Chapter 27

Metabolism of Iron and Heme

Key Points

1. Heme, an iron–porphyrin complex, is the prosthetic group of hemoglobin, myoglobin, cytochromes, and many other proteins.
2. Iron is required for the biosynthesis of heme and other nonheme-iron-containing proteins.
3. Acquisition of iron by entry into the enterocytes, its storage in the liver and macrophages, its utilization by erythroid cells for erythropoiesis and its requirement for other cellular functions are regulated by a number of proteins. Hepcidin, synthesized in the liver, regulates a number of steps in iron homeostasis. Loss of function in these regulatory proteins leads to hemochromatosis.
4. Iron deficiency anemia is the most prevalent nutritional disorder. It is due to inadequate dietary intake of iron and/or chronic blood loss.
5. Heme biosynthesis in the hematopoietic tissues and the liver requires eight enzymes that are present in both the cytosol and the mitochondria. The regulatory step is catalyzed by ALA synthase and is inhibited at its allosteric site by heme. Inherited disorders of porphyrin biosynthesis lead to porphyrias with various clinical manifestations.
6. Catabolism of red blood cells in the macrophages leads to the production of bilirubin, a waste product of porphyrin. Iron is sequestered and reutilized.
7. Bilirubin is transported to the liver bound to albumin, conjugated with glucuronic acid using UDP-glucuronic acid, and eliminated via the biliary system.
8. Elevated serum bilirubin levels (jaundice) can be due to liver disease, excessive hemolysis, and various genetic defects in its metabolism in the liver. Neonatal jaundice, if not corrected promptly, can cause damage to the CNS. Phototherapy reduces serum bilirubin levels in neonates.

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 26. 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). Fe-S cluster assembly occurs in mitochondria mediated by frataxin. In the inherited disease Friedreich's ataxia, frataxin deficiency has been observed. This autosomal recessive disease is most often caused by expansion of the GAA repeat in the first intron of the gene and is accompanied by sensory loss and impairment of gait. 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 H_2O_2 in a reaction catalyzed by iron. Thus, iron homeostasis in the body is in a delicate balance: either a deficiency or an excess of iron results in abnormalities and presents as a common cause of human diseases.

IRON METABOLISM

The total body iron of a 70 kg adult is about 4.2–4.4 g. The distribution of iron in various body compartments is shown in Table 27.1. Several specific proteins participate in the orchestration of iron metabolism (discussed later). Iron metabolism consists of the absorption of dietary iron from the gastrointestinal tract, transport in the blood, storage in the liver and macrophages, and utilization in the cells requiring synthesis of iron-containing proteins (e.g., hemoglobin in erythroblasts). Iron deficiency causes **anemia**, and iron excess causes iron accumulation diseases known as **hemochromatosis**.

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Iron Absorption, Transport, Utilization, and Storage

See Figure 27.1 for an overview of iron metabolism.

Absorption

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. At the apical membrane of the enterocyte, Fe^{3+} is converted to Fe^{2+} by **ferrireductase** followed by its uptake mediated by divalent transporter 1 (DMT1). DMT1 also transports other metal ions (e.g., Cu^{2+}, Zn^{2+}, and cobalt). The internalized Fe^{2+} is either temporarily stored after conversion to Fe^{3+} as ferritin or transported across the cell for transport to the portal capillary blood circulation. The exit of iron from the enterocytes at the basolateral surface requires the participation of **HFE protein** and a copper-containing ferroxidase

TABLE 27.1 Distribution of Iron in a 70 kg Adult[1]

Circulating erythrocytes	1800 mg[2]
Bone marrow (erythroid)	300 mg
Muscle myoglobin	300 mg
Heme and nonheme enzymes	180 mg
Liver parenchyma[3]	1000 mg
Reticuloendothelial macrophages[4]	600 mg
Plasma transferrin[5]	3 mg

[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 mmol.
[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.

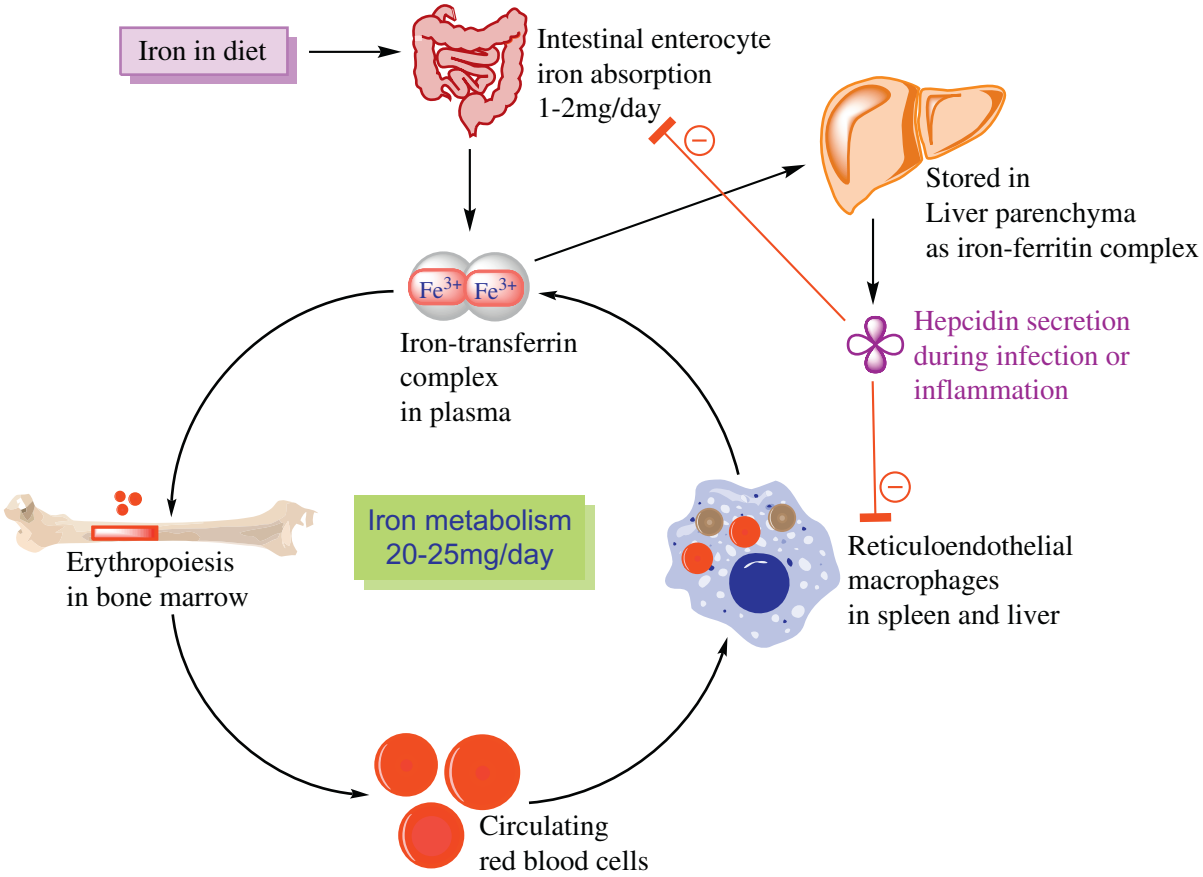


FIGURE 27.1 An overview of iron metabolism. Intestinal enterocytes uptake 1–2 mg/day of iron. Transferrin in blood carries iron to erythroid precursor cells for heme production and hepatocytes in the liver for iron storage. Hepcidin mainly released from the liver inhibits iron release from enterocytes and reticuloendothelial macrophages.

known as **hephaestin**, which converts Fe^{2+} to Fe^{3+}. Plasma **transferrin** transports iron in the ferric state to its target sites. 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 is removed for further processing.

Plasma Iron Transport

Over 95% of plasma iron is in the Fe^{3+} state bound to the glycoprotein transferrin, a monomeric β_1-globulin (M.W. 80,000). Transferrin is synthesized primarily in the liver. Each molecule of transferrin can bind two Fe^{3+} ions. The binding is extremely strong under physiological conditions. Its half-life in humans is about 8 days.

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 clathrin-coated pits with the binding of diferric transferrin to specific plasma membrane transferrin receptors. The next step is the internalization of the transferrin–transferrin receptor-HFE protein complex with formation of endosomes. In the endosomes, a proton pump acidifies the complex to pH 5.4, and by altering the conformation of proteins, iron is released from transferrin bound to transferrin receptors. In the acidified endosomes, DMT1 facilitates iron transport into the cytosol. Both apotransferrin (and a fraction of ironbound transferrin) and transferrin receptors 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 18). In the erythroid cells, most of the iron released from the endosomes is transported into mitochondria for heme synthesis in nonerythroid cells, and the iron is stored predominantly as ferritin.

Storage of Iron

Iron is stored in the apoferritin shell in the ferric state as a polynuclear hydrated ferric oxide–phosphate complex. 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). H chains possess ferroxidase activity and convert Fe^{2+} to Fe^{3+}.

Coordinate Regulation of Iron Uptake and Storage in Nonerythroid Cells

Iron uptake is regulated by transferrin receptors 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 iron regulatory elements (IREs) and iron regulatory proteins (IRPs) 1 and 2. One copy of each IRE has been identified in the 5′ untranslated region (UTR) of H and L ferritin mRNAs and five copies in the 3′ untranslated region 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.

When there are low levels of intracellular chelatable iron, iron storage declines due to inhibition of ferritin synthesis, and cellular entry of iron increases due to enhanced transferrin receptor synthesis. An opposing set of events occurs during intracellular chelatable iron excess or iron-replete states. 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 27.2). 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 a TCA cycle enzyme (Chapter 12).

Measurement of serum ferritin levels has diagnostic utility. In iron deficiency anemia, 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 (discussed later). It is customary to measure transferrin concentration indirectly from the maximum (or total) iron-binding capacity (TIBC) of plasma (reference interval for adults, 250–400 μg/dL). It can also be measured directly by immunological methods (reference interval for adults, 220–400 mg/dL). **Hypertransferrinemia** (or increased TIBC) can occur with diminished body iron stores, as in iron deficiency anemia or

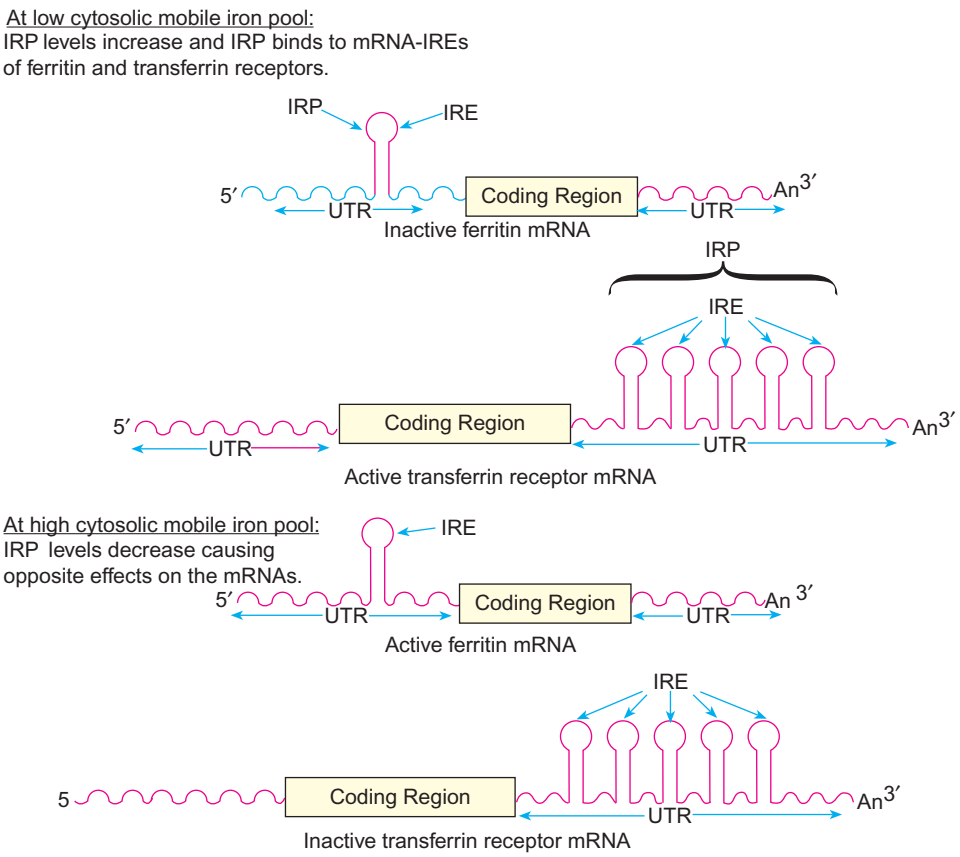


FIGURE 27.2 Coordinated translational regulation of ferritin mRNA and transferrin receptor mRNA in nonerythroid cells. Iron regulatory proteins (IRPs) are RNA-binding proteins that bind to iron regulatory elements (IREs). IREs are hairpin structures with loops consisting of CAGUGN sequences and are located at the 5′ untranslated region (UTR) and 3′ UTR for ferritin mRNA and transfer mRNA, respectively.

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 15). Hypotransferrinemia also results from excessive renal loss of plasma proteins (e.g., in nephrotic syndrome).

Regulation of Iron Metabolism

Gastrointestinal Tract

The enterocyte, the first entry portal for iron absorption, plays the predominant role in the iron metabolism. In the enterocytes, iron absorption is increased during iron deficiency and decreased during the body's iron excess. The molecular circuitry signals that mediate iron absorption involve a network of signals. Hypoxia-inducible factors (HIFs), which are transcription factors, are involved in the enterocyte iron absorption. During hypoxic conditions, the HIF signaling cascade is upregulated, promoting iron entry into the enterocytes. Thus, in iron deficiency, anemia HIF promotes absorption. However, inappropriate HIF stimulation can lead to chronic iron accumulation [1]. It should also be noted that HIF upregulation stimulates erythropoietin production in renal cells, which promotes erythropoiesis in the bone marrow (see "Erythropoietin" in Chapter 26).

Liver

Hepcidin, a 25-amino acid protein containing eight cysteine residues, is synthesized in the liver [2] and plays a central role in iron homeostasis [3]. It is a negative modulator of iron levels in the body; it decreases iron absorption and release of iron from macrophages by inactivating ferroportins. The hepcidin gene is transcriptionally activated by **hemojuvelin** (**HJV**), **HFE protein**, and **transferrin receptor 2** (**TFR2**). Thus, the inactivating mutations in the genes of hepcidin—HJV, HFE, and TFR2—are related to hereditary hemochromatosis. Activation of the hepcidin gene by interleukin-6 (IL-6), which is released during inflammation, results in anemia [4]. Action of IL-6 is mediated by the activation of signal transducer and activator of the transcription-3 (STAT-3) pathway. Under physiological conditions, hepcidin synthesis is positively regulated by bone morphogenetic proteins via

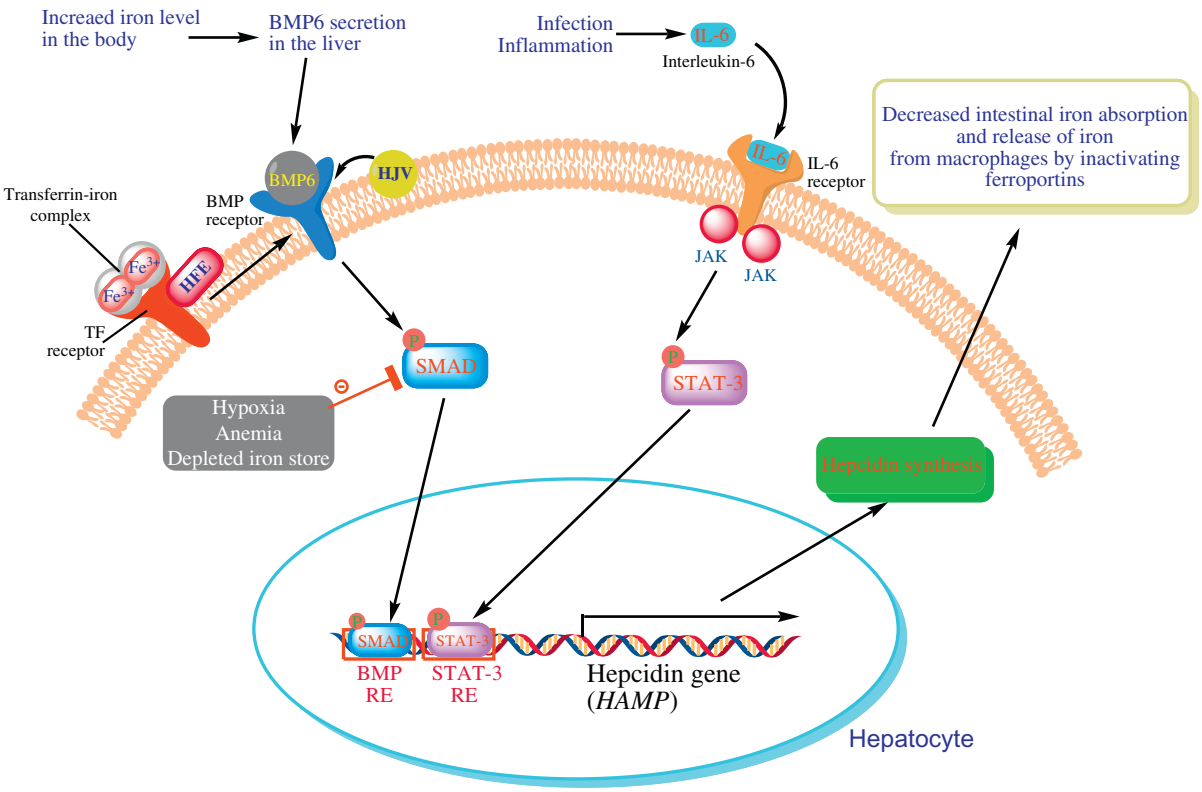


FIGURE 27.3 Hepcidin expression in the hepatocyte. The two pathways of hepcidin regulation consist of the iron homeostasis and the inflammation and infection (see text for details). HAMP = hepcidin gene, HJV = hemojuvelin, STAT-3 = signal transducer and activator of the transcription-3, BMP RE = BMP response element, STAT-3 RE = STAT-3 response element, JAK = Janus-associated kinase, TF = transferrin.

phosphorylation of downstream transcription factors. Anemia, hypoxia, and depleted iron stores inhibit the synthesis of hepcidin, promoting iron absorption (Figure 27.3).

Macrophage

Senescent red blood cells are catabolized in the macrophages (discussed later), and the iron is reclaimed and temporarily stored. The exit of Fe^{2+} from macrophages requires cell-membrane-bound **ferroportin 1** and **glycosylphosphatidylinositol (GPI)-linked ceruloplasmin**. Ceruloplasmin, like enterocyte hephaestin, is a copper-containing ferro-oxidase. It converts Fe^{2+} to Fe^{3+} for transferrin-mediated transport. Thus, copper deficiency causes anemia.

Disorders of Iron Metabolism

Iron Deficiency Anemia

Iron deficiency anemia is the most prevalent nutritional disorder [5,6]. 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 a gastrointestinal tract peptic ulceration, hemorrhoids, cancer, colonic ulceration, or hookworm infestation of 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 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 atransferrinemia** 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. There are reports of a rare congenital defect in uptake of iron by red cell precursors that leads to severe hypochromic anemia with normal plasma iron and transferrin levels.

Microcytic anemia occurs frequently in thalassemia syndromes (Chapter 26), but these patients do not require iron supplementation unless they have concurrent iron deficiency as assessed by measurement of serum iron levels and TIBC. 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 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 numbers reach 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 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

Excessive accumulation of iron (chronic iron overload) can result from the following (see also Clinical Case Study 27.1):

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 due to vitamin B_6 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 defects that lead to decreased hepcidin production (discussed earlier), in which there is an increased rate of absorption of iron in the presence of normal or enlarged iron stores and normal hematopoiesis.
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 a significant extent. Thus, the "mucosal block" responsible for keeping out unnecessary 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 excess 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 [7,8].

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, deferiprone, and deferasirox are used as iron chelators in the transfusion overload disorders [9]. In disorders of intravascular hemolysis (e.g., transfusion, sepsis, sickle cell disease), free hemoglobin and hemin are released. Both hemoglobin and hemin are toxic and produce adverse clinical conditions involving vascular, hepatic, and renal systems. Two plasma proteins, namely haptoglobin and hemopexin, function as scavenger molecules for free hemoglobin and hemin, respectively [10].

HEME BIOSYNTHESIS

The principal tissues involved in heme biosynthesis are the hematopoietic tissues and the liver. Biosynthesis

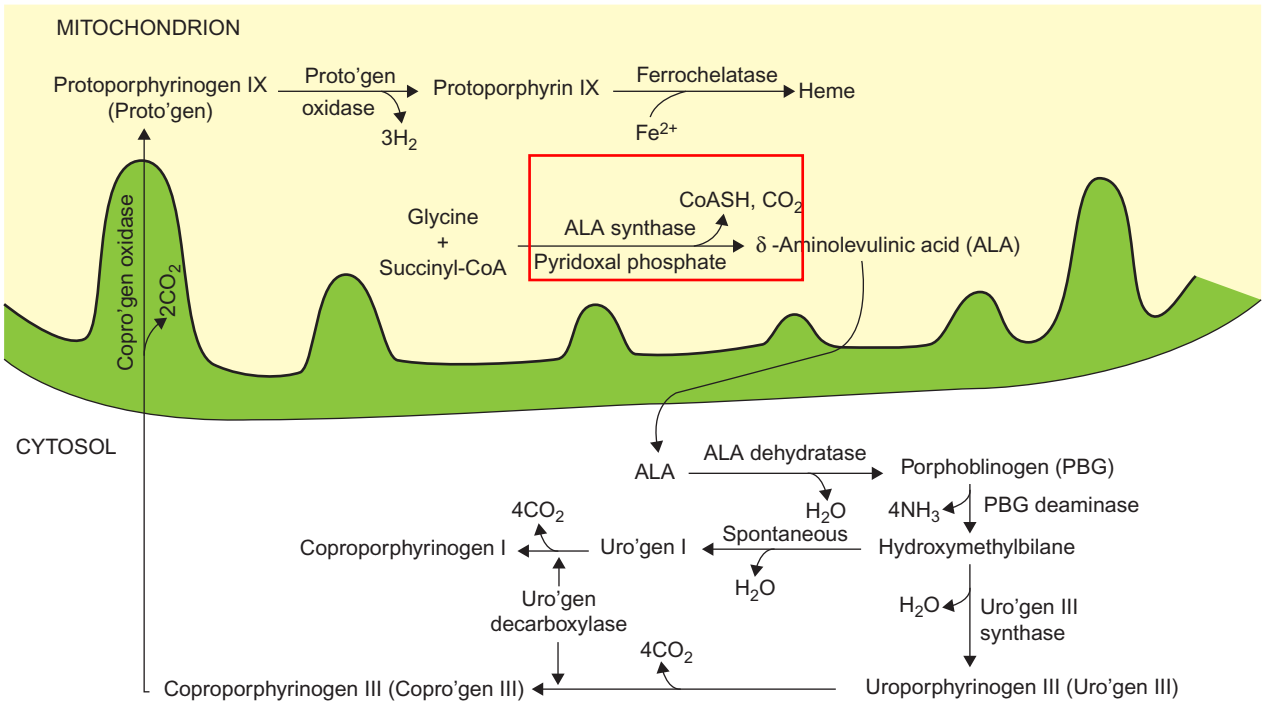
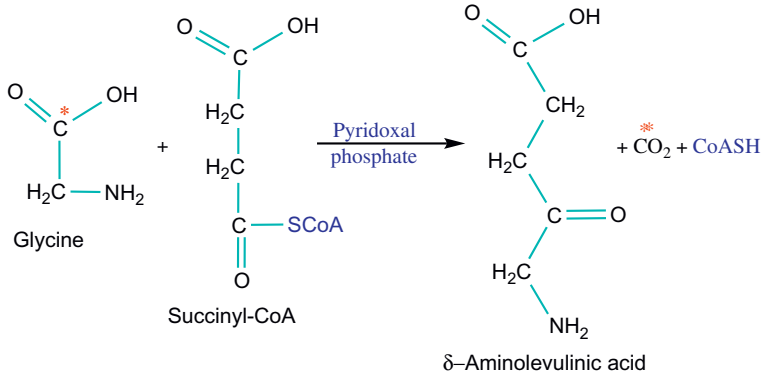


FIGURE 27.4 Biosynthetic pathway of heme. The pathway consists of eight irreversible reactions, four each in the mitochondrion and the cytosol. The primary site of regulation is the ALA synthase step.

requires the participation of eight conserved enzymes, of which four (the first and the last three) are mitochondrial and the rest are cytosolic (Figure 27.4). The reactions are irreversible. Glycine and succinate are the precursors of porphyrins. Some of these enzymes are coded by erythroid-specific and housekeeping genes [11].

Formation of δ-Aminolevulinic Acid

δ-Aminolevulinic acid (ALA) formation is catalyzed by mitochondrial ALA synthase, which condenses glycine and succinyl-CoA to ALA:



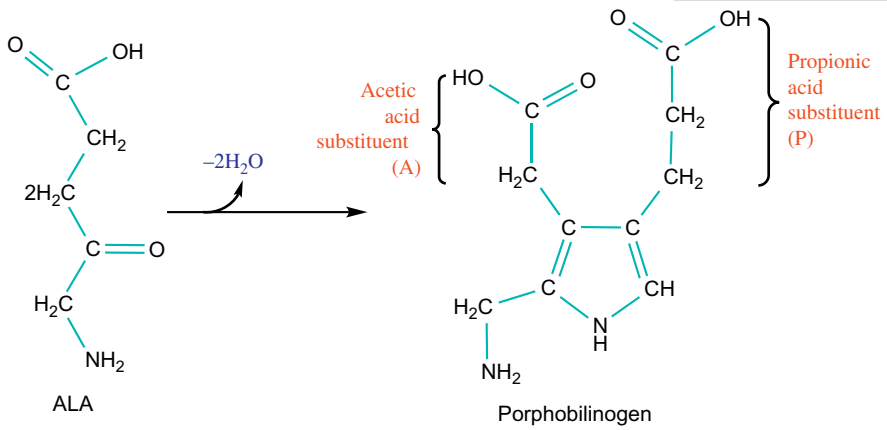
The ALA synthase is located on the matrix side of the inner mitochondrial membrane. It is encoded by a nuclear gene.

Heme synthesis also requires a functional tricarboxylic acid (TCA) 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 that needed for production of heme proteins, is oxidized to **hematin**, which contains a hydroxyl group attached to the Fe^{3+} atom. Replacement of the hydroxyl group by a chloride ion produces **hemin**. Hemin and

heme inhibit ALA synthase allosterically. Induction of ALA synthase 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 a change in the requirements for synthesis of heme. The differentiation and proliferation are initiated by erythropoietin (Chapter 26).

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 octamer of the enzyme, and they are bound via the reduced thiol groups. Zinc is required for enzyme activity. Lead is a potent inhibitor of ALA dehydratase (see Clinical Case Study 27.2).

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 27.5) 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 27.5).

Formation of Coproporphyrinogen III

Cytosolic uroporphyrinogen decarboxylase catalyzes successive decarboxylation of the four acetic groups to yield four methyl groups (Figure 27.6).

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 27.7).

Formation of Protoporphyrin IX and Heme

Both of these steps occur in mitochondria (Figure 27.8). 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. 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 [11]. They are inherited and acquired disorders characterized by excessive accumulation and excretion of porphyrins or their precursors.

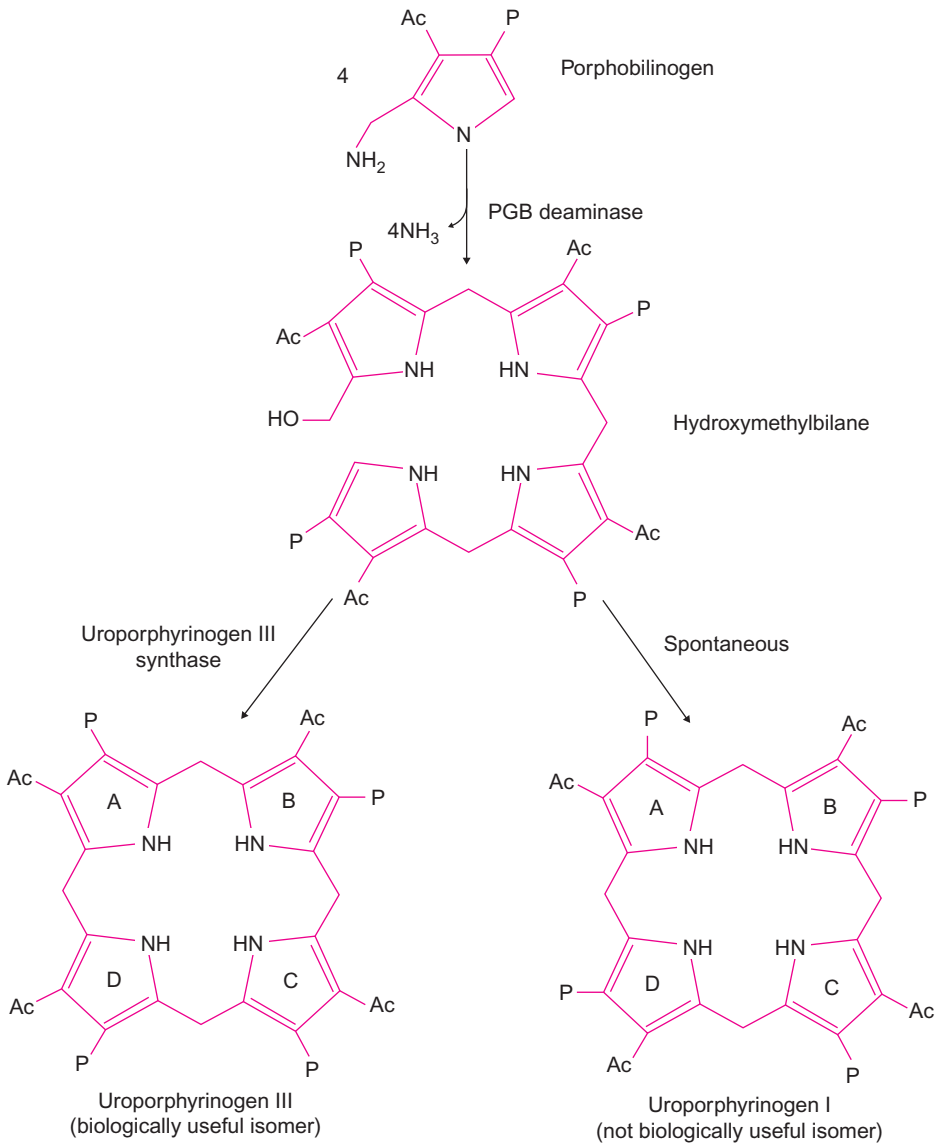


FIGURE 27.5 Synthesis of uroporphyrinogen I and III. The latter is the biologically useful isomer, and its formation requires the action of uroporphyrinogen-III synthase. Ac = $-CH_2COOH$; P = $-CH_2CH_2COOH$.

Ac = acetate
P = proprionate

Defects in any one of the eight enzymes involved in heme biosynthesis may cause inherited porphyrin-related disorders (Figure 27.9). 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 (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.

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

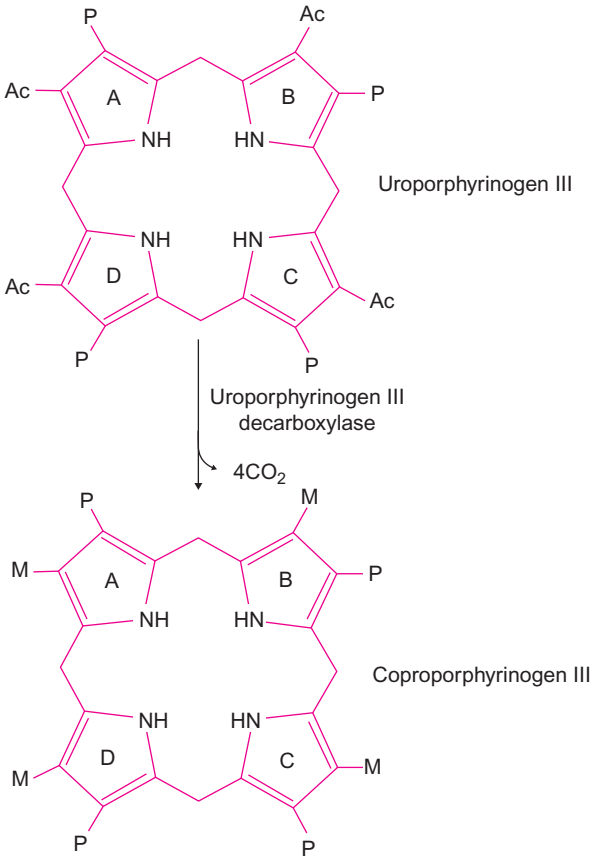


FIGURE 27.6 Formation of coproporphyrinogen III from uroporphyrinogen III. Acetic acid side chains (Ac) are decarboxylated to methyl groups (M), sequentially, starting clockwise from ring D. P = $-CH_2CH_2COOH$.

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**.

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 increased plasma concentration, it diffuses into tissues and produces jaundice. 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

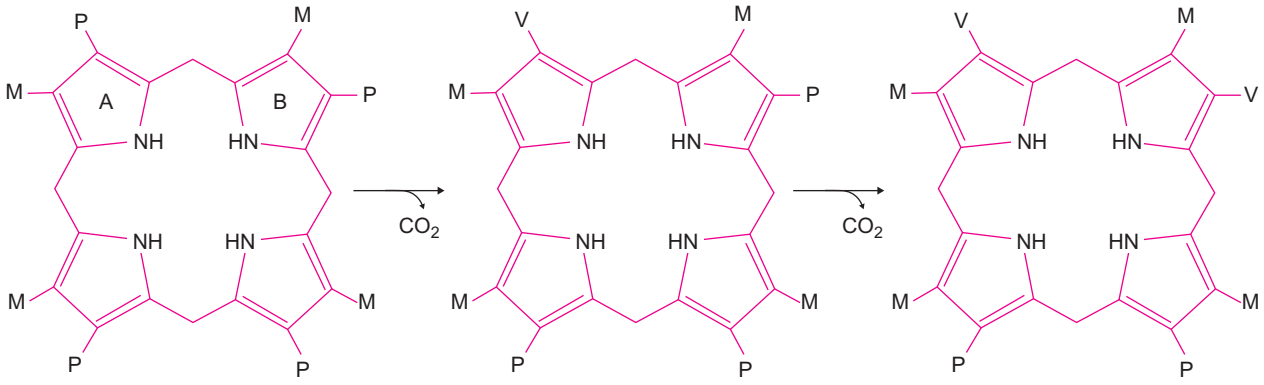


FIGURE 27.7 Formation of protoporphyrinogen IX from coproporphyrinogen III by coproporphyrinogen oxidase. Sequential oxidative decarboxylation of the propionic acid (P) side chains of rings A and B produces vinyl (V) groups (V = $-CH_2 = CH_2$). The reaction proceeds via the stereospecific loss of one hydrogen atom and decarboxylation of the propionic acid group. Molecular oxygen is the oxidant, and β-hydroxypropionate is a probable intermediate. M = CH_3.

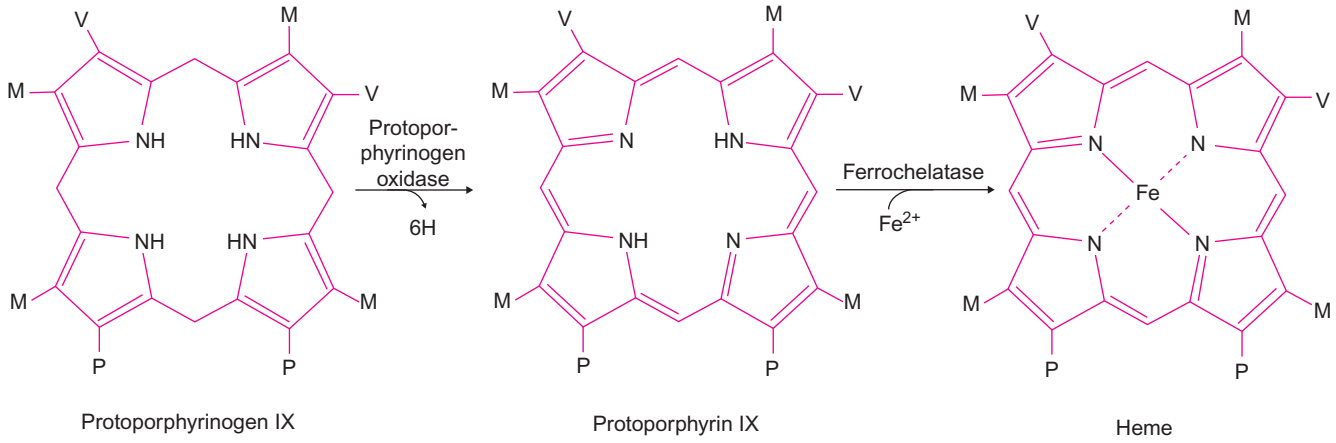


FIGURE 27.8 Formation of heme. In the reaction catalyzed by protoporphyrinogen oxidase, six hydrogens are removed and the primary electron acceptor is not known, but oxygen is required for enzyme activity. In the terminal step of heme synthesis, only Fe^{2+} is incorporated into protoporphyrin. (For key to letters see Figure 27.7.)

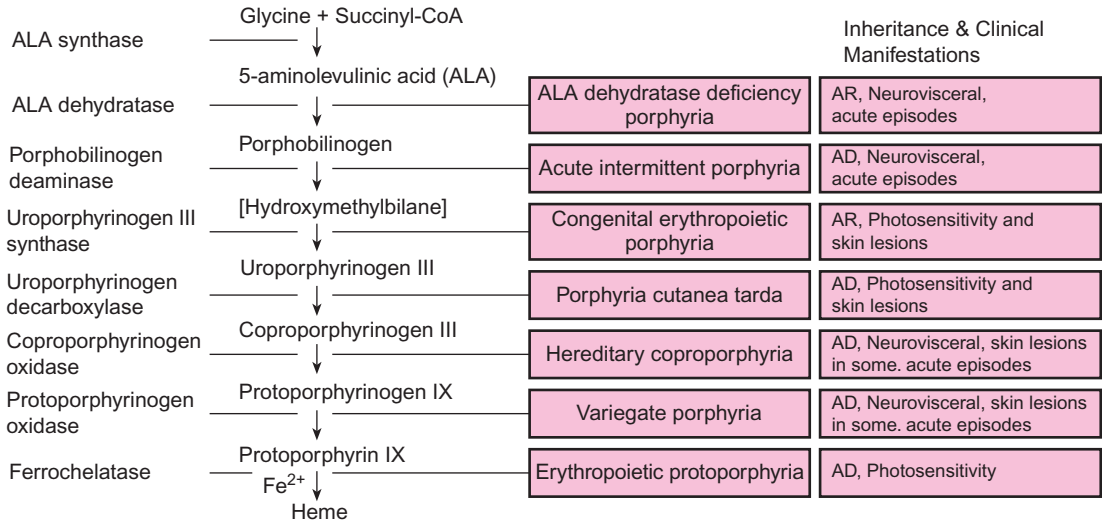


FIGURE 27.9 Heme biosynthesis pathway and the enzyme defects in various porphyrias. AD = autosomal dominant; AR = autosomal recessive.

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 (4275–5130 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 b_5, catalase), ineffective hemopoiesis (erythrocytes that never leave the marrow), and "free" heme (heme never incorporated into protein) in the liver. Hemoglobin heme has a lifespan 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. 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).

Formation of Bilirubin

A summary of the pathway for bilirubin metabolism and excretion is shown in Figure 27.10. Release of heme from heme proteins and its conversion to bilirubin occur predominantly in the mononuclear phagocytes of liver, spleen, and bone marrow (previously known as the **reticuloendothelial system**), sites where sequestration of

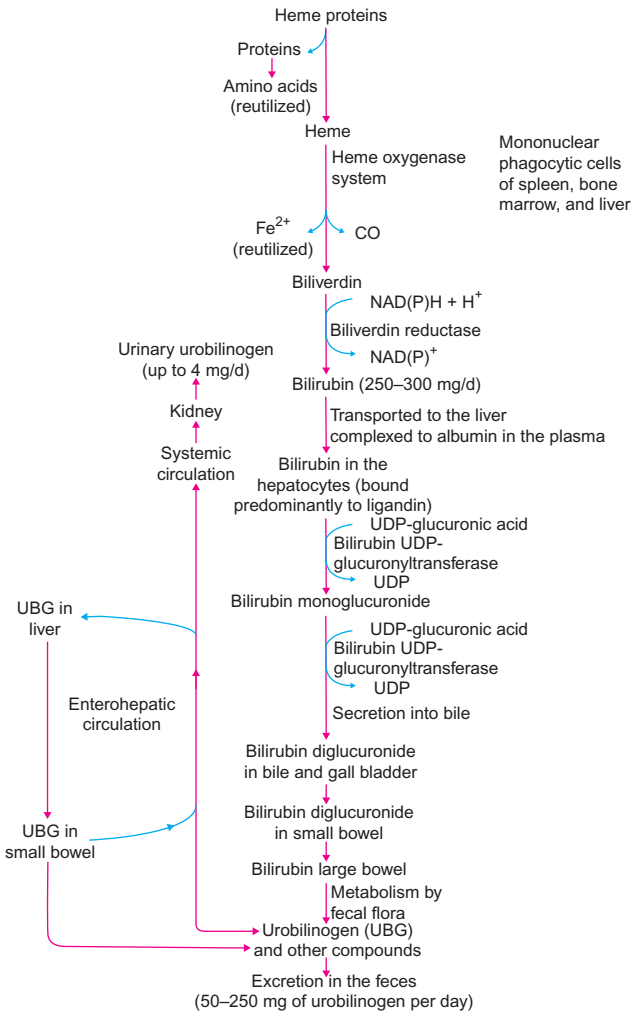


FIGURE 27.10 Catabolic pathway for the heme group from hemoproteins (predominantly hemoglobin).

aging red cells occurs. Renal tubular epithelial cells, hepatocytes, and macrophages may also contribute to bilirubin formation under some conditions. Structures of the intermediates in the conversion of heme to bilirubin are shown in Figure 27.11. The initial step after the release of heme is its binding to heme oxygenase, a microsomal enzyme distinct from the microsomal P-450 oxygenases. Heme oxygenase catalyzes what appears to be the rate-limiting step in catabolism of heme. It is induced by heme and requires O_2 and NADPH for activity. The activity of the inducible isoenzyme form of heme oxygenase is highest in the spleen, which is involved in the sequestration of senescent erythrocytes. The constitutive form of heme oxygenase is mainly localized in the liver and brain. After binding, the α-methene carbon of heme is oxidized (hydroxylated) to α-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 α-methene bridge). Since CO production in mammals occurs primarily by this pathway, measurement of expired CO has been used to estimate heme turnover. A potent competitive synthetic inhibitor of heme oxygenase is tin (Sn) protoporphyrin, which has potential therapeutic use in the treatment of neonatal jaundice (see later).

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 carboxylic groups, the two lactam carbonyl oxygens, and four pyrrolenone ring nitrogens, and thus prevents these groups from hydrogen-bonding with water (Figure 27.12). Esterification of the propionyl side chains of bilirubin with glucuronic acid disrupts the hydrogen bonds and increases its solubility. Phototherapy for neonatal jaundice also acts 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 **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 α_2-globulin and an acute-phase reactant [10].

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 per volume of plasma and minimizes the diffusion of bilirubin into extrahepatic tissues, thereby preventing bilirubin toxicity. Because of the formation of this complex, bilirubin does not normally appear in urine. Urinary bilirubin is almost invariably conjugated bilirubin (see later) 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

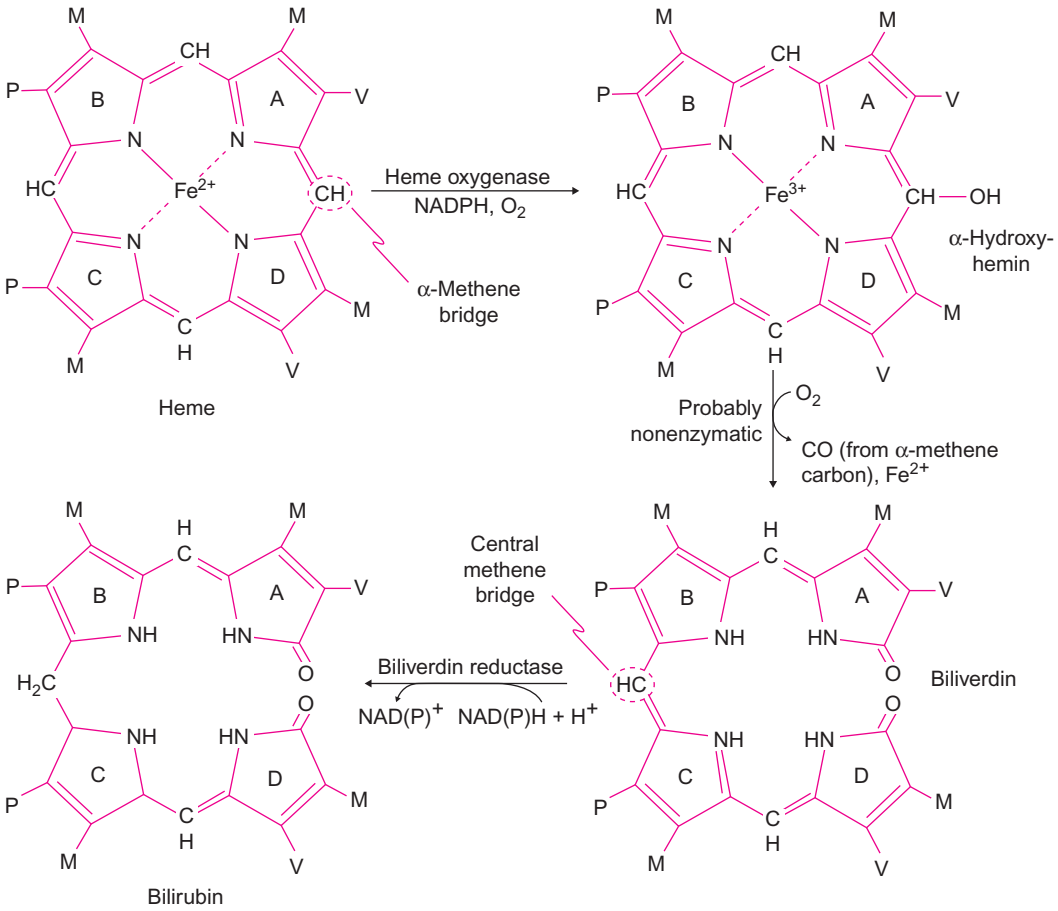


FIGURE 27.11 Conversion of heme to bilirubin in the monocytic phagocytic cells. Carbon monoxide and bilirubin are generated. The Fe^{3+} released is conserved and reutilized. Biliverdin and bilirubin are lactams. P = propionic acid; M = methyl; V = vinyl.

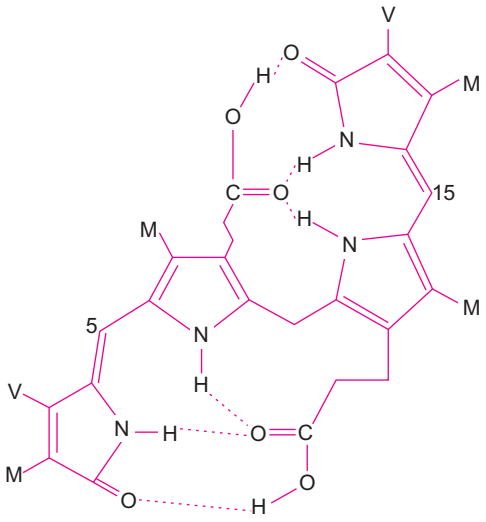


FIGURE 27.12 Conformation of bilirubin showing involuted hydrogen-bonded structure between NH/O and OH/O groups. Despite the presence of polar carboxyl groups, bilirubin is nonpolar and lipophilic. Glucuronidation disrupts hydrogen bonds and provides polar groups to yield water-soluble pigments. (For key to letters see Figure 27.11.)

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 later). 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.

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. Under normal conditions, ligandin is probably the principal hepatic bilirubin-binding protein and may serve the same protective and transport functions intracellularly as does 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 27.13). 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 14.) 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 isomers. These are known as **photobilirubins** and are formed when bilirubin is exposed to blue light of 400–500 nm wavelength. Photobilirubins cannot form the intramolecular hydrogen bonds characteristic of the natural isomer of bilirubin (Figure 27.12). 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

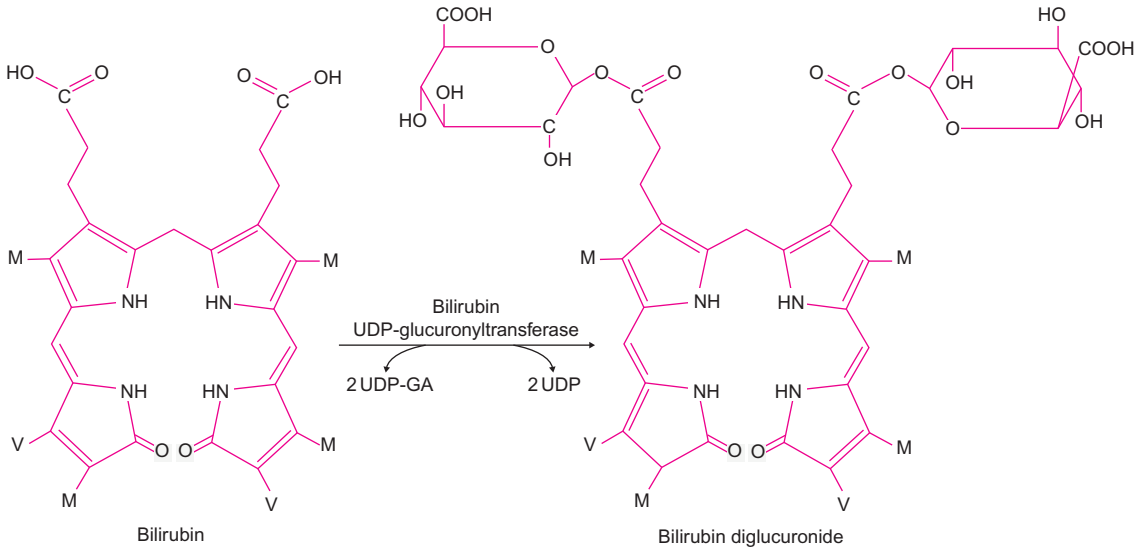


FIGURE 27.13 Formation of bilirubin diglucuronide. Glucuronidation occurs in two steps via formation of monoglucuronide. Mono- and diglucuronides are more water-soluble and less lipophilic than bilirubin. Conversion of bilirubin to water-soluble products is obligatory for excretion of bilirubin from hepatocytes. M = methyl; V = vinyl; UPD-GA = UDP-glucuronic acid.

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.

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 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 the 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**.

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 diseases. 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 $A[TA]_6TAA$ is mutated to $A[TA]_7TAA$. Mutations affecting the coding region of the enzyme, although rare, also occur.

In **Crigler−Najjar syndrome type I**, 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. Orthotopic liver transplantation is the definitive treatment, and it normalizes serum bilirubin levels.

Crigler−Najjar syndrome type II (Arias syndrome) is milder, usually benign, and is 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 I, as opposed to type II Crigler−Najjar syndrome, is essential since orthotopic liver transplantation is an important therapy for type I patients.

Conjugated Hyperbilirubinemias

Conjugated hyperbilirubinemias are due to intra- or extrahepatic reduction in 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 can also elevate serum bilirubin. Nonmechanical cholestasis can be caused by bacterial infection, pregnancy, 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**. All three disorders are uncommon or rare, and all are benign.

Neonatal Hyperbilirubinemia

Normal neonates are frequently hyperbilirubinemic [12,13]. 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 a 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 Ca^{2+}-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 I) 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 14). 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, α_1-antitrypsin deficiency, Dubin−Johnson and Rotor's syndromes, and other genetic diseases. 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 [14]. A potent competitive inhibitor of heme oxygenase is the synthetic heme analogue tin (Sn^{4+}) protoporphyrin (see Clinical Case Study 27.3). When administered parenterally, tin protoporphyrin safely decreases bilirubin formation. Exchange transfusions also rapidly decrease plasma bilirubin levels.

CLINICAL CASE STUDY 27.1 Hereditary Hemochromatosis, an Iron Storage Disorder

This case was abstracted from: R.T. Chung, J. Misdraji, D.V. Sahani, Case 33-2006: a 43-year-old man with diabetes, hypogonadism, cirrhosis, arthralgias, and fatigue, N. Engl. J. Med. 355 (2006) 1812–1819.

Synopsis

A 43-year-old Caucasian male presented with a chief complaint of fatigue, decreased libido, and erectile dysfunction. His physical examination revealed tanned skin. Comprehensive imaging and laboratory testing showed that the subject had hypogonadotropic hypogonadism, recent onset diabetes type 2, arthralgias, and hemochromatosis. The initial diagnosis of hemochromatosis was based on highly elevated serum iron, percent iron saturation and ferritin, and aminotransferase levels. Liver biopsy revealed marked iron deposition with cirrhosis. Genetic testing showed homozygous mutation in the HFE gene, resulting in the expression of C282Y-mutant HFE protein. The patient was placed on a therapeutic phlebotomy schedule to reduce iron overload, treatment with insulin for diabetes, and testosterone supplementation for decreased libido. Lifestyle modification of avoidance of ethanol intake, iron and vitamin C supplements, and decreased consumption of red meat were implemented.

Teaching Points

1. Either iron deficiency or iron excess leads to severe diseases. The most common nutritional disorder is due to iron deficiency, which leads to hypochromic, microcytic anemia. The accumulation of iron in the body, either due to frequent blood transfusions (e.g., in the management of sickle cell disease and thalassemias) or due to genetic causes because of mutations in iron regulatory proteins, leads to a pathological condition known as hemochromatosis. The toxicity of iron is due to the production of superoxide anions and hydroxyl radicals, which inactivate proteins, lipids, and nucleic acids.
2. Iron absorption, storage, and utilization are orchestrated by gastrointestinal duodenal cells, macrophages of liver and spleen, and hepatocytes. The players are transport proteins: divalent metal transporter 1 (DMT1); ferroportin; storage protein ferritin; plasma transport protein transferrin; transferrin receptors 1 and 2 (tfR1 and 2); HFE protein; hemojuvelin; cytokines; and finally the conductor hepcidin.
3. Hepcidin is a 25-amino acid peptide consisting of four intra-disulfide bonds; it attenuates (inhibits) iron absorption and iron release from macrophages. The expression of hepatic hepcidin is regulated by iron regulatory proteins, hypoxia, and inflammatory mediators (cytokines) via signaling pathways involving bone morphogenetic proteins (BMP). Thus, mutation in iron regulatory proteins that result in decreased hepcidin synthesis can lead to all forms of currently known genetic hemochromatosis.
4. Genetic hemochromatosis is the most common genetic disorder in populations of European ancestry, and among the types of genetic hemochromatosis, HFE mutation (C282Y) is the most common type (type 1). The homozygous C282Y mutation does not always lead to genetic hemochromatosis. Clinical penetrance of the homozygous C282Y mutation is incomplete and is probably affected by modifier genes.
5. Timely diagnosis of genetic hemochromatosis in subjects with high serum iron, percent iron saturation, and ferritin levels, and unexplained elevated serum aminotransferase levels is vital to prevent multiorgan iron damage. Initiation of iron removal by regular therapeutic phlebotomy can ameliorate symptoms. Iron removal by chelation therapy is utilized in some forms of genetic hemochromatosis associated with anemia and in transfusion-dependent secondary hemochromatosis.
6. Anemia of chronic disease that occurs in acute and chronic inflammatory immune disorders is caused by a cytokine-mediated increase of hepcidin synthesis, leading to decreased availability of iron required for heme biosynthesis. In addition to changes in iron homeostasis, anemia of chronic disease affects erythropoietin synthesis and proliferation of erythroid precursor cells. In anemia due to chronic renal disease, the primary cause has been attributed to decreased erythropoietin production in the kidneys. Thus, administration of recombinant erythropoietin is employed in correcting anemia of chronic renal disease.

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CLINICAL CASE STUDY 27.2 Lead Poisoning

This case was abstracted from: L.S. Friedman, L.H. Simmons, R.H. Goldman, A.R. Sohani, Case 12-2014: a 59-year-old man with fatigue, abdominal pain, anemia, and abnormal liver function, N. Engl. J. Med. 370 (2014) 1542–1550.

Synopsis

A 59-year-old man presented to the clinic with a 3-day history of fatigue, epigastric pain, nausea, and ankle swelling. His physical examination was unremarkable, but laboratory studies revealed anemia and elevated liver enzymes. Review of a peripheral blood smear showed microcytic anemia, polychromasia (red cell enlargement with a purplish hue), and basophilic stippling (punctate basophilic inclusions that are evenly distributed throughout the cytoplasm). He was sent home with omeprazole for the treatment of peptic-ulcer disease and bleeding ulcer. However, within one week his abdominal pain worsened, and he developed an unusual constellation of symptoms raising concerns for lead poisoning, such as behavioral changes and dysgeusia (altered sense of taste). The patient's blood lead level was found to be markedly elevated. A thorough occupational history did not reveal a definitive source for the patient's lead poisoning, except perhaps the daily usage of an Italian mug and spoon containing lead-based paint. The patient's condition was considered severe, so chelating therapy was administered (calcium disodium EDTA and 2,3-dimercaptosuccinic acid).

Teaching Points

1. The most common source of lead poisoning in the United States is from workplace exposures (e.g., lead-based paint, building renovations). A blood lead level of 10 μg/dL or higher is considered elevated. In acute lead poisoning, blood lead levels may reach as high as 100 μg/dL or more.
2. At a blood lead level of about 55 μg/dL, heme synthesis becomes impaired. Lead is a potent inhibitor of heme synthesis by binding to the enzyme 5-aminolevulinic acid (ALA) dehydratase. Lead also inhibits the enzyme heme chelatase in the final step of heme synthesis.
3. Patients with lead poisoning may present clinically like patients with porphyria, a disorder in which there is a deficiency of ALA dehydratase. The resulting overproduction of ALA in both disorders can cause symptoms of nausea, abdominal pain, constipation, restlessness, and pain in the arms and legs.
4. Basophilic stippling is a hallmark feature of lead poisoning and sideroblastic anemia, but it may not be seen in all cases of lead poisoning. Coarse basophilic stippling is a result of impaired hemoglobin synthesis or impaired incorporation of iron into heme, causing abnormal ribosomal structure and incomplete RNA degradation, which appear as punctate inclusions evenly distributed throughout the cytoplasm.
5. The treatment of lead poisoning is administration of a chelating agent, such as calcium disodium EDTA or 2,3-dimercaptosuccinic acid. Removal of the lead source is also an important part of the treatment.

CLINICAL CASE STUDY 27.3 Hyperbilirubinemia

This case was abstracted from: G.J. Mizejewski, K.A. Pass, Sn-Mesoporphyrin interdiction of severe hyperbilirubinemia in Jehovah's Witness' newborns as an alternative to exchange transfusion, Pediatrics 108 (2001) 1374–1377.

Synopsis

Two unrelated newborn infants at the same hospital were found to have progressive hyperbilirubinemia despite phototherapy. Their hyperbilirubinemia was due to immune hemolytic disease of the newborn. Exchange transfusion therapy was considered when their plasma unconjugated bilirubin had reached a level of 19.5 mg/dL. However, the families of both infants refused exchange transfusion therapy due to religious concerns. Alternative therapy with Sn-mesoporphyrin (Sn-MP) was necessary. Sn-MP is a powerful inhibitor of heme oxygenase. Intramuscular administration of a single dose of Sn-MP led to a sustained decrease in the infants' plasma bilirubin levels and the resolution of hyperbilirubinemia.

Teaching Points

1. Bilirubin is a catabolic product of heme synthesized in the macrophages in two enzymatic steps. The first step is catalyzed by the rate-limiting enzyme heme oxygenase. Elimination of bilirubin requires albumin-bound transport to the liver, conjugation with glucuronic acid, and eventual removal via the biliary-gastrointestinal tract.
2. Newborn unconjugated hyperbilirubinemia, a common condition, is usually treated with phototherapy. Note that the photoisomers of bilirubin are water-soluble, do not require glucuronidation, and are eliminated in the urine. Severe hyperbilirubinemia can occur due to prematurity, isoimmune hemolytic disease, glucose-6-phosphate dehydrogenase deficiency, asphyxia, acidosis, and hypoalbuminemia. Genetic defects in bilirubin glucuronidation also result in hyperbilirubinemia.
3. High plasma levels of bilirubin cause brain damage (e.g., bilirubin encephalopathy evolving into kernicterus). The precise plasma levels of bilirubin that cause abnormal neurologic manifestations are not understood (see references [2] and [3]). A determining factor for the management of hyperbilirubinemia is its progressive increase, unresponsiveness to phototherapy, and the presence of risk factors.

(*Continued*)

CLINICAL CASE STUDY 27.3 (Continued)

4. In the treatment of neonatal hyperbilirubinemia, exchange blood transfusion therapy is effective for rapid elimination of bilirubin. However, inhibition of its production via heme oxygenase by Sn-MP is also effective.
5. While the primary management consideration for neonatal jaundice requires incorporation of methods that decrease plasma bilirubin levels, the management of adult jaundice requires the diagnosis of diseases of the hepatic-biliary system (e.g., cancer, cirrhosis, infection), followed by their treatment.

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Chapter 28

Endocrine Metabolism I: Introduction and Signal Transduction[1]

Key Points

1. Hormones and neurotransmitters are integrated, and they coordinate cellular functions in the body.
2. The physiologic response to hormones can be autocrine, paracrine, or endocrine.
3. Hormones can be amino acid-derived amines, peptides, proteins or glycoproteins, steroids, or eicosanoids.
4. The nervous and endocrine systems function in a coordinated manner to promote growth, homeostasis, and reproductive competence.
5. Feedback regulation of an endocrine system (usually negative) involves both simple feedback loops (e.g., insulin secretion regulated by plasma glucose levels) and complex feedback loops (e.g., hypothalamic, pituitary, thyroid in the secretion of thyroxine). Some of the feedback loops can be positive.
6. Hormone secretion can be pulsatile, episodic, daily, monthly, or seasonal.
7. Steroid hormones and thyroid hormones (T_3 and T_4) require transport proteins in the blood to reach the target sites.
8. Some hormones have multiple physiologic effects at a given target site (e.g., insulin), and some hormones have different physiological effects at different sites (e.g., testosterone).
9. The physiological response to a hormone (ligand) is determined by the presence of a specific receptor at the target cell. The receptors may be located on the plasma cell membrane, in the cytosol, or in the nucleus.
10. Hormone recognition and binding at their specific receptor binding site initiate the signal transduction amplification pathways, which culminates in an appropriate biological response. This chapter discusses only a few selected pathways.
11. Nuclear receptors are responsible for the action of the thyroid hormone tetraiodothyronine (T_3). T_3 binding to the nonhistone receptor proteins stimulates transcription at the target sites. However, it can also inhibit transcription of thyroid stimulating hormone (TSH) at the pituitary (a negative feedback process).
12. All steroid hormones, like T_3, mediate their action via nuclear receptors. However, glucocorticoids and aldosterone initially bind to cytosolic receptors.
13. Amine, polypeptide, and protein hormones (either growth promoting or inhibiting) initiate their action by binding to plasma membrane receptors on the cell surface.
14. Cell surface receptors can be G-protein-coupled receptors (GPCR), tyrosine kinase receptors, or guanylyl kinase receptors. GPCR pathways include the heterotrimeric G-protein-coupled adenylate cyclase–cAMP system and the G-protein-coupled phosphatidyl-inositol-Ca^{2+} pathway.
15. G-protein-coupled receptor signaling pathways affect diverse metabolic processes and are regulated by several different mechanisms involving the regulation of GTPase activity of the $G\alpha$ subunit. Some bacterial toxins act by activating or inhibiting $G\alpha$ subunit activity.
16. Monomeric G-proteins anchored to the inner cytoplasmic membrane also participate in the normal cellular functions when activated by external stimuli. Some of these are proto-oncogenes and, when mutated, become oncogenes that promote cancer.
17. Tyrosine kinase receptors are of two types. The first type contains an extracellular receptor domain and an intracellular domain with tyrosine kinase activity (e.g., insulin). The second type consists of the extracellular hormone-induced dimerization with resultant activation of constitutively associated tyrosine kinases known as Janus kinases (JAK) and involves cytosolic signal transducer and activator of transcription pathway (STAT). These signal amplifications of JAK–STAT pathways are involved in several other pathways of the cytokines of growth factors (e.g., growth hormone, prolactin).
18. Nonreceptor tyrosine kinases are found in the cytosol and are involved in the signal transduction pathways of normal cellular processes. Abnormalities in these pathways can lead to cancer (e.g., chronic myelogenous leukemia, BCR-ABL tyrosine kinase).
19. Specific inhibitions at the receptor sites using monoclonal antibodies or tyrosine kinase inhibitors are therapeutic targets used in the management of some cancers.

1. Endocrine topics not discussed in Chapters 28 through 32 that are covered elsewhere in the text are as follows: gastrointestinal hormones, Chapter 11; eicosanoids, Chapter 16; pancreatic hormones, Chapter 20; parathyroid hormone and vitamin D, Chapter 35; renin–angiotensin system and antidiuretic hormone, Chapters 30 and 37. A list of expanded acronyms appears in the appendix.

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