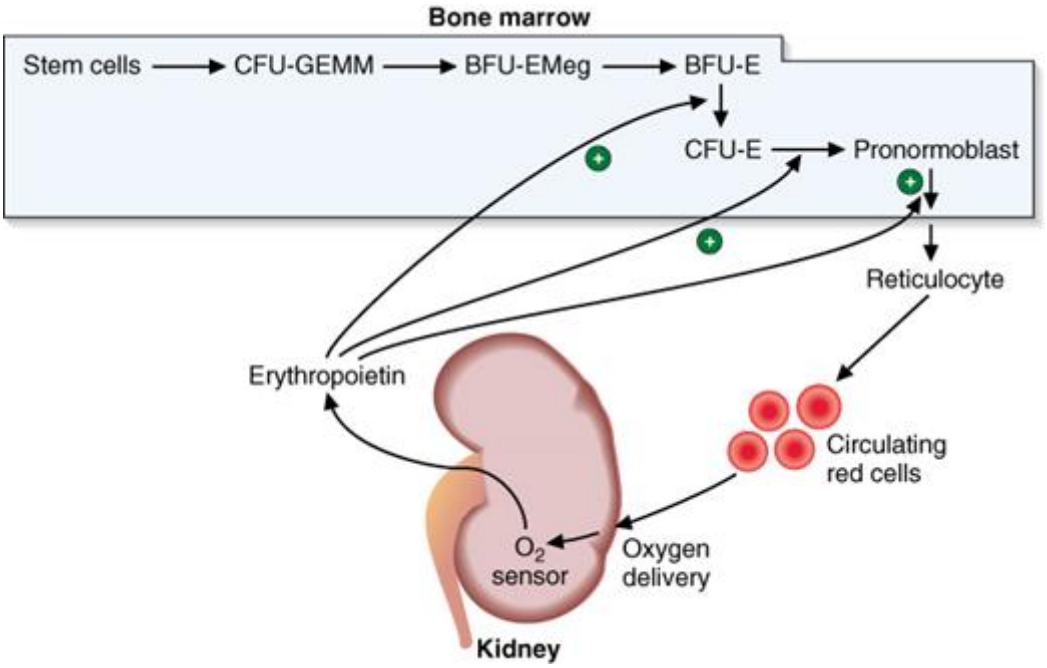
The production of red cells is regulated by the demands of oxygen delivery to the tissues. In response to reduced tissue oxygenation, the kidney releases the hormone erythropoietin, which stimulates the multiplication and maturation of erythroid progenitors. The progression along the erythroid pathway begins with the stem cell and passes through the mixed myeloid progenitor cell (colony-forming unit–granulocyte, erythroid, monocyte, megakaryocyte [CFU-GEMM]), burst-forming unit–erythroid (BFU-E), 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 messenger RNA (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. 42.13**).

FIGURE 42.13

Erythropoietin stimulation of erythrocyte maturation.

The abbreviations are described further in the text. BFU, burst-forming unit; CFU, colony-forming unit; CFU-GEMM, colony-forming unit–granulocyte, erythroid, monocyte, megakaryocyte.



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.

Nutritional Anemias

Each person produces approximately 10^{12} RBCs per day. Because so many cells must be produced, nutritional deficiencies in iron, vitamin B_{12}, and folate prevent adequate RBC 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 RBCs continue dividing past their normal stopping point, resulting in small (microcytic) red cells. The cells are also pale because of the lack of hemoglobin, compared with normal cells (thus, a pale microcytic anemia results).

A complication of sickle cell disease is an increased formation of gallstones. A sickle cell crisis accompanied by the intravascular destruction of RBCs (hemolysis) experienced by patients with sickle cell disease, such as **Will S.**, 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 in the blood increase. More unconjugated bilirubin is then 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.

Deficiencies of folate or vitamin B_{12} can cause megaloblastic anemia, in which the cells are larger than normal. Folate and vitamin B_{12} are required for DNA synthesis (see **Chapters 38** and **39**). 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.

Hemoglobinopathies, Hereditary Persistence of Fetal Hemoglobin, and Hemoglobin Switching

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 S.** 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.

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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 tends to precipitate, 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.

Thalassemias

For optimal 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 because 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. The α-thalassemias usually arise from complete gene deletions. Two copies of the α-globin gene are found on each chromosome 16, for a total of four α-globin genes per precursor cell. If one copy of the gene is deleted, the size and hemoglobin concentration of the individual RBCs is minimally reduced. If two copies are deleted, the RBCs are of decreased size (microcytic) and reduced hemoglobin concentration (hypochromic). However, the individual usually does not have an anemia. The loss of three α-globin genes causes a moderately severe microcytic hypochromic anemia (hemoglobin, 7 to 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 might have two α-globin genes deleted. In one case, one copy of chromosome 16 might have both α-globin genes deleted, whereas the other copy had two functional α-globin genes. In the second case, both chromosomes might have lost one of their two copies of the α-globin gene. The former possibility is more common among Asians, the latter among Africans.

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As discussed in **Chapter 14**, β-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, although they typically have microcytic, hypochromic RBCs and may have mild anemia. β^+/β^+ homozygotes have 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 ineffective for delivering oxygen to the tissues because of its high oxygen affinity. As RBCs age, HbH precipitates in the cells, forming inclusion bodies. RBCs with inclusion bodies have shortened life spans 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 RBC membranes through the heme-facilitated lipid oxidation by ROS. Both lipids and proteins, particularly band 4.1, are damaged.

Hereditary Persistence of Fetal Hemoglobin

Fetal hemoglobin (HbF), the predominant hemoglobin of the fetal period, consists of two α-chains and two γ-chains, whereas adult Hb consists of two α-chains and two β-chains. The process that regulates the conversion of HbF to HbA is called hemoglobin switching. Hemoglobin 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 HbF in place of HbA. Patients with hemoglobinopathies such as β-thalassemia or sickle cell anemia frequently have less severe illnesses if their levels of HbF 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 HbF past birth have hereditary persistence of HbF (HPFH).

The difference in amino acid composition between the β-chains of HbA and the γ-chains of HbF results in structural changes that cause HbF to have a lower affinity for 2,3-BPG than 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.

Nondeletion Forms of HPFH

The nondeletion 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 β-thalassemia mutations, they have an ameliorating effect on the disease because of the increased production of γ-chains.

Deletion Forms of HPFH

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In deletion HPFH, both the entire δ- and β-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 individual is clinically normal. Other individuals with similar deletions that remove the entire δ- and β-genes do not produce enough HbF 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 so that they activate the γ-promoters. In individuals with $\delta^0\beta^0$-thalassemia, the enhancer sequences have not been relocated so that they can interact with the γ-promoters.

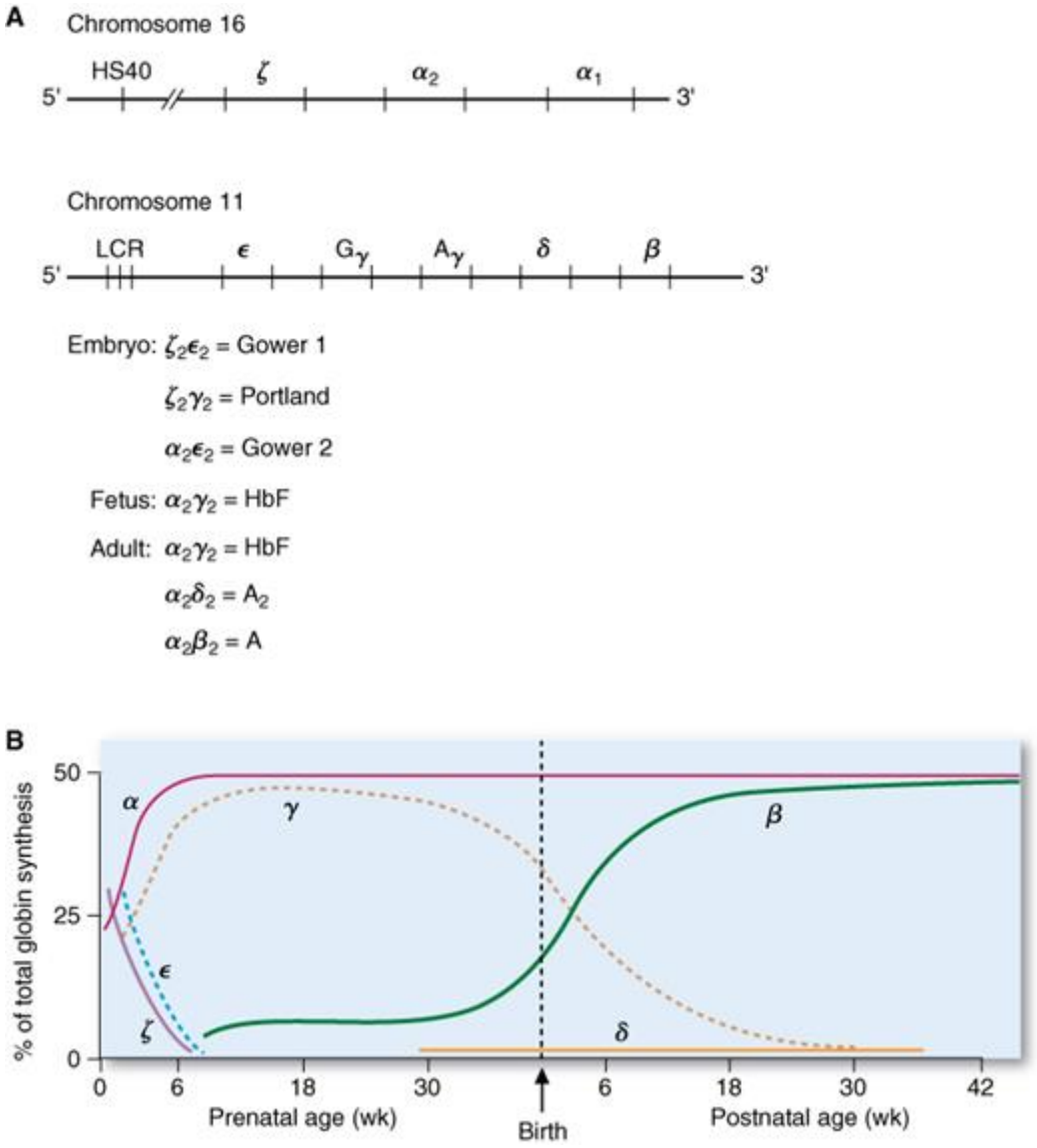
Hemoglobin Switching: A Developmental Process Controlled by Transcription Factors

In humans, embryonic megaloblasts (the embryonic RBC 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 RBCs. 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 (**Fig. 42.14**).

FIGURE 42.14

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. HbF, fetal hemoglobin; LCR, locus control region. **B.** The switching of globin chain synthesis during development.



Structure and Transcriptional Regulation of the α- and β-Globin Gene Loci

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The α-globin locus on chromosome 16 contains the embryonic ζ (zeta) gene and two copies of the α-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, Gγ and Aγ (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. 42.14**). The embryonic hemoglobins are $\zeta_2\varepsilon_2$ (Gower 1), $\zeta_2\gamma_2$ (Portland), and $\alpha_2\gamma_2$ (Gower 2). HbF is predominantly $\alpha_2G\gamma_2$. The major adult species is $\alpha_2\beta_2$ (hemoglobin A); the minor adult species is $\alpha_2\delta_2$ (hemoglobin A_2). The HbF found in adult cells is $\alpha_2A\gamma_2$. The timing of hemoglobin switching is controlled by a developmental clock that is 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

Edward R. Edward R.'s RBCs 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, Edward R.'s RBCs become spherical and unable to deform. His spleen is enlarged because of the large number of RBCs 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 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 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. **Edward R.** underwent a splenectomy. Because the spleen was the major site of destruction of his RBCs, his anemia improved significantly after surgery. He was discharged with the recommendation to take a folate supplement daily. It was explained to Mr. R. 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.

Lisa N. Lisa N. 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, the result is a β^+-thalassemia.

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Biochemical Comments

Control of Hemoglobin Switching. 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 approximately 100 kb (kilobases). The major regulatory element, HS40, is a nuclease-sensitive region of DNA that lies 5′ of the ζ-gene (see **Fig. 42.14**). HS40 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 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 mRNA 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 approximately 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 that control 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. 42.15**). 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 (out of many) 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 (consisting of the transcription factors CP2 and NFE4) 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.

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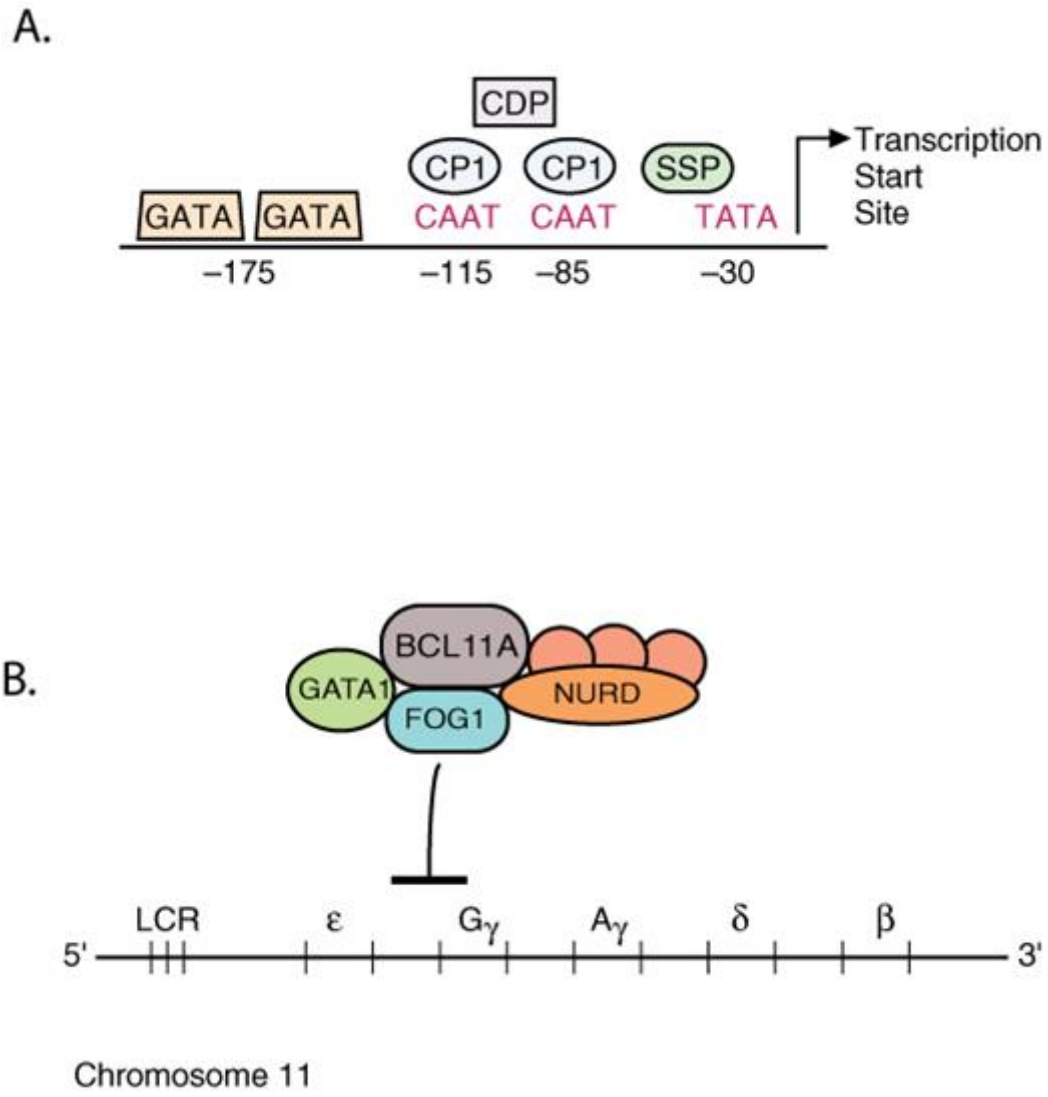
FIGURE 42.15

A. The γ-globin gene promoter, indicating some of the transcription factor–binding sites associated with hereditary persistence of fetal hemoglobin. **B.** The β-globin gene locus. When BCL11A is expressed, the interaction between the locus control region (LCR) and the γ-gene promoter is blocked, turning off γ-gene expression. NURD is a chromatin-remodeling complex containing histone deacetylase activity. CDP, CAAT displacement protein; SSP, stage-selector protein-binding.



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.

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Further insights into the control of hemoglobin switching indicate that the transcription factor BCL11A is a strong repressor of γ-globin gene expression. BCL11A interacts with a variety of other transcription factors (GATA-1, FOG1, and the NURD [nucleosome remodeling and histone deacetylase] repressor complex) to repress γ-globin expression. This appears to be caused by BCL11A interfering with LCR interactions with the γ-globin gene promoter. Experiments that reduce, or eliminate, BCL11A expression lead to an increase in γ-globin synthesis. BCL11A expression is regulated by the transcription factor KLF1, which is essential for β-globin expression. KLF1 increases BCL11A expression, which blocks γ-globin gene expression, whereas KLF1 stimulates β-globin gene expression. These recent results suggest that drugs which interfere with KLF1 action would enhance and activate fetal globin gene expression in individuals with either β-thalassemia or sickle cell disease (see **Fig. 42.15B**).

Key Concepts

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- The blood contains a wide variety of distinct cell types, each of whose function is necessary for maintaining the body's internal environment.
- Erythrocytes transport oxygen throughout the body and return carbon dioxide back to the lung.
  - Erythrocytes lack nuclei and carry out limited metabolic reactions.
    - Glycolysis provides energy and NADH.
    - The NADH maintains the iron in hemoglobin in the ferrous state.
    - The hexose monophosphate shunt provides NADPH to regenerate reduced glutathione to protect the membrane from oxidative damage.
    - 1,3-Bisphosphoglycerate (1,3-BPG) is converted to 2,3-BPG as a by-product of glycolysis in order to regulate oxygen binding to hemoglobin.
  - Heme synthesis occurs in the erythrocyte precursor, using succinyl-CoA and glycine. Inherited defects in heme synthesis lead to porphyrias.
  - Iron, a critical part of heme, is carried throughout the body on protein carriers, because free iron is toxic.
  - The erythrocyte membrane is flexible as a result of its unique cytoskeletal structure, which allows erythrocytes to deform in order to travel through narrow capillaries.
- Hematopoiesis is the generation of the unique blood cell types from a single precursor stem cell in the bone marrow.
- Polymorphonuclear leukocytes consist of a variety of cells types that release chemical signals when activated (granulocytes), phagocytose foreign bodies (neutrophils), destroy parasites (eosinophils), and are involved in the allergic response (basophils).
- Mononuclear leukocytes include the lymphocytes (necessary for the immune response) and monocytes (which develop into macrophages, which engulf debris left behind after granulocytes attack foreign material).
- A wide variety of mutations can lead to alterations in hemoglobin function (hemoglobinopathies):
  - Sickle cell anemia
  - Thalassemias
  - Hereditary persistence of HbF (hemoglobin switching and its regulation)
- Diseases discussed in this chapter are summarized in **Table 42.4**.

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TABLE 42.4

Diseases Discussed in Chapter 42

DISEASE OR DISORDER	ENVIRONMENTAL OR GENETIC	COMMENTS
Thalassemias	Genetic	Unbalanced synthesis of α- and β-chains of hemoglobin, leading to anemia
Pyruvate kinase deficiency	Genetic	Red cell hemolysis, leading to fewer red cells. An increase in 2,3-bisphosphoglycerate levels often masks the effects of the anemia.
Congenital methemoglobinemia	Genetic	Oxidation of the iron in heme to the ferric state, which will not bind oxygen, although many individuals with this disorder are asymptomatic
Glucose 6-phosphate dehydrogenase deficiency	Genetic	Affects red blood cell (RBC) membrane stability through an inability to protect membrane proteins and lipids against oxidation
Porphyrias	Genetic	Inherited defects in almost any step of heme synthesis, leading to a series of diseases with different symptoms and outcomes
Iron deficiency	Both	Reduced iron leads to reduced heme synthesis and reduced oxygen delivery to the tissues
X-linked severe combined immunodeficiency syndrome	Genetic	Loss of a cytokine receptor subunit, leading to a complete loss of B- and T-cell maturation and proliferation and no functional immune system
Defective erythropoietin receptor	Genetic	RBC formation is reduced under conditions in which RBC production should be increased (such as reduced oxygen delivery to the tissues).
Hemoglobin C	Genetic	A point mutation in hemoglobin, leading to a lysine for a glutamic acid at position 6 of the β-chain (E6K), leading to hemolytic anemia in the homozygous state
Hereditary persistence of fetal hemoglobin	Genetic	Mutations in promoter and enhancer regions, leading to misexpression of the globin γ-gene and constant expression of the gene
Spherocytosis	Genetic	Mutations in any of several RBC membrane proteins (such as spectrin), leading to instability of the red cells, destruction of the RBCs, and an anemia

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Review Questions—Chapter 42

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

A. An increase in 2,3-BPG synthesis by the red cell

B. A decrease in 2,3-BPG 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 anemia, with microcytic hypochromic red cells. The boy lives in a 100-year-old apartment building and has been seen 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 one of the following?

A. Iron

B. Vitamin B_{12}

C. Folate

D. Heme

E. Vitamin B_6

3. Drugs are being developed that will induce the transcription of certain 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 one of the following?

A. The α_1-gene

B. The α_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 one of the following?

A. Lymphocyte

B. Basophil

C. Eosinophil

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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 that requires 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 expresses only 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 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.

6. An individual displays an anemic condition and upon molecular analysis is shown to be a compound heterozygote for HbS/HbC. The symptoms exhibited by the patient are more severe than those exhibited by patients with sickle cell trait (HbA/HbS) owing primarily to which one of the following?

A. Increased concentration of HbC molecules in the patient's RBCs

B. Increased volume of the patient's RBCs

C. Increased concentration of HbS in the patient's RBCs

D. Alterations in the patient's RBC morphology

E. Precipitation of HbS molecules within the patient's RBCs

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7. A young boy has been observed to have bluish fingertips and toes. Blood work indicates a mild anemia, and molecular analysis indicates the child has an inherited erythrocyte pyruvate kinase deficiency. This enzyme mutation leads to an increase in the 2,3-BPG levels in the erythrocyte, which helps to ameliorate the effects of the mutation. The increase in 2,3-BPG levels occurs because of which one of the following?

A. The lack of pyruvate kinase leads to an increase in 1,3-BPG levels, which is used to form 2,3-BPG by the Rapoport-Luebering shunt pathway.

B. The increase in phosphoenolpyruvate levels leads to the phosphorylation of 3-phosphoglycerate, forming 2,3-BPG.

C. The increase in phosphoenolpyruvate levels leads to the phosphorylation of 2-phosphoglycerate, forming 2,3-BPG.

D. The increase in phosphoenolpyruvate levels leads to an increase in 3-phosphoglycerate, which is phosphorylated by ATP to produce 2,3-BPG.

E. The lack of pyruvate kinase activity leads to an increase of glyceraldehyde 3-phosphate, which is oxidized by an isozyme of glyceraldehyde 3-phosphate dehydrogenase to form 2,3-BPG.

8. A young boy was recently diagnosed with anemia, and further analysis demonstrated that he had hereditary spherocytosis. This disease leads to anemia through which one of the following mechanisms?

A. Lack of NADPH to protect cell membrane lipids and proteins from oxidation

B. Nutritional deficiency of iron, folate, or vitamin B_{12}

C. Inability to reduce ferric hemoglobin to the normal ferrous state

D. Improper formation of the RBC membrane cytoskeleton

E. A mutation in heme synthesis

9. A patient is a strict vegetarian and, as such, is concerned about getting sufficient iron in his diet. Which suggestion below could increase his dietary iron absorption?

A. Never peel potatoes when preparing a potato dish.

B. Squeeze fresh lemon juice on spinach salad.

C. Reassure him that iron in plants is readily absorbed.

D. Meat is the only dietary source of iron.

E. Taking a vitamin with vitamin B_{12} would help iron absorption.

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10. The pluripotent stem cell of the bone marrow produces all blood cells through different lineages via induction of different differentiation pathways. Which one of the following is produced from the same cell line as RBCs?

A. NK-cells

B. B-lymphocytes

C. T-lymphocytes

D. Basophils

E. Platelets

Answers

1. **The answer is A.** Increased 2,3-BPG in the red cell will favor the deoxy conformation of hemoglobin and thus allow more oxygen to be released in the tissues. This is useful because the hemoglobin is not as saturated at high altitudes as at low elevations because of the lower concentration of oxygen at high altitudes. Answers C and D are incorrect because the red cells do not synthesize proteins. Answer E is incorrect because reducing the blood pH will not aid in oxygen delivery; the Bohr effect works best when tissue pH is lower than blood pH in order to stabilize the deoxy form of hemoglobin. If the pH of both the blood and the tissue are the same, the Bohr effect will not be able to occur.

2. **The answer is D.** The boy is suffering from lead poisoning, which interrupts heme synthesis at the ALA dehydratase and ferrochelatase steps. Without heme, the oxygen-carrying capability of blood is reduced, and the flow of electrons through the electron-transfer chain is reduced because of the lack of functional cytochromes. Together, these lead to an inability to generate energy, and fatigue results. Answer A is incorrect because although an iron deficiency would lead to the same symptoms, this would not be expected in the patient because of his daily vitamin uptake. Lead ingestion will not lead to an iron loss. Answers B and C are incorrect because vitamin B_{12} and folate deficiencies will lead to macrocytic anemia, owing to disruption of DNA synthesis. Answer E is incorrect because the ingestion of paint chips is unlikely to lead to a vitamin B_6 deficiency in a child, particularly one who is taking daily vitamins.

3. **The answer is C.** Turning on a gene that would provide a functional alternative to the β-gene would enable the defective β-protein to be bypassed. Only the γ-chain can do this, but it is normally only found in HbF. The δ-chain is also a β-replacement globin, but it was not listed as a potential answer. Answer D is incorrect because it is the β-chain that is mutated, and it is already being expressed. Unlike the α-gene, of which there are two copies per chromosome, there is only one copy of the β-gene per chromosome. The other genes listed (answers A, B, and E) are α-chain replacements, and expression of these genes will not alleviate the problem inherent in the β-gene.

4. **The answer is D.** The only two types of blood cells that lack nuclei are the mature RBC and the platelet. Platelets arise from membrane budding from megakaryocytes and are essentially membrane sacs containing the cytoplasmic contents of their precursor cell. All of the other cell types listed contain a nucleus.

5. **The answer is D.** Thalassemias result from an imbalance in the synthesis of α- and β-chains. Excessive synthesis of α-chains results in their precipitation in developing red cells, which often kills the developing cell. The more severely affected child has an α/β ratio of 1:5, whereas the less severely affected child has a ratio of 1:2.5. When β-chains are in excess, they form stable tetramers that bind but do not release oxygen, thus reducing the red cell's ability to deliver oxygen. Thus, this difference in chain ratio makes an important difference in the functioning of the red cell.

6. **The answer is C.** HbC forms an insoluble tetramer that precipitates in RBCs. Because of this, the concentration of HbS is increased in the RBCs, leading to enhanced sickling as compared to someone who has a mixture of HbS and HbA molecules. The enhanced sickling is not directly the result of the precipitation of the HbC molecules, nor is it a result of an increased volume of RBCs (an increased volume would reduce the concentration of HbS, which would reduce sickling). The HbS molecules will form rods under low-oxygen conditions, but they do not precipitate in the cell as do the HbC molecules. Alterations in RBC morphology are a result of the sickling, not a cause of the sickling.

7. **The answer is A.** When pyruvate kinase activity is reduced, phosphoenolpyruvate (PEP) will accumulate. PEP is in equilibrium with 2-phosphoglycerate, which is in equilibrium with 3-phosphoglycerate (3-PG). As 3-PG accumulates, the phosphoglycerate kinase reaction will be inhibited, increasing the levels of 1,3-BPG. As 1,3-BPG levels accumulate, some of it will be shunted to produce 2,3-BPG via 1,3-BPG mutase. The increase in 2,3-BPG levels will then affect the release of oxygen to the tissues from hemoglobin. PEP is not used as a phosphate donor in phosphorylation reactions in the generation of higher levels of 2,3-BPG. ATP is used in the conversion of 3-PG to 1,3-BPG but not to form 2,3-BPG. The oxidation of glyceraldehyde 3-phosphate (glyceraldehyde 3-P) does not generate 2,3-BPG; there is no isozyme of glyceraldehyde 3-P dehydrogenase which will produce 2,3-BPG from glyceraldehyde 3-P.

8. **The answer is D.** Mutations in spectrin, ankyrin, and band 3 proteins lead to improper formation of the RBC membrane cytoskeleton, resulting in malformed RBCs (spherocytes) which have a shortened life span, thus leading to anemia. Mutations in heme synthesis lead to porphyrias. Lack of NAPDH (such as in glucose 6-P dehydrogenase deficiency) leads to anemia via cell membrane oxidation and lysis. Oxidation of the heme iron to the ferric state results in reduced oxygen binding to hemoglobin. Nutritional deficiencies of iron, folate, or vitamin B_{12} would lead to a megaloblastic anemia (folate and vitamin B_{12} deficiencies) or a microcytic anemia (iron deficiency).

9. **The answer is B.** Meats, green leafy vegetables, and fortified cereals and grains are all sources of dietary iron, whereas potatoes are not. Plant nonheme iron is not readily absorbed from the diet, but the presence of vitamin C (citrus fruits) increases the uptake of nonheme iron from the digestive tract. Vitamin B_{12} is not involved in iron absorption.

10. **The answer is E.** Both platelets and RBCs are derived from the BFU-EMeg lineage. Basophils are derived from the CFU-Ba lineage, B- and T-lymphocytes from the lymphoid stem cell lineage, and NK-cells from NK-precursor.

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