Heme, an iron-porphyrin complex, is the prosthetic group of many important proteins. The central role of hemoglobin and myoglobin in oxygen transport and storage was discussed in Chapter 28. Heme proteins or enzymes are involved in redox reactions (e.g., cytochromes) and participate in many oxidation reactions needed for synthesis of metabolically important compounds as well as for degradation and detoxification of waste products and environmental toxins.

Ionic forms of iron (referred to hereafter as iron) also participate in a variety of enzymatic reactions as nonheme irons, which are present as iron-sulfur clusters (e.g., mitochondrial electron transport). There are also both storage and transportable forms of iron that are bound to proteins. Under normal physiological conditions only trace amounts of free iron exist. In the body, if iron exceeds the sequestration capacity of the iron-binding proteins present in different physiological compartments, the free iron can cause tissue damage. Cellular injury is caused by reactive oxygen species that are produced from \( \text{H}_2\text{O}_2 \) in a reaction catalyzed by iron. Thus, iron homeostasis in the body is in a delicate balance. Either the deficiency or the excess results in abnormalities and presents as a common cause of human diseases.

29.1 Iron Metabolism

Total-body iron of a 70-kg adult is about 4.2–4.4 g. The distribution of iron in various body compartments is given in Table 29-1. The key players of iron metabolism include iron-responsive elements of appropriate mRNAs, iron regulatory proteins divalent metal transporter 1, major histocompatibility complex (MHC) class I-like protein designated as HFE protein, \( \beta_2 \)-microglobulin, transferrin, transferrin receptor, and ferritin.

Absorption of Iron from the Diet

The dietary requirement for iron depends on the amount and composition of the food, the amount of iron lost from the body, and variations in physiological state such as growth, onset of menses, and pregnancy. The average North American diet contains about 6 mg of iron per 1000 calories and supplies about 10–15 mg/d. Of that ingested, 8–10% (1–1.5 mg/d) is absorbed. Thus, dietary factors that affect absorption are more important than the iron content of the diet and may be more important for correction of iron deficiency than addition of iron to the diet.
TABLE 29-1
Distribution of Iron in a 70-kg Adult

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating erythrocytes</td>
<td>1800</td>
</tr>
<tr>
<td>Bone marrow (erythroid)</td>
<td>300</td>
</tr>
<tr>
<td>Muscle myoglobin</td>
<td>300</td>
</tr>
<tr>
<td>Heme and non-heme enzymes</td>
<td>180</td>
</tr>
<tr>
<td>Liver parenchyma</td>
<td>1000</td>
</tr>
<tr>
<td>Reticuloendothelial macrophages</td>
<td>600</td>
</tr>
<tr>
<td>Plasma transferrin</td>
<td>3</td>
</tr>
</tbody>
</table>

1 These are approximate values. Premenopausal women have lower iron stores due to periodic blood loss through menstruation. Iron balance in the body is maintained by intestinal absorption of 1–2 mg/day and by loss of 1–2 mg/day.
2 1 mg = 17.9 umol
3 Primarily storage forms of iron.
4 Senescent red blood cells are catabolized by the macrophages, the salvaged iron is temporarily stored, and made available via transferrin for erythron and for hemoglobin synthesis.
5 Transportable form of iron.

Iron in food exists mainly in the ferric (Fe³⁺) state, complexed to proteins, amino acids, organic acids, or heme. It is absorbed in the ferrous state, reduction being accomplished in the gastrointestinal tract by ascorbate, succinate, and amino acids. Gastric acid potentiates iron absorption by aiding in formation of soluble and absorbable ferrous chelates. In achlorhydria or after partial gastrectomy, absorption is subnormal but is increased by administration of hydrochloric acid. Carbonates, tannates, phosphates, phytates, and oxalates may decrease iron absorption by formation of insoluble complexes, but their effect can be prevented by adequate dietary calcium, which complexes with them and makes them unavailable for reaction with iron. Absorption of heme iron is not affected by these agents. Heme is absorbed intact from food and more effectively than inorganic iron. Antacids, such as aluminum hydroxide and magnesium hydroxide, also decrease iron absorption.

In general, foods of animal origin provide more assimilable iron than foods of vegetable origin, since on a weight basis, vegetables contain less iron and more substances (e.g., phytates) that inhibit iron absorption. Foods that contain more than 5 mg of iron per 100 g include organ meats (liver, heart), wheat germ, brewer's yeast, oysters, and certain dried beans. Foods that contain 1–5 mg of iron per 100 g include muscle meats, fish, fowl, some fruits (prunes, raisins), most green vegetables, and most cereals. Foods that contain less than 1 mg of iron per 100 g include milk and milk products and most nongreen vegetables.

Ferrous iron is absorbed principally from the mature enterocytes lining the absorptive villi of the duodenum. The amount of iron absorption by these enterocytes is determined by the prior programming of the duodenal crypt cells based on iron requirements of the body as they undergo maturation. The regulation of intestinal iron absorption is critical because iron excretion from the body is a limiting physiological process (discussed later). The small intestine is also an excretory organ for iron, since that stored as ferritin in the epithelial cells is lost when they are shed and replaced every 3–5 days. Heme iron is transported intact into the mucosal cells and the iron removed for further processing.

The mechanism of entry of ferrous iron from the intestinal lumen into the enterocytes and its eventual transport into the portal blood is beginning to be understood. The first step is the programming of the duodenal undifferentiated deep-crypt cells with regard to sensing the iron requirements of the body. The programming of the crypt cells for capacity to absorb iron is thought to occur as follows. A protein (HFE) that spans the cell membrane like an HLA molecule associates with β₂-microglobulin like an HLA protein. The HFE protein is coded for by a gene (HFE) located on the short arm of chromosome 6 near the MHC gene loci. Mutations in the HFE gene are associated with an inherited disorder of excessive dietary iron absorption that is known as hereditary hemochromatosis (discussed later). HFE protein spans the cell membrane of the crypt enterocytes with its N-terminal domain projecting outside. Near the cell membrane a segment of HFE protein, like an HLA protein, associates with β₂-microglobulin (Figure 29-1) and stabilizes the HFE protein.

Plasma transferrin transports iron in the ferric state and is an indicator of iron stores in the body. Each molecule of transferrin binds with two ferric ions (diferric transferrin) and undergoes receptor-mediated endocytosis when bound to transferrin receptors (discussed later) with the aid of HFE protein β₂-microglobulin complex. In the cytosol, iron is released from the endosomes. The level of cytoplasmic iron regulates the translation of mRNA of a protein known as divalent metal transporter-1 (DMT1), which participates in iron entry into the enterocytes located on the villus tip (Figure 29-2). Regulation of DMT1 synthesis is coupled to cytoplasmic iron levels and involves the presence of a stem-loop hairpin structure in the 3'-untranslated region that resembles an iron regulatory element (IRE) and its interaction with an iron regulatory protein (IRP1). In the iron-deficient state, IRP binds to IRE and stabilizes the mRNA of DMT1. This stabilization of mRNA leads to increased production of DMT1 and its eventual localization on the cell surface. The transport of ferrous iron across the apical membrane of the villus enterocyte that is mediated by DMT1 occurs through a proton-coupled process. DMT1 also transports a number
FIGURE 29-1
A diagrammatic representation of the transmembrane HFE protein. The extracellular portion of the HFE protein consists of three α domains. The β2-microglobulin is noncovalently associated with the α3 domain of HFE protein, stabilizing its structure. The extracellular missense mutations H63D and C282Y are identified in patients with hereditary hemochromatosis. HFE protein is involved in sensing circulating iron concentration and participates in the regulation of gene expression of products involved in iron absorption, transport, or storage. [Reproduced with permission from: A novel MHC class I-like is mutated in patients with hereditary hemochromatosis. J. N. Feder, A. Gnirke, W. Thomas et al. Nature Genetics 13, 399 (1996).]

of divalent metal ions, including Mn2+, Cu2+, Zn2+, Cd2+, and Pb2+.

At normal levels of iron intake, absorption requires uptake from the intestinal lumen by the mucosa and transfer from the mucosa to the portal blood. Both events are inversely affected by the state of body iron stores. In iron deficiency states, nonferrous metals such as cobalt and manganese, which have an ionic radius similar to that of iron and form octahedral complexes with six-coordinate covalent bonds, also are absorbed at an increased rate. Oral administration of a large dose of iron reduces (or temporarily inhibits) the absorption of a second dose of iron by the absorptive enterocytes even in the presence of systemic iron deficiency. The mechanism of mucosal block, which resists acquiring additional iron by the enterocytes with high amounts of intracellular iron, is not yet understood. It probably involves set points established in the enterocytes for iron recently consumed in the diet (dietary regulator).

Iron absorption also is affected by erythropoiesis. When erythropoiesis is accelerated by bleeding, hemolysis, or hypoxia, iron absorption is increased. Conversely, diminished erythropoiesis due to starvation, blood transfusion, or return to sea level from a high altitude decreases iron absorption. How the size of body iron stores and the rate of erythropoiesis transmit information to the duodenum is not known. Feedback control seems to be weak or absent, since in iron-deficient subjects enhanced iron absorption continues long after hemoglobin is restored to normal levels. Furthermore, in chronic hemolytic anemia, iron absorption is increased, persists for prolonged periods, and leads to iron overload.

The need for dietary iron is ultimately determined by the rate of iron loss from the body and the amount required for maintenance and growth. Iron is tightly conserved once it is absorbed. Its excretion is minimal and unregulated, and facilitated by normal exfoliation from the surfaces of the body (dermal, intestinal, pulmonary, urinary), loss of blood by gastrointestinal bleeding, and loss in bile and sweat. Insignificant amounts are lost in urine, since iron in plasma is complexed with proteins that are too large to pass through the kidney glomerular membrane. Iron in feces is primarily unabsorbed dietary iron. Obligatory iron
FIGURE 29-2
Intestinal absorption of dietary iron. Ferrous iron is absorbed by the duodenal villus tip enterocytes mediated by divalent metal transporter-1 (DMT1). Iron transport mediated by DMT1 of the apical surface and the basolateral transporter at the basolateral surface are coupled to ferric reductase and ferroxidase that change the iron oxidation state, respectively. The degree of iron entry is determined by the level of DMT1 and its level of expression is programmed in the crypt cells. The programming of the crypt cells is coupled to the body iron stores via transferrin-mediated and HFE protein-modulated iron transport. [Modified and reprinted with permission from B. R. Bacon, L. W. Powell, P. C. Adams, et al. Molecular medicine and hemochromatosis: at the cross roads. Gastroenterology 116, 193 (1999).]

loss for a 70-kg man is 0.5–1 mg/d, an amount equal to that normally absorbed from the diet. During growth, menstruation, and pregnancy, the requirement reaches about 2–2.5 mg/d. Recommended daily iron intake for various groups is shown in Appendix IV.

The principal loss of iron in nonpregnant women during the reproducing years is through menstruation. In one study, the mean menstrual blood loss was 43.4 ± 2.3 mL. Since each milliliter of blood from a normal woman contains about 0.5 mg of iron, the amount lost every 27 days is
about 20–23 mg. Increased menstrual flow (menorrhagia) significantly augments iron loss and leads to iron deficiency anemia (see below). In pregnancy use of supplemental iron is recommended. A newborn has about a 3- to 6-month supply of iron in its liver and may require iron-rich foods from the sixth month onward, since milk is poor in iron.

**Plasma Iron Transport**

Over 95% of plasma iron is in the Fe$^{3+}$ state bound to the glycoprotein transferrin, a monomeric $\beta_1$-globulin (M.W. 80,000). Electrophoretic studies have revealed the existence of 21 genetic variants. In some, single-amino-acid substitutions account for variation in electrophoretic mobility. Transferrin is synthesized primarily in the liver and appears at the end of the first month of fetal development. Its half-life in humans is about 8 days. Desialylation may be a requirement for its removal from plasma by the liver, as it is for other plasma proteins (Chapter 10). In fact, asialotransferrin is more rapidly cleared from plasma than transferrin. It is not required for intestinal absorption of iron.

Each molecule of transferrin can bind two Fe$^{3+}$ ions. The binding is extremely strong under physiological conditions, and the binding constants of the two sites are not significantly different. For each Fe$^{3+}$ bound, one HCO$_3^-$ ion is also bound and three H$^+$ ions are released from the protein.

Thus, diferric transferrin gains two net negative charges.

$$2\text{Fe}^{3+} + \text{apotransferrin} + 2\text{HCO}_3^- \rightleftharpoons [\text{Fe}_2\text{-transferrin-(HCO}_3^-)_2]^{2^-} + 6\text{H}^+$$

The metal binding sites are located in N- and C-terminal domains. The protons released upon binding of each Fe$^{3+}$ ion are probably derived from ionization of two tyrosyl residues and of a water molecule bound to Fe$^{3+}$ ion.

The bulk of transferrin iron is delivered to immature erythroid cells for utilization in heme synthesis. Iron in excess of this requirement is stored as ferritin and hemosiderin. Unloading of iron to immature erythroid cells is by receptor-mediated endocytosis. The process begins in the clathrin-coated pits with the binding of diferric transferrin to specific plasma membrane transferrin receptors that are associated with the HFE protein complex. The next step is the internalization of the transferrin-transferrin receptor-HFE protein complex with formation of endosomes. The iron transporter DMT1 present in the cell membrane is also internalized into the endosomes. In the endosomes, a proton pump acidifies the complex to pH 5.4, and by altering conformation of proteins, iron is released from transferrin bound to transferrin receptor and HFE protein. This process of iron release from the complex is inhibited by HFE protein. Thus, dysfunctional HFE protein can cause excessive release of iron from the transferrin-transferrin receptor-HFE protein complex. In the acidified endosomes, DMT1 facilitates iron transport into the cytosol. Both apotransferrin (and a fraction of iron-bound transferrin) and transferrin receptor are returned to cell surfaces for reuse. In this type of receptor-mediated endocytosis of transferrin-transferrin receptor complex, the endosomes do not come into contact with lysosomes. The process is therefore unlike that of low-density lipoprotein receptor-mediated internalization (Chapter 20).

In the erythroid cells, most of the iron released from the endosomes is transported into mitochondria for heme synthesis (discussed later); in nonerythroid cells, the iron is stored predominantly as ferritin and to some extent as hemosiderin.

**Storage of Iron**

There are two storage forms of iron, ferritin and hemosiderin. Ferritin is the predominant storage form and contains diffusible, soluble, and mobile fractions of iron. Hemosiderin is aggregated deposits resulting from the breakdown of ferritin in secondary lysosomes and its level increases progressively with increasing levels of iron overload. Apoferritin is a protein shell consisting of 24 subunits of two types; a light (L) subunit (M.W. 19,000) and a heavy (H) subunit (M.W. 21,000). The H subunit has ferroxidase activity and the L subunit facilitates nucleation and mineralization of the core made up of hydrated ferric oxide phosphate complex.

**Coordinate Regulation of Iron Uptake and Storage in Non-Erythroid Cells**

Iron uptake is regulated by transferrin receptor and storage of iron as ferritin which occurs post-transcriptionally for these two proteins. The regulation maintains an optimal intracellular-transit-chelatable iron pool for normal functioning in the body. The regulatory process consists of an interaction between IREs and IRPs 1 and 2. One copy of each IRE has been identified in the 5'-untranslated region of H and L ferritin mRNAs and five copies in the 3'-untranslated region (UTR) of transferrin receptor mRNA. IRE sequences are highly conserved and have a stem-loop structure with a CAGUGN sequence at the tip of the loop. IRPs are RNA-binding proteins that bind to IREs and regulate the translation of the respective mRNAs.

During low levels of intracellular chelatable iron, iron storage declines due to inhibition of ferritin synthesis; cellular entry of iron increases due to enhanced transferrin receptor synthesis. An opposing set of events occurs...
CHAPTER 29 Metabolism of Iron and Heme

At low cytosolic mobile iron pool:
IRP levels increase and bind to mRNA-IREs of ferritin and transferrin receptors.

At high cytosolic mobile iron pool:
IRP levels decrease causing opposite effects on the mRNAs.

FIGURE 29-3
Coordinate translational regulation of ferritin mRNA and transferrin receptor mRNA in nonerythroid cells. Iron regulatory proteins (IRP) are RNA-binding proteins that bind to iron regulatory elements (IREs). IREs are hairpin structures with loops consisting of CAGUGN sequences and are located at the 5'-untranslated region (UTR) and 3'-UTR for ferritin mRNA and transferrin mRNA, respectively.

During intracellular chelatable iron excess or iron-replete states, a coordinated control occurs when IRP binds to IRE at the 5'-UTR of ferritin mRNAs inhibiting ferritin synthesis; simultaneously, the binding of IRP to IRE at the 3'-UTR of transferrin receptor mRNA stimulates transferrin receptor synthesis (Figure 29-3). Intracellular iron regulates the level of IRPs. During the expansion of the iron pool, IRPs are inactivated, leading to efficient translation of ferritin mRNA and rapid degradation of transferrin receptor mRNA. In iron-replete cells, IRP1 acquires iron by the formation of iron-sulfur clusters (4Fe-4S) that bind to IREs with low affinity. During iron deficiency states, IRP1 lacks a 4Fe-4S cluster and binds to IREs with high affinity. IRP1, when it possesses an iron-sulfur cluster, has aconitase activity, normally TCA cycle enzyme (Chapter 14). Mutations that change IREs can lead to constitutive ferritin biosynthesis. An autosomal dominant disorder of IRE leads to hyperferritinemia without any iron overload. Patients with this inherited disorder also exhibit early onset of cataract that may also be cotransmitted as an autosomal dominant trait. Other factors also regulate ferritin synthesis. For example, nitric oxide enhances binding of IRP to IRE and inhibits ferritin synthesis. One of the causes of anemia of chronic inflammatory diseases may be due to increased ferritin synthesis in the reticuloendothelial macrophages by inflammatory cytokines interleukins 1 and 6 (IL-1 and IL-6), which act by preventing efficient release of iron.

Measurement of serum ferritin levels has diagnostic utility. In iron deficiency anemia (discussed later), serum ferritin levels are low; in iron storage disease, the levels are high. However, serum ferritin levels can also be elevated under many other circumstances, including liver diseases and chronic inflammatory diseases.

Alterations of Plasma Transferrin Concentration
Plasma transferrin levels are commonly measured in the evaluation of disorders of iron metabolism (see below). It is customary to measure transferrin concentration...
indirectly from the maximum (or total) iron binding capacity (TIBC) of plasma (reference range for adults, 250–400 μg/dL). It can also be measured directly by immunological methods (reference range for adults, 220–400 mg/dL). **Hypertransferrinemia** (or increased TIBC) can occur with diminished body iron stores as in iron deficiency anemia or during pregnancy (because of enhanced mobilization of storage iron to supply maternal and fetal demands). Hypertransferrinemia of iron deficiency is corrected by oral iron supplementation, whereas that due to pregnancy is not. Exogenous administration of estrogens (e.g., oral contraceptives) also causes hypertransferrinemia.

**Hypotransferrinemia** can result from protein malnutrition and accompanies hypoalbuminemia. Since transferrin has a much shorter half-life (8 days) than albumin (19 days), measurement of the transferrin level may be a more sensitive indicator of protein malnutrition than albumin measurement (see also chapter 17). Hypotransferrinemia also results from excessive renal loss of plasma proteins (e.g., in nephrotic syndrome).

**Disorders of Iron Metabolism**

**Iron Deficiency Anemia**

Iron deficiency anemia is the most prevalent nutritional disorder. Its cause may comprise many overlapping factors: dietary iron deficiency; absence of substances that favor iron absorption (ascorbate, amino acids, succinate); presence of compounds that limit iron absorption (phytates, oxalates, excess phosphates, tannates); lack of iron absorption due to gastrointestinal disorders (malabsorption syndrome, gastrectomy); loss of iron due to menstruation, pregnancy, parturition, lactation, chronic bleeding from the gastrointestinal tract peptic ulceration, hemorrhoids, cancer, colonic ulceration, or hookworm infestation or the genitourinary tract (uterine fibroids); enhanced demand for growth or new blood formation; deficiency of iron transport from mother to fetus; abnormalities in iron storage; deficiencies in release of iron from the reticuloendothelial system (infection, cancer); inhibition of incorporation of iron into hemoglobin (lead toxicity); and rare genetic conditions (transferrin deficiency, impaired cellular uptake of iron by erythroid precursors).

In the initial phase of depletion of the iron content of the body, the iron stores maintain normal levels of hemoglobin and of other iron proteins. With exhaustion of storage iron, hypochromic and microcytic anemia becomes manifest.

The clinical characteristics of iron deficiency anemia are nonspecific and include pallor, rapid exhaustion, muscular weakness, anorexia, lassitude, difficulty in concentrating, headache, palpitations, dyspnea on exertion, angina on effort, peculiar craving for unnatural foods (pica), ankle edema, and abnormalities involving all proliferating tissues, especially mucous membranes and the nails. The onset is insidious and may progress slowly over many months or years.

Physiological adjustments take place during the gradual progression of the disorder, so that even a severe hemoglobin deficiency may produce few symptoms. Iron deficiency may affect the proper development of the central nervous system. Early childhood iron deficiency anemia may lead to cognitive abnormalities.

Individuals who have **congenital transferrinemia** lack apotransferrin and suffer from severe hypochromic anemia in the presence of excess iron stores in many body sites, susceptibility to infection (transferrin inhibits bacterial, viral, and fungal growth, probably by binding the iron required for growth of these organisms), and retardation of growth. This condition does not respond to administration of iron. Intravenous administration of transferrin normalizes the iron kinetics. A rare congenital defect in uptake of iron by red cell precursors has been reported that leads to severe hypochromic anemia with normal plasma iron and transferrin levels.

Microcytic anemia occurs frequently in thalassemia syndromes (Chapter 28), but these patients do not require iron supplementation unless they have concurrent iron deficiency as assessed by measurement of serum iron levels and TIBC. Serum iron concentration exhibits a morning peak and an evening nadir; this pattern is reversed in night-shift workers. The circadian variation is primarily due to differences in rate of release of iron by the reticuloendothelial system. Transferrin levels do not show circadian fluctuation. Iron deficiency anemia can also be assessed from the plasma ferritin concentration (which when decreased reflects depleted iron stores), red cell protoporphyrin concentration (increased because of lack of conversion to heme), and the number of sideroblasts in the bone marrow (which parallels iron stores). Sideroblasts are erythrocyte precursors (normoblasts) containing free ferritin–iron granules in the cytoplasm that stain blue with the Prussian blue reagent. There is a close correlation between plasma iron levels, TIBC, and the proportion of sideroblasts in bone marrow. In hemolytic anemias, pernicious anemia, and hemochromatosis, the serum iron level increases and sideroblast number reaches 70% (normal range, 30–50% of total cells). In iron deficiency, the sideroblasts are decreased in number or absent.

Before treatment is initiated, the cause of the negative iron balance must be established. Treatment should correct the underlying cause of anemia and improve the iron balance. In general, oral therapy with ferrous salts is
satisfactory; however, sometimes parenteral therapy is preferred, e.g., in proven malabsorption problems, gastrointestinal disease and excessive blood loss, and for patients who cannot be relied on to take oral medication.

Iron-Storage Disorders

A type of iron storage disorder characterized by general increase in tissue iron levels without damage to parenchymal cells is known as hemosiderosis. Hemosiderin is a storage form of iron in which ferric hydroxide is present as micelles. It appears as insoluble granules that contain denatured aggregated ferritin, nonferritin proteins, lipids, heme, and other pigments. Hemosiderosis results when iron is present in excessive quantities in a diet that permits maximum iron absorption. For example, the African Bantu eat a diet high in corn (low in phosphate) cooked in iron pots, drink an indigenous beer brewed in iron pots, and suffer from Bantu siderosis. Hemosiderosis can progress to hemochromatosis with hepatic cirrhosis and diabetes mellitus.

Excessive accumulation of iron (chronic iron overload) can result from the following.

1. Defective erythropoiesis (dyserythropoiesis); impaired hemoglobin synthesis leading to lack of utilization and consequent accumulation of iron in mitochondria, e.g., from inhibition of ALA synthase activity by dietary vitamin B6 deficiency; inhibition of heme synthesis by lead; impairment of pyridoxine metabolism in alcoholic patients; familial sideroblastic anemias; and Cooley's anemia.
2. Repeated blood transfusions, e.g., in Cooley's anemia or sickle cell disease.
3. Hereditary hemochromatosis, an autosomal recessive defect in which there is increased rate of absorption of iron in the presence of normal or enlarged iron stores and normal hematopoiesis (discussed later).
4. High dietary iron and substances that enhance its absorption (e.g., Bantu siderosis).
5. Hereditary atransferrinemia.

In all of these disorders, the gastrointestinal tract cannot limit absorption of iron to significant extent. Thus, the “mucosal block” responsible for keeping out unneeded iron on a daily basis is susceptible to disruption, perhaps at more than one point. Iron overload leads to progressive deterioration in pancreatic, hepatic, gonadal, and cardiac function. Clinical manifestations include cirrhosis, diabetes mellitus, life-threatening arrhythmias, and intractable heart failure. Removal of excess iron produces clinical improvement, particularly of diabetes and congestive heart failure.

In iron storage diseases accompanied by normal erythropoiesis (e.g., hereditary hemochromatosis), removal of excessive iron is accomplished by repeated bloodletting (phlebotomy). Therapeutic phlebotomy of a unit of blood (which contains about 250 mg of iron) may be performed up to three times per week. When the iron stores become depleted, reaccumulation of iron is prevented by four to six phlebotomies per year. In asymptomatic patients, periodic determination of serum ferritin provides a measure of storage of iron.

In hemochromatosis secondary to refractory anemias (e.g., Cooley's anemia, sickle cell anemia), patients require repeated blood transfusions to survive childhood and adulthood. Therapy consists of administration of iron-chelating agents. Deferoxamine, an iron chelator isolated from Streptomyces pilosus, has the structure:

\[
\begin{align*}
H_2N-&(CH_2)_5-N-C-(CH_2)_2-C-N-(CH_2)_5-N-C-(CH_2)_2-C-N-(CH_2)_5-N-C-CH_3 \\
\end{align*}
\]

It contains six nitrogen atoms separated by fairly long, flexible stretches of methylene groups. Since each iron atom can bind six ligands, one molecule of deferoxamine is probably capable of occupying all six coordination sites and producing a 1:1 iron-deferoxamine complex.

For ferric iron, the \(K_{assoc}\) of deferoxamine is about \(10^{30}\), while the \(K_{assoc}\) for \(Ca^{2+}\) is about \(10^2\). Iron in hemoproteins is not affected by this agent, while the ferric iron of ferritin and hemosiderin is chelated in preference to that found in transferrin. Such selectivity makes the compound useful in treatment of iron storage problems and acute iron poisoning.

The deferoxamine-iron complex is excreted in urine. (Iron is not normally excreted by this route.)

Deferoxamine given orally complexes with dietary iron, making the drug and the iron unavailable for absorption. The preferred route of administration is by intramuscular injection. Irritation and pain at the site of administration and the need for daily injections make the treatment unpopular. In addition, even with coadministration of large amounts of ascorbic acid, the iron loss produced is far below that necessary to remove all of the iron accumulated during chronic transfusion therapy.

Slow, continuous intravenous infusion or continuous subcutaneous administration may be more effective in
establishing negative iron balance and eliminating stored iron. A small, labile (chelatable) iron pool may be in slow equilibrium with a much larger (storage) pool. When deferoxamine is administered, the labile pool is rapidly emptied. Any deferoxamine that remains in the body or is administered thereafter finds no iron to bind. Thus, most of a single intramuscular dose is simply excreted unchanged. If the chelator is given as a continuous infusion, the labile pool is initially depleted and kept empty. As iron is released from storage sites, it is immediately chelated and removed. Removal of up to 180 mg of iron per day has been accomplished by this method, making it as effective as phlebotomy. Massive intravenous injections of deferoxamine have also been reported to produce excretion of large amounts of iron in iron-overloaded patients.

**Hereditary Hemochromatosis**

Hereditary hemochromatosis is a common inherited autosomal recessive disorder of excessive iron accumulation in parenchymal cells of liver, heart, pancreas, endocrine organs, skin, and joints. The term hemochromatosis is used when organ damage has occurred in the presence of impaired function. It occurs predominately in Caucasian populations; about 1 in 200–400 Caucasians are at risk for developing clinical symptoms. Individuals with hereditary hemochromatosis absorb about 3–4 mg/d of iron as compared with a normal rate of about 1–2 mg/d. This excess iron, absorbed over several years, causes accumulation of as much as 20–40 g as compared to normal amounts of about 4 g. In untreated patients, progressive iron accumulation can cause organ damage resulting in hepatic dysfunction, diabetes, cardiomyopathy, hypogonadism with infertility, arthritis, and skin hyperpigmentation. Death can occur due to cirrhosis, diabetes, cardiomyopathy or hepatocellular carcinoma.

Hereditary hemochromatosis is associated with a gene on the short arm of chromosome 6 near the MHC gene complex. This gene is known as HFE and codes for HFE protein. The roles of HFE protein along with β₂-microglobulin in the regulation of intestinal iron absorption and iron sequestration in the form of ferritin and hemosiderin have been discussed previously. Gene knockout mice for either HFE protein or β₂-microglobulin develop an iron overload disorder similar to human hemochromatosis, thus substantiating the roles of HFE protein and β₂-microglobulin in iron homeostasis.

Two missense mutations (C282Y and H63D) in the HFE gene have been identified in hereditary hemochromatosis. The substitution of a tyrosyl residue for a cysteiny1 residue at position 282 results in the loss of formation of a disulfide linkage necessary for the proper association with β₂-microglobulin (Figure 29-1). In the absence of this disulfide linkage, HFE protein fails to reach the normal membrane location and is rapidly degraded. Thus, C282Y is a loss of function (knockout) mutation. The H63D mutation has no effect on the HFE protein’s association with β₂-microglobulin. However, this mutation may compromise the protein regulation of the interaction between transferrin and its receptor.

Population studies among Caucasians have shown that about 1 in 10 are heterozygous for the C282Y mutation. Homozygosity of C282Y has been observed in 85–90% of hereditary hemochromatosis patients. In about 4% of the hereditary hemochromatosis patients, heterozygosity of C282Y and H63D has been observed. There are still unanswered questions concerning hereditary hemochromatosis. For example, some C282Y homozygotes exhibit neither biochemical nor clinical evidence of iron accumulation. On the contrary, some hemochromatosis patients do not possess a C282Y mutation. Thus, other yet-to-be-identified genetic and environmental factors must play a role in the development of hemochromatosis.

Other forms of hemochromatosis include neonatal and juvenile types in which biochemical defects have not yet been identified. Patients with aceruloplasminemia resulting from mutations in the ceruloplasmin gene exhibit accumulation of iron in neural and glial cells in the brain, in hepatocytes, and in pancreatic islet cells. Ceruloplasmin, a copper-containing protein, has ferroxidase activity and participates in the release of iron from cells. Aceruloplasminemia associated with iron overload is a different disorder from that of Wilson’s disease (hepatolenticular degeneration) in which biliary excretion of copper and incorporation of copper into ceruloplasmin are defective (Chapter 37). Porphyria cutanea tarda, a disorder of porphyrin biosynthesis (discussed later), usually is accompanied by iron accumulation. Thirty percent of patients with porphyria cutanea tarda are either homozygous or heterozygous for the C282Y mutation affecting the HFE protein.

Treatment of hereditary hemochromatosis is therapeutic phlebotomy (discussed earlier). This method is safe, effective, and life saving, and ideally should begin before symptoms develop. Serum ferritin levels are used as a surrogate marker for estimating total-body iron stores. Morphologic studies and quantitative determination of iron in liver tissue obtained by biopsy have been used in the assessment of early hereditary hemochromatosis and the degree of liver injury.

Finally, hereditary hemochromatosis is a treatable disease. Biochemical screening for the identification of the
disease in the general population has been suggested. The biochemical tests include the measurement of serum levels of iron, transferrin saturation, and ferritin.

29.2 Heme Biosynthesis

The principal tissues involved in heme biosynthesis are the hematopoietic tissues and the liver. Biosynthesis requires participation of eight enzymes, of which four (the first and the last three) are mitochondrial and the rest are cytosolic (Figure 29-4). The reactions are irreversible. Glycine and succinate are the precursors of porphyrins.

Formation of δ-Aminolevulinic Acid

δ-Aminolevulinic acid (ALA) formation is catalyzed by mitochondrial ALA synthase, which condenses glycine and succinyl-CoA to ALA. The enzyme is located on the matrix side of the inner mitochondrial membrane. It is encoded by a nuclear gene and is synthesized in the cytosol on the free polyribosomes as a precursor. The precursor protein is processed to active form during its translocation into mitochondria (Chapter 25). Pyridoxal phosphate is the required coenzyme.

The reaction mechanism consists of formation of a Schiff base by pyridoxal phosphate with a reactive amino group of the enzyme; entry of glycine and formation of an enzyme-pyridoxal phosphate-glycine-Schiff base complex; loss of a proton from the α carbon of glycine with the generation of a carbanion; condensation of the carbanion with succinyl-CoA to yield an enzyme-bound intermediate (α-amino-β-ketoacidic acid); decarboxylation of this intermediate to ALA; and liberation of the bound ALA by hydrolysis. ALA synthesis does not occur in mature erythrocytes.

In experimental animals, deficiency of pantothenic acid (needed for CoASH and, hence, succinyl-CoA synthesis), lack of vitamin B₆, or the presence of compounds that block the functioning of pyridoxal phosphate (e.g., isonicotinic acid hydrazide; Chapter 17) can prevent heme synthesis and cause anemia. Heme synthesis also requires a functional tricarboxylic acid cycle and an oxygen supply.

The primary regulatory step of heme synthesis in the liver is apparently that catalyzed by ALA synthase. The regulatory effects are multiple. The normal end product, heme, when in excess of need for production of heme proteins, is oxidized to hematin, which contains a hydroxyl group attached to the Fe³⁺ atom. Replacement of the hydroxyl group by a chloride ion produces hemin. Hemin and heme inhibit ALA synthase allosterically. Hemin also inhibits the transport of cytosolic ALA synthase precursor protein into mitochondria.

ALA synthase has a turnover rate of 70 minutes in adult rat liver and is inducible. Its induction is suppressed by hemin and increased by a variety of xenobiotics (e.g., environmental pollutants) and natural steroids. In erythropoietic tissues, where the largest amount of heme is synthesized, regulation of heme biosynthesis may also involve the process of cell differentiation and proliferation of the erythron, which occurs to meet change in requirements for the synthesis of heme. The differentiation and proliferation are initiated by erythropoietin.

Formation of Porphobilinogen

Two molecules of ALA are condensed by cytosolic zinc containing ALA dehydratase to yield porphobilinogen (PBG). There are four zinc ions per
FIGURE 29-4
Biosynthetic pathway of heme. The pathway consists of eight irreversible reactions, four each in the mitochondrion and the cytosol. The primary site of regulation is the ALA synthase step.

Formation of Uroporphyrinogen III
Uroporphyrinogen III formation occurs in the cytosol and requires the successive action of porphobilinogen deaminase (or methylbilane synthase) and uroporphyrinogen III synthase. Porphobilinogen deaminase catalyzes condensation of four porphobilinogen molecules in a symmetrical head-to-tail arrangement to form a straight-chain tetrapyrrole, hydroxymethylbilane. Uroporphyrinogen III synthase catalyzes the rearrangement of one of the pyrrole rings (ring D in Figure 29-5) to form an asymmetrical tetrapyrrole, followed by its cyclization to form uroporphyrinogen III. In the absence of uroporphyrinogen III synthase (e.g., in congenital erythropoietic porphyria), the hydroxymethylbilane cyclizes spontaneously to the uroporphyrinogen I isomer, which is not a precursor of heme (Figure 29-5).

Formation of Coproporphyrinogen III
Cytosolic uroporphyrinogen decarboxylase catalyzes successive decarboxylation of the four acetic groups to yield four methyl groups (Figure 29-6).

Formation of Protoporphyrinogen IX
Mitochondrial coproporphyrinogen oxidase is localized in the intermembrane space and is probably loosely bound to the outer surface of the inner membrane. It catalyzes the successive conversion of propionic acid groups of ring A and ring B to vinyl groups (Figure 29-7).

Formation of Protoporphyrin IX and Heme
Both of these steps occur in mitochondria (Figure 29-8). Porphyrinogen oxidase removes six hydrogen atoms (four from methane bridge carbons and two from pyrrole nitrogens) from protoporphyrinogen to yield protoporphyrin. The oxidase has an absolute requirement for oxygen. Protoporphyrinogen can also be oxidized nonenzymatically to protoporphyrin at physiological pH, temperature, and aerobic conditions. Protoporphyrin oxidase is bound to the
inner mitochondrial membrane, and its active site faces the cytosolic side of the membrane. Formation of heme is accomplished by ferrochelatase (or heme synthase), which incorporates Fe$^{2+}$ into protoporphyrin and is inhibited by lead. Zinc can function as a substrate in the absence of iron.

**Disorders of Heme Biosynthesis**

The *porphyrias* are a group of disorders caused by abnormalities in heme biosynthesis. They are inherited and acquired disorders characterized by excessive accumulation and excretion of porphyrins or their precursors. Defects in any one of the eight enzymes involved in heme biosynthesis may cause inherited porphyrin-related disorders (Figure 29-9). Porphyrins have a deep red or purple color (Greek *porphyra* = purple). Porphyrins are excreted by different routes, depending on their water solubility. For example, uroporphyrin with its eight carboxylic group substituents is more water-soluble than the porphyrins derived from it and is eliminated in the urine, whereas protoporphyrin (which contains two carboxylic groups) is excreted exclusively in bile. Coproporphyrin has four carboxylic groups and is found in bile and urine.

These disorders are associated with acute or cutaneous manifestations (or both). In the acute state, the presentation may include abdominal pain, constipation, hypertension, tachycardia, and neuropsychiatric manifestations. Cutaneous problems consist of photosensitivity (itching, burning, redness, swelling, and scarring), hyperpigmentation, and sometimes hypertrichosis.
Porphyria may be classified as hepatic or erythropoietic. However, enzyme defects are sometimes common to both tissues. Porphyrias can be induced by alcohol, stress, infection, starvation, hormonal changes (e.g., menstruation), and certain drugs. These drugs presumably precipitate acute manifestations in susceptible subjects since they are inducers of cytochrome P-450 and increase the need for synthesis of heme as they deplete the mitochondrial pool of free heme. Major hepatic porphyrias include acute intermittent porphyria, variegate porphyria, hereditary coproporphyria, and porphyria cutanea tarda. The principal erythropoietic porphyrias are hereditary erythropoietic porphyria and erythropoietic protoporphyria.

**Hepatic Porphyrias**

**Acute intermittent porphyria** is associated with excessive urinary excretion of ALA and porphobilinogen. The lack of polymerization of porphobilinogen is due to deficiency of porphobilinogen deaminase in several cell types (e.g., hepatocytes, erythrocytes, fibroblasts, lymphocytes). Acute clinical manifestations include neuropsychiatric disorders and abdominal pain. The cause of these manifestations is not clear, but accumulation of porphyrin precursors (ALA and porphobilinogen) in pharmacological amounts has been implicated. Since afflicted subjects cannot make porphyrins to any great extent, they are not photosensitive. This disorder is inherited as an autosomal dominant trait.

**Porphyria cutanea tarda** is the most common form. It is inherited as an autosomal dominant trait and is due to deficiency of uroporphyrinogen III decarboxylase. Clinical

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**Figure 29-6**

Formation of coproporphyrinogen III from uroporphyrinogen III. Acetic acid side chains (Ac) are decarboxylated to methyl groups (M), sequentially, starting clockwise from ring D. P, -CH$_2$CH$_2$COOH.

(an abnormally excessive growth of hair). Four porphyrias can manifest as acute disorders: δ-ALA dehydratase deficiency porphyria, acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria.
manifestations are mild to severe photosensitivity and liver disease. Most affected individuals have increased hepatic iron stores, which can be successfully decreased by phlebotomy. Acute episodes can be precipitated by overindulgence of alcohol or, less frequently, by therapy with estrogen.

**Hereditary coproporphyria** and **variegate porphyria** are inherited as autosomal dominant traits. They are caused by deficiency of coproporphyrinogen oxidase and protoporphyrinogen oxidase, respectively.

In most of these disorders, increased hepatic ALA synthase activity is due to decreased heme synthesis. There are also increased amounts of ALA and porphobilinogen in liver, plasma, and urine and specific metabolites produced before the metabolic block. ALA synthase is regulated by the heme by a feedback process and by gene repression. Hematin has been used to treat acute attacks with marked success. Although often inadequate, carbohydrate feeding has been associated with improvement in acute intermittent porphyria. This “glucose effect” may depend on repression of the gene for ALA synthase, but the mechanism is not known.

**Erythropoietic Porphyrias**

A defect in synthesis of type III isomers from hydroxymethylbilane, due to deficiency of uroporphyrinogen III
synthase, produces congenital erythropoietic porphyria. Type I porphyrins (principally uroporphyrin I) are formed, accumulate in the tissues, and are excreted in the urine. The deficiency in production of the type III isomer further increases levels of the type I isomers by reducing the regulatory effect on ALA synthase. Excessive amounts of porphyrins in erythrocytes may produce hemolysis. A compensatory increase in hemoglobin formation can then exaggerate the already increased production of type I porphyrins. Their accumulation produces a pink to dark red color in teeth, bones, and urine. Red-brown teeth and urine are pathognomonic. Patients are sensitive to long-wave ultraviolet light and sunlight. The abnormality is transmitted as an autosomal recessive trait.

Erythropoietic protoporphyrin results from deficiency of ferrochelatase in reticulocytes in bone marrow. It is transmitted as an autosomal dominant trait with variable penetrance and expressivity. In general, it is a benign disorder whose most prominent symptom is photosensitivity. Occasionally, it leads to liver disease. Reduced ferrochelatase activity results in accumulation of protoporphyrin in maturing reticulocytes and young erythrocytes. When a smear of these cells is exposed to fluorescent light, they exhibit red fluorescence. The protoporphyrin appears in the plasma, is picked up by the liver, and is excreted into the bile. Protoporphyrin accumulation in the liver can lead to severe liver disease. In contrast to individuals afflicted by other porphyrias, these patients have normal urinary porphyrin levels. High levels of protoporphyrin are found in erythrocytes, plasma, and feces. The photosensitivity may be caused by stimulation of protoporphyrin in dermal capillaries to an excited (triplet) state by visible light. This in turn converts molecular oxygen to singlet oxygen, which produces cell damage. Oral administration of β-carotene decreases the photosensitivity, possibly owing to a quenching effect on singlet oxygen and free-radical intermediates.

29.3 Heme Catabolism

When heme proteins are degraded in mammals, the polypeptides are hydrolyzed to amino acids while the heme groups are freed of their iron, which is salvaged, and are converted to bilirubin. After transport to the liver, bilirubin is coupled to glucuronic acid and the conjugated bilirubin is excreted into bile as the principal bile pigment. When increased production or decreased excretion of bilirubin causes its plasma concentration to exceed 0.1–1.0 mg/dL (2–17 μmol/L), it diffuses into tissues and produces jaundice. Although jaundice is relatively harmless unless due to extremely high concentrations of unconjugated bilirubin, it indicates the presence of a disease process that requires medical attention. The yellow coloration of jaundiced skin and sclerae has aroused much interest and has made bilirubin the subject of extensive research. Fractionation and quantitation of serum bilirubin are now widely used for diagnosis and prognosis of hepatobiliary disease.

Bilirubin is a waste product and has no known beneficial physiological function. However, both the conjugated and the unconjugated forms of bilirubin show antioxidative properties (e.g., inhibition of lipid peroxidation). The physiological role of the antioxidative property of bilirubin is not known.

Bilirubin is a yellow-orange pigment that in its unconjugated form is strongly lipophilic and cytotoxic. It is virtually insoluble in aqueous solutions below pH 8 but readily dissolves in lipids and organic solvents and diffuses freely across cell membranes. Bilirubin toxicity is normally prevented by tight binding to serum albumin. Only when the binding capacity of albumin is exceeded can a significant amount of unconjugated bilirubin enter cells and cause damage. Conjugated bilirubin is hydrophilic and does not readily cross cell membranes, even at high concentrations. Of the 250–300 mg (4,275–5,130 μmol) of bilirubin normally produced in 24 hours, about 70–80% is derived from hemoglobin. The remainder comes from several sources, including other heme proteins (e.g., cytochromes P-450 and bs, catalase), ineffective hemopoiesis (erythrocytes that never leave the marrow), and “free” heme (heme never incorporated into protein) in the liver. Hemoglobin heme has a life span equal to that of the red cell (about 125 days), whereas heme from other sources (with the exception of myoglobin, which is also quite stable) turns over much more rapidly. Hepatic P-450 enzymes have half-lives of 1–2 days. When radioactively labeled glycine or ALA is injected and radioactivity in fecal bile pigments is monitored, two peaks are seen. The rapidly labeled bilirubin (early bilirubin) peak appears 3–5 days after injection and contains about 15–20% of the injected label. It is increased by drugs that induce hepatic P-450 oxygenases and in erythropoietic porphyria and anemias associated with ineffective erythropoiesis (lead poisoning, thalassemias, and some hemoglobinopathies). Thus, the bilirubin in the early peak is partly derived from these sources. The slowly labeled bilirubin (late bilirubin) peak appears at approximately 120 days, contains about 80–85% of the label, and is due to heme released from senescent erythrocytes.
rate-limiting step in catabolism of heme. It is induced by nases. Heme oxygenase catalyzes what appears to be the release of heme is its binding to heme oxygenase, a microsomal enzyme distinct from the microsomal P-450 oxidase (hydroxylated to $\alpha$-hydroxyhemin, which undergoes autoxidation to biliverdin (a blue-green pigment) with consumption of $O_2$ and release of iron and carbon monoxide (derived from oxidation of the $\alpha$-methene bridge). Since CO production in mammals occurs primarily by this pathway, measurement of expired CO has been used to estimate heme turnover. Values obtained exceed those derived from plasma bilirubin measurements by about 15%, probably because of bilirubin produced in the liver and excreted into the bile without entering the circulation. A potent competitive synthetic inhibitor of heme oxygenase is tin (Sn) protoporphyrin, which has a potential therapeutic use in treatment of neonatal jaundice (see below).

In nonmammalian vertebrates, biliverdin is the final metabolite in heme catabolism. Transport of biliverdin is much easier than that of bilirubin because biliverdin is water-soluble. Conversion of biliverdin to bilirubin may have evolved in mammals because, unlike biliverdin, bilirubin readily crosses the placenta. In this way, the fetus can eliminate heme catabolites via the mother’s circulation. However, this explanation may not be complete, since the rabbit (a placental mammal) excretes biliverdin as the major bile pigment.

Biliverdin is reduced to bilirubin by NAD(P)H-dependent biliverdin reductase, a cytosolic enzyme that acts at the central methene bridge. Although both molecules have two propionic acid groups, the polarity of biliverdin is greater than that of bilirubin. Bilirubin can form six internal hydrogen bonds between the carboxyl groups, the two lactam carbonyl oxygens, and four pyrrolenone ring nitrogens, and thus prevents these groups from hydrogen-bonding with water (Figure 29-12). Biliverdin cannot form these hydrogen bonds because of the lack of free rotation imposed by the double bond at the central methene bridge. Esterification of the propionyl side chains of bilirubin with glucuronic acid disrupts the hydrogen bonds and increases its solubility and liability. “Activators,” such as ethanol and methanol, used in the van den Bergh test to measure “indirect bilirubin,” and phototherapy for neonatal jaundice also act by disrupting the hydrogen-bonded structure of unconjugated bilirubin.

Hemoglobin and heme released from intravascular hemolysis or blood extravasations (e.g., subcutaneous hematomas) are bound, respectively, by haptoglobin and...
SECTION 29.3 Heme Catabolism

M

P

V

p

V

N

Heme oxygenase

NADPH, O2

α-Methene bridge

Fe

P

V

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Heme

α-Hydroxy-hemin

CO (from α-methene carbon), Fe

Heme

Biliverdin

Bilirubin

FIGURE 29-11
Conversion of heme to bilirubin in the monocytic phagocytic cells. Carbon monoxide and bilirubin are generated.
Fe released is conserved and reutilized. Biliverdin and bilirubin are lactams. P, Propionic acid; M, methyl; V, vinyl.

hemopexin to form complexes that cannot be filtered by the kidney. This action prevents renal loss of the heme iron and protects the renal tubules from possible damage by precipitated hemoglobin. Haptoglobin-hemoglobin and hemopexin-heme complexes are processed in mononuclear phagocytic cells in a way similar to that for hemoglobin. Haptoglobin and hemopexin are glycoproteins synthesized in the liver. The former is an α2-globulin and an acute-phase reactant (i.e., its synthesis and release into the circulation are augmented during an acute insult to the body); the latter is a β1-globulin but not an acute-phase protein (see also Appendix VI).

Circulatory Transport of Bilirubin

Bilirubin formed in extrahepatic tissues is transported to the liver for excretion in bile. Since bilirubin is virtually insoluble in aqueous media, it is transported to the liver bound noncovalently to serum albumin. The bilirubin-albumin complex increases the amount of bilirubin carried

FIGURE 29-12
Conformation of bilirubin showing invovled hydrogen bonded-structure between NH/O and OH/O groups. Despite the presence of polar carboxyl groups, bilirubin is nonpolar and lipophilic. Disruption of hydrogen bonds by glucuronidation or by conversion of bilirubin to configurational or structural isomers yields water-soluble pigments.
per volume of plasma and minimizes diffusion of bilirubin into extrahepatic tissues, thereby preventing bilirubin toxicity. Because of formation of this complex, bilirubin does not normally appear in urine. Urinary bilirubin is almost invariably conjugated bilirubin (see below) and signifies the presence of a pathological process. An albumin molecule binds two molecules of bilirubin at one high-affinity site and at one to three secondary sites. Bilirubin conjugated with glucuronic acid also binds to albumin but with much lower affinity. Another form of bilirubin (probably conjugated), very tightly (probably covalently) bound to albumin, has been described. The mechanism of its formation is not known, although blockage of biliary flow associated with an intact hepatic conjugating system releases a chemically reactive form of bilirubin into the circulation.

If the capacity of albumin to bind bilirubin is exceeded because of increased amounts of unconjugated bilirubin or decreased concentration of albumin, bilirubin readily enters extrahepatic tissues. In neonates, this can cause kernicterus, a serious condition associated with permanent neurological damage (see below). Bilirubin can be displaced from binding to albumin by sulfonamides, salicylates (notably aspirin), and cholangiographic contrast media. Use of these substances in jaundiced newborn infants increases the risk of occurrence of kernicterus. Medium-chain fatty acids increase and short-chain fatty acids decrease bilirubin binding to albumin. Binding, at least to the primary site, is independent of pH. Estimation of reserve bilirubin binding capacity has been used to evaluate the risk of bilirubin toxicity in icteric patients.

Hepatic Uptake, Conjugation, and Secretion of Bilirubin

Hepatocytes take up bilirubin from the sinusoidal plasma and excrete it after conjugation with glucuronic acid across the canalicular membrane into the bile. The entry and exit steps and the transport of bilirubin within the cell are not completely understood. The following is a plausible interpretation of the available data.

Since binding of bilirubin to albumin is usually reversible, a small amount of free bilirubin is present in plasma in equilibrium with albumin-bound bilirubin. It is probably this free bilirubin that is taken up at a rate determined by its plasma concentration. As this free bilirubin concentration decreases, more bilirubin is released from albumin and becomes available for uptake. Alternatively, the albumin-bilirubin complex may bind to specific hepatocyte plasma membrane receptors, and thereby bilirubin is released to enter the cell. Both models are consistent with the finding that albumin does not accompany bilirubin into the hepatocyte.

The entry step seems to be carrier-mediated, is saturable, is reversible, and is competitively inhibited by sulfobromophthalein, indocyanine green, cholecystographic agents, and several drugs. Bile salts do not compete with bilirubin for hepatic uptake.

After it enters hepatocytes, bilirubin is transported to the smooth endoplasmic reticulum for glucuronidation bound to a protein. Two cytosolic proteins, Z protein (fatty acid–binding protein) and ligandin (Y protein), bind bilirubin and other organic anions. Ligandin, which constitutes about 2–5% of the total soluble protein in rat and human liver, has lower capacity but higher affinity for bilirubin than Z protein. Ligandin (M.W. 47,000) has two subunits, A and B, which appear to be identical except for a 30-amino-acid extension at the carboxyl terminus of the B subunit. Bilirubin is bound entirely to the A subunit (two molecules per A subunit). Ligandin also has glutathione S-transferase, glutathione peroxidase, and ketosteroid isomerase activities, which depend on both subunits. Glutathione S-transferases catalyze detoxification reactions for a number of substances. Binding of bilirubin and other organic anions to ligandin occurs at sites unrelated to its enzyme activities.

Under normal conditions, ligandin is probably the principal hepatic bilirubin-binding protein and may serve intracellularly the same protective and transport functions as albumin in plasma. It may also help limit reflux of bilirubin into plasma, since its affinity for bilirubin is at least five times greater than that of albumin. Z protein (M.W. 11,000) becomes important at high plasma bilirubin concentrations. The concentration of ligandin in the liver does not reach adult levels until several weeks after birth, whereas neonatal and adult levels of Z protein are the same. This lack of ligandin, together with low hepatic glucuronyltransferase activity, is the probable cause of transient, “physiological,” nonhemolytic, neonatal jaundice.

Glucuronidation of bilirubin in the endoplasmic reticulum by UDP–glucuronyltransferase produces an ester between the 1-hydroxyl group of glucuronic acid and the carboxyl group of a propionic acid side chain of bilirubin (Figure 29-13). In bile, about 85% of bilirubin is in the diglucuronide form and the remainder is in the monoglucuronide form. Glucuronidation increases the water solubility of several lipophilic substances. There appear to be many UDP–glucuronyltransferases in the endoplasmic reticulum, which differ in substrate specificity. (Biosynthesis of UDP-glucuronic acid was described in Chapter 15.)
SECTION 29.3  Heme Catabolism

SECRETION ACROSS THE CANALICULAR MEMBRANE INTO BILE APPEARS TO BE THE RATE-LIMITING STEP IN HEPATIC BILIRUBIN METABOLISM. IT IS PROBABLY CARRIER-MEDIATED, REQUIRES ENERGY, IS SATURABLE, AND IS UNAFFECTED BY BILE SALTS. BILIRUBIN CAN BE MADE WATER-SOLUBLE BY CONVERSION TO ITS CONFIGURATIONAL ISOMERS. THESE PHOTOBILIRUBINS ARE FORMED WHEN BILIRUBIN IS EXPOSED TO BLUE LIGHT OF THE 400- TO 500-NM WAVELENGTH (FIGURE 29-14). PHOTOBILIRUBINS CANNOT FORM THE INTRAMOLECULAR HYDROGEN BONDS CHARACTERISTIC OF THE NATURAL ISOMER OF BILIRUBIN (FIGURE 29-12). THUS, THEY ARE MORE POLAR AND READILY EXCRETED IN THE BILE WITHOUT THE REQUIREMENT FOR GLUCURONIDATION. LUMIRUBIN, A STRUCTURAL ISOMER OF BILIRUBIN, IS FORMED BY LIGHT-INDUCED INTRAMOLECULAR CYCLIZATION OF THE VINYL SIDE GROUP OF C-3. IT CONTAINS A SEVEN-MEMBERED RING, IS STABLE, IS POLAR, AND IS EXCRETED WITHOUT CONJUGATION. THESE OBSERVATIONS EXPLAIN THE MECHANISM OF PHOTOThERAPY COMMONLY USED FOR TREATMENT OF NEONATAL HYPERBILIRUBINEMIA.

FIGURE 29-13
Formation of bilirubin diglucuronide. Glucuronidation occurs in two steps via formation of monoglucuronide. Mono- and diglucuronides are more water-soluble and less lipophilic than bilirubin. Conversion of bilirubin to water-soluble products is obligatory for excretion of bilirubin from hepatocytes. M, Methyl; V, vinyl; UDP-GA, UDP-glucuronic acid.

FIGURE 29-14
Photoisomers of bilirubin. The presence of two methene bridges containing double bonds (colored areas) gives rise to configurational (geometrical) isomers of bilirubin. Each double bond can exist in the Z or E configuration. The naturally occurring, most stable, water-insoluble form is the Z, Z isomer. It undergoes photoisomerization to configurational isomers (Z, E; E, Z; and E, E), which are more polar owing to inability to form intramolecular hydrogen bonds and are excretable from the liver without glucuronidation. Some excretion of photoisomers in urine also occurs.
Bilirubin in the Intestinal Tract

Most bilirubin entering the intestine in bile is in the diglucuronide form, which is very poorly absorbed in the small and large intestines. In the lower small intestine and colon, bacteria remove glucuronic acid residues and reduce bilirubin to the colorless urobilinogen and stercobilinogen. Exposure to air oxidizes these to urobilin and stercobilin, respectively, (i.e., red-orange pigments that contribute to the normal color of stool and urine). Other degradation products of bilirubin are present in minor amounts in feces.

Urobilinogen is excreted mostly in the feces, but a small fraction is absorbed from the colon, enters the portal circulation, is removed by the liver, and is secreted into bile. That which is not removed from the portal blood by the liver enters the systemic circulation and is excreted by the kidneys. Urobilinogen excretion in urine normally amounts to 1–4 mg per 24 hours, as opposed to the 40–280 mg (67–470 μmol) excreted in feces.

Lack of urobilinogen in the urine and feces indicates biliary obstruction; stools are whitish (“clay-colored”) owing to the absence of bile pigment. Urinary and fecal urobilinogen excretion increases in hemolytic anemia.

Disorders of Bilirubin Metabolism

The plasma of normal subjects contains 0.1–1 mg of bilirubin per deciliter (2–17 μmol/L), mostly in the unconjugated form. Unconjugated bilirubin is known as indirect-reacting bilirubin and conjugated bilirubin as direct-reacting bilirubin (see Table 29-2).

Jaundice occurs when plasma becomes supersaturated with bilirubin (>2–2.5 mg/dL) and the excess diffuses into the skin, sclera, and other tissues. The sclera is particularly affected because it is rich in elastin, which has a high affinity for bilirubin. Reddish yellow pigments, particularly carotene and lycopene, may give a yellowish tinge to the skin but they do not usually produce scleral coloration. Hyperbilirubinemia may result from elevation of unconjugated or conjugated bilirubin levels.

Unconjugated Hyperbilirubinemias

Unconjugated hyperbilirubinemias result from imbalance between the rates of production of pigment and of its uptake or conjugation in the liver. Because of the large reserve capacity of the liver for conjugation and excretion of bilirubin, increased production seldom elevates unconjugated serum bilirubin to more than 3–4 mg/dL. If a greater increase occurs, some degree of liver dysfunction probably also occurs. These disorders are usually due to decreased uptake of pigment by hepatocytes or to failure of these cells to store, transport, or conjugate bilirubin. Bilirubinuria does not accompany these disorders. Except in infancy or when pigment gallstones form, unconjugated hyperbilirubinemias are benign.

Gilbert’s syndrome may be the most common cause of mild, persistent, nonhemolytic, unconjugated hyperbilirubinemia. Serum bilirubin concentration rarely exceeds 5 mg/dL and usually fluctuates between 1.3 and 3 mg/dL. Other liver function tests are normal. The syndrome is usually asymptomatic and is detected during routine laboratory testing or examination for other disease. Family studies suggest that Gilbert’s syndrome is an autosomal dominant disorder. The unconjugated hyperbilirubinemia in Gilbert’s syndrome is due to decreased UDP-glucuronyltransferase activity resulting from an insertion mutation found in the promoter region of the enzyme. The wild-type promoter [TA]6TAA is mutated to [TA]4TAA. Mutations affecting the coding region of the enzyme, although rare, also occur.

In Crigler-Najjar syndrome type 1, activity of hepatic bilirubin UDP-glucuronyltransferase is undetectable and bilirubin conjugates are absent from the serum, bile, and urine, but biliary secretion of sulfobromophthalein and indocyanine green is normal. The disease is apparent shortly after birth, kernicterus develops, and death commonly occurs during the neonatal period. The effectiveness of phototherapy is often transient. The enzyme is not inducible by phenobarbital. This autosomal recessive defect occurs in all races. The Gunn strain of Wister rats has a similar genetic defect and has been used to study the syndrome.

Crigler-Najjar syndrome type II (Arias syndrome) is milder, usually benign, and caused by partial deficiency of bilirubin UDP-glucuronyltransferase. Jaundice may not appear until the second or third decade of life. The monoglucuronide is the predominant pigment in bile. Phenobarbital induces the enzyme. Dominant and recessive inheritance patterns have been described. An accurate diagnosis of type 1, as opposed to type 2 Crigler-Najjar syndrome, is essential since orthotopic liver transplantation is an important therapy for type 1 patients.

Conjugated Hyperbilirubinemias

Conjugated hyperbilirubinemias are due to intra- or extrahepatic reduction to bile flow (cholestasis) with spillage of conjugated bilirubin into the bloodstream, which may occur from injury to the endothelial cells lining bile ductules or from reverse pinocytosis, by the hepatocytes. Since the serum bilirubin is mostly the water-soluble glucuronide, bilirubinuria is usually present.

Abdominal tumors, gallstones, strictures, hepatitis, and cirrhosis can mechanically block the biliary canaliculi or
ducts. If obstruction affects only intrahepatic bile flow, hyperbilirubinemia occurs when 50% or more of the liver is involved. Extrahepatic obstruction elevates serum bilirubin only if it increases the pressure in the canaliculi above the maximum secretion pressure of the hepatocytes (about 250 mm Hg). Nonmechanical cholestasis can be caused by bacterial infection, pregnancy, and sex steroids and other drugs, or it may be genetically determined.

In cholestasis, bile salts and bile pigments are retained and appear in the circulation, and steatorrhea and deficiencies of fat-soluble vitamins may occur. These deficiencies are often manifested as hypoprothrombinemia (from lack of vitamin K) and osteomalacia (from lack of vitamin D). The magnitude depends on the degree of obstruction. If blockage is complete, urinary urobilinogen will be absent and the stools will have a pale, clay-like color.

Familial diseases include Dubin-Johnson syndrome, Rotor’s syndrome, and benign familial recurrent cholestasis. Serum bilirubin values in Dubin-Johnson syndrome are the same as those found in Rotor’s syndrome (Table 29-2). Very little is known about benign familial recurrent cholestasis. All three disorders are uncommon or rare, and all are benign. The liver in Dubin-Johnson syndrome appears black on direct examination. The pigment is not identical to melanin but may be a catecholamine (perhaps epinephrine) polymer. The total urinary coproporphyrin excretion is normal but about 80% is the I isomer and the rest is the III isomer, the reverse of the normal ratio. This abnormality seems to be diagnostic for the syndrome, provided the history and physical examination are consistent with the diagnosis. Obligate heterozygotes have urinary coproporphyrin patterns intermediate between those of normal and affected individuals, indicating autosomal recessive inheritance. The hyperbilirubinemia, hepatic pigment, and urinary coproporphyrin abnormality may result from a defect in porphyrin biosynthesis. Studies of sulfobromophthalein clearance show normal storage but greatly decreased secretion. Investigation of a mutation in Corriedale sheep, which causes a similar condition, may help clarify this poorly understood disorder.

In Rotor’s syndrome, patients lack the hepatic pigment but have urinary coproporphyrin levels 2.5-5 times greater than normal. Coproporphyrin type I is increased relative to the type III isomer but not as much as in Dubin-Johnson syndrome. Intrahepatic storage of sulfobromophthalein is

### TABLE 29-2

**Serum Bilirubin Levels in Normal and Some Abnormal Conditions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total</th>
<th>Unconjugated (indirect reacting)</th>
<th>Conjugated (direct reacting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>0.2-2.9</td>
<td>0.2-0.7</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>1 day</td>
<td>0-6</td>
<td>0-6</td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td>0.3-11</td>
<td>2-3</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>7 days</td>
<td>0.1-9.9</td>
<td>1.1-2.7</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal (adult)</td>
<td>0.1-1</td>
<td>0.2-0.7</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>Hemolytic disorders (in adults)</td>
<td>2.2-3.4</td>
<td>2-3</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>Gilbert’s disease (probably a</td>
<td>1.2-3</td>
<td>1.1-2.7</td>
<td>Normal</td>
</tr>
<tr>
<td>heterogeneous group of diseases)</td>
<td>(rarely &gt;5)</td>
<td>(rarely &gt;5)</td>
<td></td>
</tr>
<tr>
<td>Glucuronyltransferase deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I: Crigler-Najjar syndrome—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II: Crigler-Najjar syndrome—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>partial deficiency</td>
<td>6-22</td>
<td>6-22</td>
<td>Trace</td>
</tr>
<tr>
<td>(up to 40 with physiological stress)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dubin-Johnson syndrome</td>
<td>2.5-20</td>
<td>19</td>
<td>&gt;50% of total</td>
</tr>
<tr>
<td>Cholestasis (severe form)</td>
<td>10</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis (severe)</td>
<td>11</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Hepatitis (acute/severe)</td>
<td>10</td>
<td>1.5</td>
<td>8.5</td>
</tr>
</tbody>
</table>

*To convert mg/dL to μmol/L, multiply by 17.1.*
markedly decreased, but the rate of secretion is only moderately depressed. Inheritance appears to be autosomal recessive.

**Neonatal Hyperbilirubinemia**

Normal neonates are frequently hyperbilirubinemic (Table 29-2). Birth interrupts normal placental elimination of pigment, and the "immature" liver of the neonate must take over. Normally serum bilirubin levels rise on the first day of life, reaching a maximum (rarely greater than 10 mg/dL) by the third or fourth day. This type is mostly unconjugated. If the placenta is functioning normally, jaundice will not be present at birth. If jaundice is present at birth, a cause other than hepatic immaturity must be sought.

The primary blocks to bilirubin metabolism are low activity of bilirubin glucuronyltransferase and low concentration of ligandin in the liver at birth. Secretion of conjugated bilirubin into the bile is also reduced.

Hepatic immaturity may be partly due to diversion in utero of blood from the liver by the ductus venosus. When this channel closes shortly after birth and normal hepatic blood flow is established, concentrations of a number of substances rise within the hepatocytes and may induce enzymes needed for their metabolism. Accumulation of bilirubin in plasma may play an important role in hastening the maturation. Although the liver normally matures within 1–2 weeks after birth, hypothyroidism can prolong this process for weeks or months.

The neonate is at risk for kernicterus if the serum unconjugated bilirubin level is higher than 17 mg/dL. Kernicterus is characterized by yellow staining of clusters of neuronal cell bodies in the basal ganglia, cerebellum, and brain stem, leading to motor and cognitive deficits or death. Immaturity and perhaps hypoxia make the blood-brain barrier permeable to bilirubin and contribute to the likelihood of kernicterus. The biochemical basis of bilirubin encephalopathy is due to many causes: inhibition of RNA and protein synthesis, carbohydrate metabolism (both cAMP-mediated and Ca²⁺-activated), phospholipid-dependent protein kinases, enzymes involved in the electron transport system, and impaired nerve conduction.

A major complicating factor can be hemolytic anemia such as that of *erythroblastosis fetalis* caused by Rh incompatibility between mother and child. The hemolysis increases the rate of bilirubin formation, which soon overwhelms the liver and produces severe jaundice and kernicterus. Sickle cell anemia has a similar effect. Congenital absence of bilirubin UDP-glucuronyltransferase (Crigler-Najjar syndrome type 1) usually causes a kernicterus that is fatal shortly after birth. Inhibition of glucuronyltransferase by various drugs (e.g., novobiocin) or toxins can increase the severity of neonatal jaundice. "Breast milk jaundice" is due to the presence in breast milk of a substance (perhaps pregnane-3α,20β-diol) that inhibits bilirubin glucuronyltransferase, although the resulting unconjugated hyperbilirubinemia is seldom serious enough to cause neurotoxicity or to require discontinuation of breast-feeding. Other risk factors for pathologic hyperbilirubinemia include Gilbert's syndrome (discussed earlier) and glucose-6-phosphate dehydrogenase deficiency (Chapter 15).

Conjugated hyperbilirubinemia is rare during the neonatal period. It can result from impaired hepatocellular function or extrahepatic obstruction. Hepatocellular defects can be caused by bacterial, viral, or parasitic infections, cystic fibrosis, α₁-antitrypsin deficiency, Dubin-Johnson and Rotor's syndromes, and other genetic disease. Extrahepatic obstruction can be congenital (biliary atresia) or acquired.

Treatment of neonatal jaundice is usually by phototherapy. A decrease in bilirubin production in the neonatal period can also be achieved by inhibiting the rate-limiting enzyme of bilirubin formation from heme, namely, the heme oxygenase. A potent competitive inhibitor of heme oxygenase is the synthetic heme analogue tin (Sn⁴⁺) protoporphyrin. When administered parenterally, the tin protoporphyrin safely decreases bilirubin formation. Exchange transfusions also rapidly decrease plasma bilirubin levels.

**Supplemental Readings and References**


