Ascorbate can be regenerated from dehydroascorbate by dehydroascorbate reductase, which uses reduced glutathione as the reductant.

Epinephrine. Epinephrine is synthesized by methylating norepinephrine:

norepinephrine + *S*-adenosylmethionine → epinephrine + *S*-adenosylhomocysteine

The methyl donor for this reaction, *S*-adenosylmethionine (SAM), represents the activated form of methionine.

20.4.6.3 Inactivation of Catecholamines. The two enzymes that inactivate the catecholamines are catechol *O*-methyltransferase (COMT) and monoamine oxidase (MAO). COMT uses *S*-adenosylmethionine to methylate the hydroxyl group in the 2′-position on the phenyl ring. MAO catalyzes the removal of the terminal amino group of a catecholamine such as dopamine, generating an aldehyde which is then oxidized further to a carboxyl group. The reactions catalyzed by COMT and MAO can occur in either order; the resulting degradation products (i.e., vanillylmandelic acid from norepinephrine, homovanillic acid from dopamine) are excreted in the urine (Fig. 20-8).

20.4.6.4 Synthesis of Melanin. Melanin is synthesized by specialized cells called *melanocytes*, located in the skin, hair roots, and iris and retina of the eye. Melanocytes contain tyrosinase, a copper-dependent tyrosine hydroxylase that converts tyrosine first to DOPA quinone and then to a family of bicyclic molecules called *indoles*. Subsequent oxidation and polymerization of the indoles results in the formation of melanins, whose multiple aromatic rings account for the pigmentation for the skin and hair. Synthesis of tyrosinase in melanocytes is induced by exposure to UV light.

20.4.6.5 Synthesis of Thyroid Hormones. Tyrosine is also the precursor of the thyroid hormones T_4 (3,5,3′,5′-tetraiodothyronine or thyroxine) and T_3 (3,5,3′-triiodothyronine). The molecular iodine (I_2) required in this reaction is synthesized by thyroid peroxidase, which catalyzes the reaction of iodide ions with hydrogen peroxide and subsequent incorporation of iodine atoms into tyrosine residues of thyroglobulin to form mono- and diiodotyrosine (Fig. 20-9). Thyroid peroxidase also catalyzes the coupling of two diiodinated or one iodinated plus one diiodinated tyrosine residue of thyroglobulin. Subsequent lysosomal hydrolysis of the thyroglobulin releases the active hormones thyroxine and triiodothyronine.

20.4.7 Metabolism of Tryptophan

Tryptophan is the precursor of the neurotransmitter serotonin (Fig. 20-10). Serotonin, in turn, is utilized by the pineal gland to synthesize melatonin, which regulates seasonal and circadian rhythms.

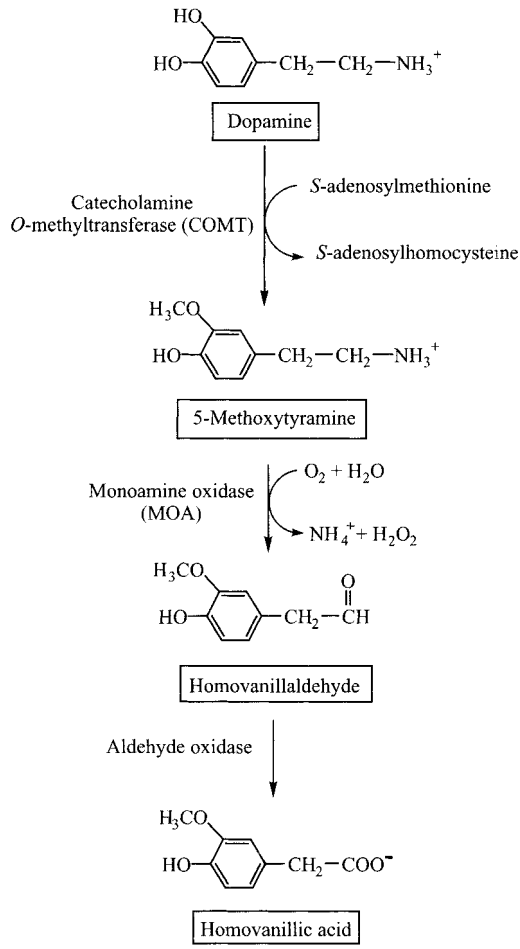

FIGURE 20-8 Inactivation of dopamine.

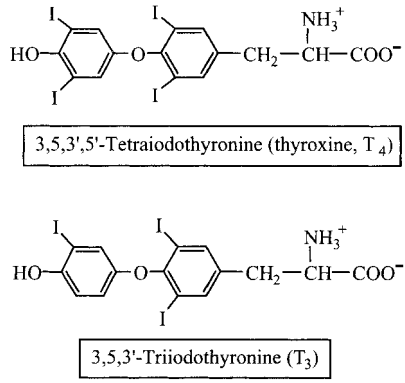

FIGURE 20-9 Structures of the thyroid hormones derived from tyrosine.

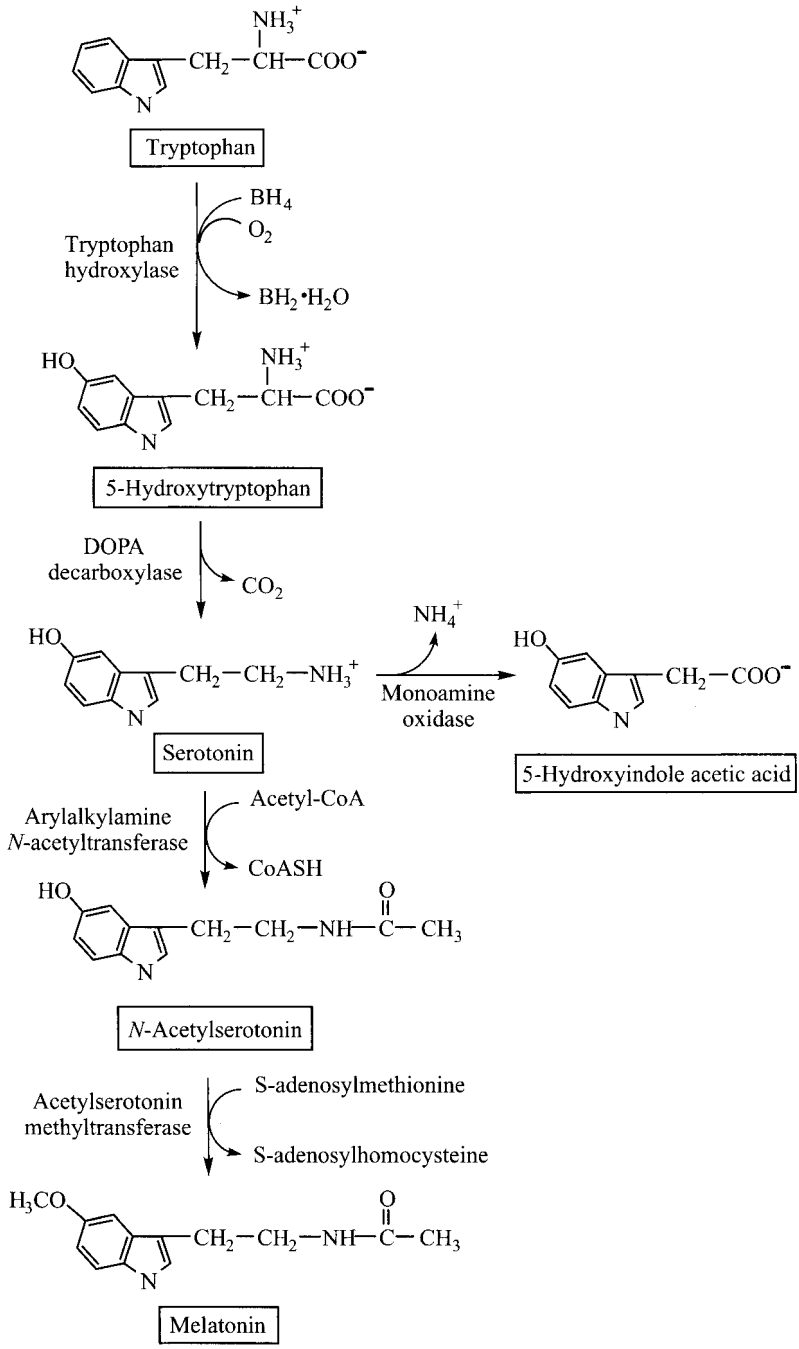

FIGURE 20-10 Synthesis of serotonin and melatonin from tryptophan.

20.4.7.1 Synthesis of Serotonin. The pathway for the synthesis of serotonin is similar to that which generates dopamine. The first step is catalyzed by tryptophan hydroxylase, which, like tyrosine hydroxylase, is a tetrahydrobiopterin (BH_4)-dependent enzyme:

$$\text{tryptophan} + O_2 + BH_4 \rightarrow \text{5-hydroxytryptophan} + BH_2 + H_2O$$

DOPA decarboxylase, the enzyme that catalyzes the decarboxylation of DOPA to produce dopamine, then decarboxylates 5-hydroxytryptophan:

$$\text{5-hydroxytryptophan} \rightarrow \text{serotonin} + CO_2$$

20.4.7.2 Inactivation of Serotonin. Monoamine oxidase, which inactivates catecholamines, also catalyzes the oxidative deamination of serotonin to produce 5-hydroxyindole acetic acid (Fig. 20-10). However, unlike the pathway that inactivates catecholamines, the hydroxyl group on the ring of serotonin is not methylated.

20.4.7.3 Synthesis of Melatonin. Melatonin is synthesized from serotonin by the following two-step reaction sequence (Fig. 20-10):

$$\text{serotonin} + \text{acetyl-CoA} \rightarrow \textit{N}\text{-acetylserotonin} + \text{CoASH}$$

$$\textit{N}\text{-acetylserotonin} + \textit{S}\text{-adenosylmethionine} \rightarrow \text{melatonin} + \textit{S}\text{-adenosylhomocysteine}$$

20.4.7.4 Synthesis of Niacin. One of the alternative pathways for tryptophan catabolism produces niacin, which is the precursor of the nicotinamide component of NAD^+ and $NADP^+$. Niacin synthesis, however, represents a minor pathway for the catabolism of tryptophan; only about 3% of the tryptophan that is metabolized actually follows the pathway to nicotinamide adenine nucleotide synthesis. Niacin is therefore an essential dietary requirement and is designated as a vitamin (B_3).

20.4.8 Decarboxylation of Other Amino Acids

We have seen that decarboxylation of tyrosine results in the synthesis of DOPA and other catecholamines (Fig. 20-7), while decarboxylation of 5-hydroxytryptophan generates serotonin (Fig. 20-10). Several other important bioactive amines are also synthesized by the pyridoxal phosphate (PLP)–dependent decarboxylation of amino acids. Among these are the neurotransmitter γ-aminobutyric acid (GABA) and histamine, which is synthesized by mast cells as part of the allergic response:

$$\text{glutamate} \rightarrow \gamma\text{-aminobutyric acid} + CO_2$$

$$\text{histidine} \rightarrow \text{histamine} + CO_2$$

20.5 ABNORMAL FUNCTIONING OF AMINO ACID METABOLISM

20.5.1 Maple Syrup Urine Disease

Maple syrup urine disease (MSUD, a.k.a. branched-chain α-ketoaciduria) results from a genetic deficiency of branched-chain α-ketoacid dehydrogenase. Thus, it is an inborn error in the metabolism that impairs the catabolism of the branched-chain amino acids leucine, isoleucine, and valine. Affected persons exhibit hyperaminoacidemia and excrete excessive amounts of branched-chain α-ketoacids and related side products in their urine. As a result, the urine has a characteristic odor, which Western physicians find reminiscent of maple syrup: Mediterranean physicians report that the odor is similar to that of fenugreek seeds.

Maple syrup urine disease can result from mutations in any of the genes that encode the enzymes of the α-ketoacid dehydrogenase enzyme complex, with the severity of the disease being inversely related to the level of residual enzyme activity. The major clinical features of MSUD are mental and physical retardation. The classic, most severe form of MSUD results in lethargy, weight loss, and encephalopathy in infancy. Untreated, MSUD can result in seizures and coma followed by death. Treatment involves restricting dietary intake of the branched-chain amino acids to the minimum amount necessary to sustain growth.

20.5.1.1 Thiamine-Responsive MSUD. Some MSUD patients have mutant forms of α-ketoacid dehydrogenase that have a lower affinity for thiamine pyrophosphate or are more subject to proteolysis when the cofactor is not bound to the enzyme. For these patients, the hyperaminoacidemia can usually be corrected with moderate levels of dietary protein restriction plus very large doses of thiamine hydrochloride.

20.5.1.2 MSUD with Lactic Acidosis. MSUD type III is the result of a deficiency in dihydrolipoamide dehydrogenase, which is the common E3 component of three mitochondrial thiamine-dependent enzyme complexes: branched-chain α-ketoacid dehydrogenase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase. Because multiple dehydrogenase activities are affected, the clinical consequences are more severe than in classic MSUD.

20.5.2 Phenylketonuria

Phenylketonuria is another relatively common inborn error of amino acid metabolism (1/20,000 in the United States). People with PKU lack phenylalanine hydroxylase, which catalyzes the reaction

$$\text{phenylalanine} + O_2 + BH_4 \rightarrow \text{tyrosine} + BH_2 + H_2O$$

Lacking hepatic phenylalanine hydroxylase activity, plasma phenylalanine increases to the point where phenylalanine is metabolized by phenylalanine transaminase, with

a resulting plasma accumulation and urinary excretion of phenylpyruvate, phenylacetate, and other aromatic metabolites of phenylalanine.

Phenylketonuria was one of the first genetic diseases for which neonatal screening was established. The initial Guthrie test employed β-2-thienylalanine, which inhibits the growth of the bacterium *Bacillus subtilis* on minimal culture medium; addition of phenylalanine restores growth. As a result, growth of a bacterial colony in response to application of a blood or urine sample is a positive indicator for PKU in a patient. The Guthrie test has now generally been replaced with tandem mass spectroscopy, which permits simultaneous screening for multiple inborn errors of metabolism.

Untreated, severe forms of PKU result in progressive and severe mental retardation, with other neurological manifestations, including seizures, gait abnormalities, and unstable temperature regulation. Fortunately, PKU is one of a number of genetic diseases in which neonatal screening and rapid medical intervention, ideally within the first week of life, result in successful outcomes. Treatment involves severe restriction of dietary phenylalanine, which requires elimination of protein-rich foods and reliance on semisynthetic food substitutes. Patients must also avoid products containing the artificial sweetener aspartame (the methyl ester of aspartylphenylalanine) since hydrolysis of aspartame in the liver generates phenylalanine.

Phenylalanine is an essential amino acid and as such should not be completely eliminated from the diet. In the absence of adequate phenylalanine hydroxylase activity, tyrosine also becomes an essential amino acid. Indeed, the hypopigmentation observed in PKU patients is due to decreased availability of tyrosine for melanin synthesis as well as competitive inhibition of tyrosinase by the elevated levels of phenylalanine.

20.5.2.1 Maternal PKU. Fetuses with genetic PKU do not develop neurological degeneration in utero because maternal metabolism of phenylalanine prevents excessive accumulation of the amino acid in fetal tissue. However, when a woman with PKU becomes pregnant, her fetus is at risk because, if maternal phenylalanine levels are not tightly controlled during pregnancy, the mother with PKU will expose her fetus to elevated levels of phenylalanine and its toxic metabolites during in utero development. Such infants are born with preexisting neurological damage, microcephaly, and congenital heart malformations. For this reason, females with PKU are strongly encouraged to maintain their phenylalanine-restricted diets into adulthood.

20.5.2.2 Variant PKU. Tetrahydrobiopterin is the cofactor for phenylalanine hydroxylase. Some people with PKU have a deficit in the ability to either synthesize or recycle the cofactor, and therefore also have impaired synthesis of catecholamines and serotonin. Administration of BH_4 is effective in treating the hyperphenylalanemia of these patients; however, since BH_4 does not cross the blood–brain barrier, this therapy does not restore neurotransmitter synthesis in the CNS. These patients therefore require treatment with L-DOPA and 5-hydroytryptophan, which do cross the blood–brain barrier and are precursors for dopamine, epinephrine, and serotonin synthesis. Therapy of variant PKU also includes administration of carboxy-DOPA, an inhibitor of dopamine decarboxylase which prevents excessive synthesis of epinephrine

by the adrenals. Carboxy-DOPA does not cross the blood–brain barrier and thus does not inhibit neural synthesis of catecholamines.

20.5.2.3 BH_4-Responsive PKU. Some people with deficiencies in phenylalanine hydroxylase deficiency also respond to BH_4 therapy. It appears that the mutant enzyme in these individuals has either a reduced affinity for BH_4 or is stabilized by the presence of additional cofactor. For such persons, supplementation with BH_4 permits some relaxation of the stringent dietary exclusion of phenylalanine. A stereoisomer of BH_4 called Kuvan has recently become available for the treatment of BH_4-responsive PKU.

20.5.3 Pyridoxine-Dependent Epilepsy

People with pyridoxine-dependent epilepsy (EPD) exhibit seizures in the first hours of life and are unresponsive to standard anticonvulsant therapy. The genetic defect had long been thought to reside in the gene for glutamate decarboxylase, the enzyme that converts glutamate to the neurotransmitter γ-aminobutyric acid (GABA). However, recent studies indicate that patients with EPD are deficient in α-aminoadipic semialdehyde dehydrogenase, an intermediate in a minor (pipecolic acid) pathway of lysine catabolism. As a result, there is accumulation of a metabolic intermediate (piperidine-6-carboxylate) that reacts with and sequesters pyridoxal phosphate (PLP). Since PLP is an essential cofactor in neurotransmitter metabolism, those affected are dependent on relatively high doses of pyridoxine hydrochloride to prevent recurrence of seizures.

20.6 PARKINSON'S DISEASE

The characteristic tremors of the shaking palsy of Parkinson's disease are the result of gradual loss of dopamine-producing neurons in the substantia nigra. Dramatic symptomatic relief is obtained by administering DOPA, which is decarboxylated to dopamine by α-amino acid decarboxylase in the brain. Side effects such as gastrointestinal disturbances are prevented by addition of a peripheral inhibitor such as carboxy-DOPA.

20.7 ALBINISM

Albinism is a genetic disorder in which production of the photoprotective pigment melanin is impaired. One cause of the disease is a defect in the gene for tyrosinase, the enzyme that catalyzes several steps in the pathway of melanin synthesis, including the conversion of tyrosine to dopaquinone. Defects in several other proteins also cause albinism, but the role of each is not well understood. Affected persons have visual impairment and are at increased risk for skin cancer.

CHAPTER 21

SULFUR AMINO ACID METABOLISM

21.1 FUNCTIONS OF METHIONINE

Methionine is unique among the essential amino acids in that it is the only one that contains a sulfur atom (Fig. 21-1). Furthermore, in addition to its role as an amino acid constituent of proteins, different portions of the methionine molecule serve as precursors for a variety of other key molecules.

21.1.1 Methionine Provides Sulfur Atoms for Other Organic Molecules in the Body

Methionine provides the sulfur atom for the synthesis of cysteine, which is not an essential amino acid. Like methionine, cysteine is a constituent of proteins; in most proteins the sulfhydryl groups of particular cysteines form intra- and interpolypeptide disulfide bridges. Cysteine is also a component of glutathione (Fig. 21-1), which in its reduced form is a major component of the intracellular defense system that protects cells against oxidative damage from hydrogen peroxide and other reactive oxygen species.

Cysteine is the precursor of taurine, an unusual amino acid whose acidic nature derives from a sulfate rather than a carboxyl group. Although not a component of proteins, taurine plays many essential roles: It is one of two amino acids (the other being glycine) used to form conjugated bile salts in adults, and the one most often used to form conjugated bile salts in neonates (see Fig. 3-5). Taurine is also an abundant

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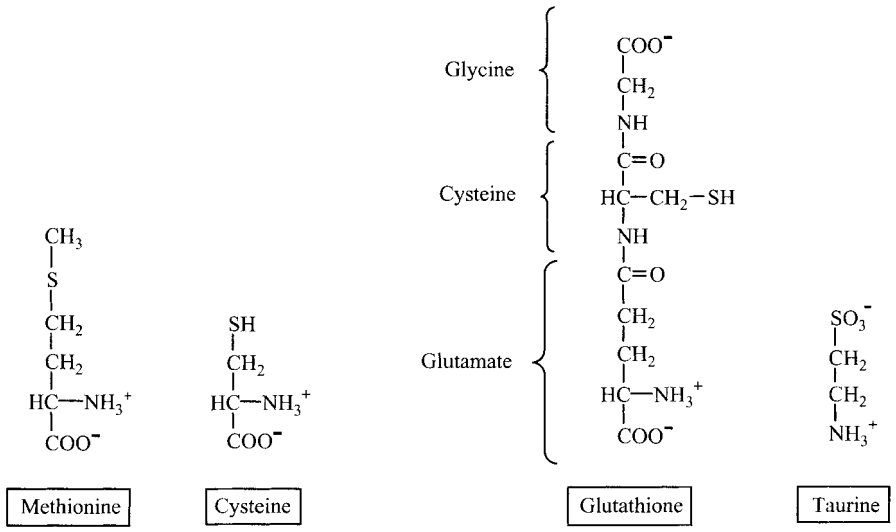

FIGURE 21-1 Methionine and other sulfur-containing molecules.

intracellular free amino acid which is involved in osmoregulation, particularly in the brain and neural retina.

Catabolism of cysteine produces free sulfate ions, which provide the sulfur moieties for the sulfation reactions required to synthesize proteoglycans and sulfated steroids. Many hormones, including catecholamines, steroids, and thyroid hormones, can be sulfated in vivo. Sulfation of both thyroxine (T_4) and triiodothyronine (T_3) inactivates these hormones. Sulfotransferases also play an important role in drug metabolism and detoxification of xenobiotics by rendering organic molecules more soluble and thus more readily excretable in the urine.

21.1.2 Methionine Donates Methyl Groups

S-Adenosylmethionine (SAM) (Fig. 21-2) is the major donor of methyl groups in a variety of biosynthetic pathways. These include biosynthesis of a number of small molecules, including epinephrine, creatine, melatonin, and phosphatidylcholine as well as methylation of proteins (see Chapter 20). In addition, SAM-dependent methylation of cytosine residues in DNA provides a mechanism for regulating gene expression, particularly during fetal development.

21.1.3 Methionine Donates Aminopropyl Groups

S-Adenosylmethionine can also donate three carbons plus an amino group to putrescine, generating spermidine and then spermine, which are two polyamines that stabilize DNA during cell growth and proliferation (Fig. 21-3).

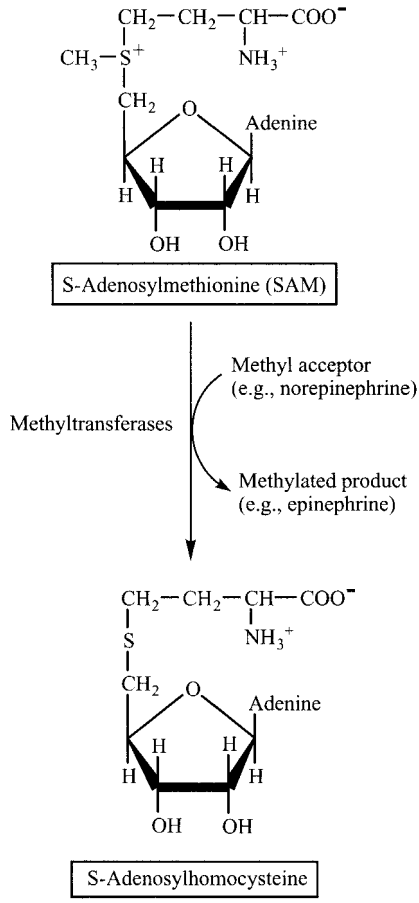

FIGURE 21-2 S-Adenosylmethionine is a methyl donor.

21.2 LOCALIZATION

Nearly all cells of the body use *S*-adenosylmethionine as a methyl donor. By contrast, the transsulfuration pathway, which utilizes the sulfur atom of methionine in the synthesis of cysteine, occurs primarily in the liver, kidney, and gastrointestinal tract.

21.3 METHIONINE METABOLISM

21.3.1 Activation of Methionine

S-Adenosylmethionine is synthesized from methionine plus ATP (Fig. 21-4). This ATP-dependent reaction is unusual in that all three of the phosphate atoms of

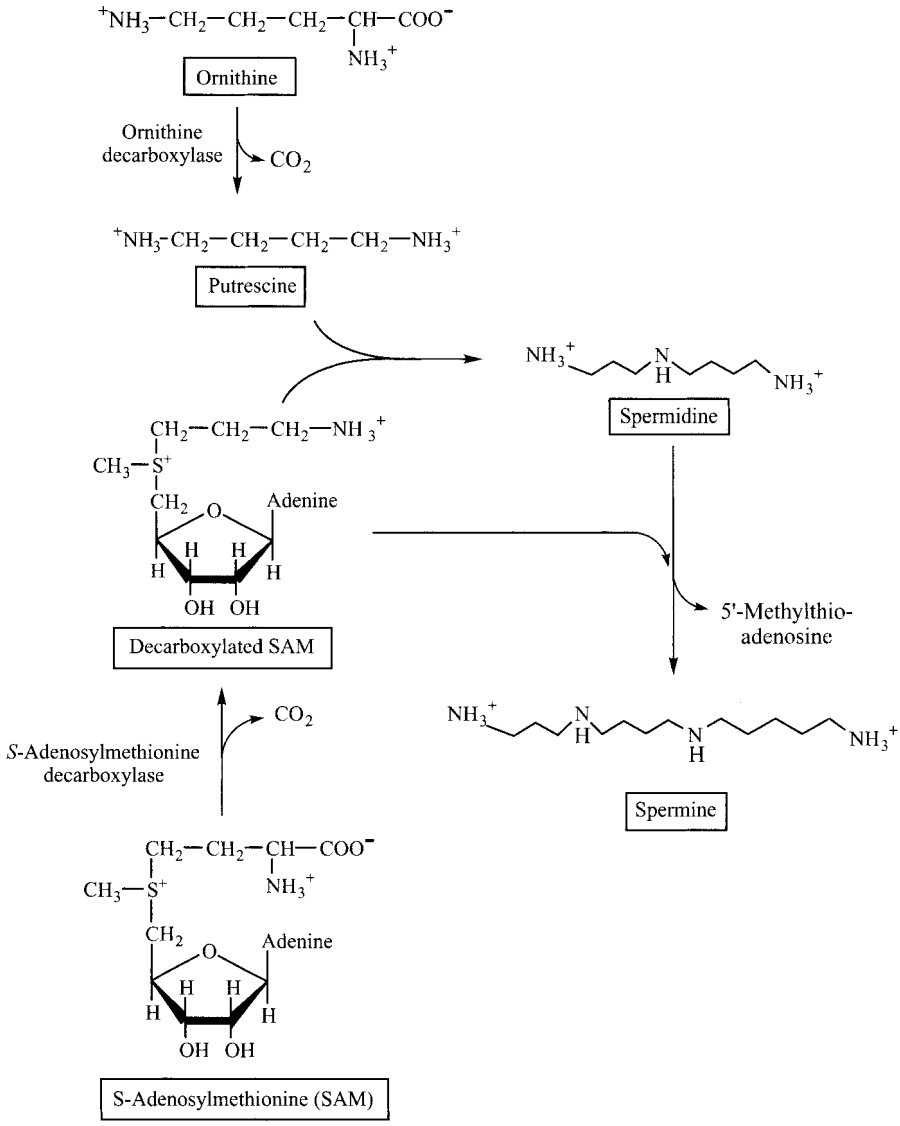

FIGURE 21-3 Structures and synthesis of polyamines.

ATP are cleaved from the ATP molecule:

$$\text{methionine} + \text{ATP} \rightarrow S\text{-adenosylmethionine} + \text{PP}_\text{i} + \text{P}_\text{i}$$

The reaction occurs in two steps: Methionine adenosyltransferase first forms SAM and a triphosphate and then the triphosphate is cleaved into $\text{PP}_\text{i} + \text{P}_\text{i}$.

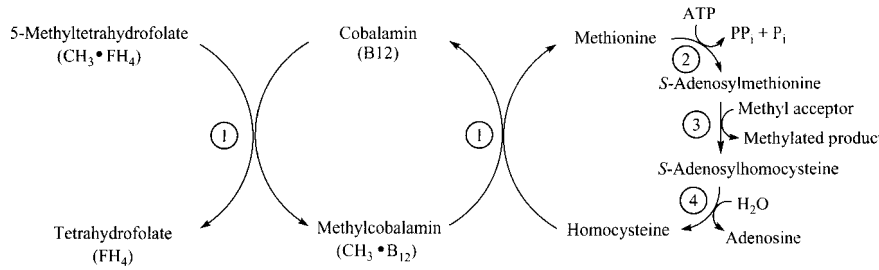

FIGURE 21-4 Regeneration of methionine from homocysteine: ① methionine synthase (methyltetrahydrofolate homocysteine methyltransferase); ② methionine adenosyltransferase; ③ SAM-dependent methyltransferase; ④ *S*-adenosylhomocysteine hydrolase.

21.3.2 Donation of Methyl Groups

SAM is the major methyl donor in a number of biosynthetic reactions, each of which is catalyzed by a specific SAM-dependent methyltransferase:

norepinephrine + *S*-adenosylmethionine → epinephrine + *S*-adenosylhomocysteine

acetylserotonin *S*-adenosylmethionine → melatonin + *S*-adenosylhomocysteine

guanidinoacetate + *S*-adenosylmethionine → creatine + *S*-adenosylhomocysteine

Three successive methyl transfer steps are involved in the synthesis of phosphatidylcholine from phosphatidylethanolamine (see Fig 14-10):

phosphatidylethanolamine + 3*S*-adenosylmethionine →
phosphatidylcholine + 3*S*-adenosylhomocysteine

SAM-dependent methyltransferases also methylate both DNA and RNA, and catalyze the methylation of specific lysine, arginine, and glutamine residues in proteins. Subsequent proteolysis of trimethyllysine-containing proteins releases the trimethyllysine, which is a precursor for the synthesis of carnitine, the molecule that serves to transport long-chain fatty acids into the mitochondrion.

In all cases, the donation of a methyl group from SAM produces *S*-adenosylhomocysteine, which is subsequently hydrolyzed by *S*-adenosylhomocysteine hydrolase to release free homocysteine (Fig. 21-3). Homocysteine is a homolog of cysteine in that it has an additional $-CH_2-$ group in its carbon backbone; however, homocysteine is not utilized in protein synthesis. There are two pathways that metabolize homocysteine: remethylation and transsulfuration.

21.3.3 Remethylation of Homocysteine

Homocysteine is remethylated by methionine synthase (Fig. 21-4):

homocysteine + methylcobalamin → methionine + cobalamin

The major source of the methyl group of methylcobalamin is 5-methyltetrahydrofolate ($FH_4 \cdot CH_3$), which is discussed in greater detail in Chapter 22. A minor pathway for remethylating homocysteine, which is present only in liver and kidney, utilizes betaine instead of 5-methyltetrahydrofolate as the methyl donor. Betaine (*N*-trimethylglycine) is not an essential nutrient since it can be synthesized in the liver from choline. Remethylation of homocysteine regenerates methionine, which can be activated at the expense of ATP to provide *S*-adenosylmethionine. The remethylation pathway thus completes a metabolic cycle in which *S*-adenosylmethionine plays a catalytic role in methylation reactions.

21.3.4 The Transsulfuration Pathway and Cysteine Synthesis

The other route for metabolizing homocysteine is the transsulfuration pathway, which transfers the sulfur atom of homocysteine to serine, generating cysteine in the process (Fig. 21-5). This pathway consists of two reactions, which form and then cleave cystathionine. The first reaction is catalyzed by cystathionine synthase and utilizes pyridoxal phosphate (PLP) as its cofactor:

$$\text{homocysteine} + \text{serine} \rightarrow \text{cystathionine} + H_2O$$

Cystathionase, another PLP-dependent enzyme, then catalyzes the hydrolytic deamination of cystathionine.

$$\text{cystathionine} + H_2O \rightarrow \text{cysteine} + \alpha\text{—ketobutyrate} + NH_4^+$$

Note in Figure 21-5 that the newly formed cysteine molecule contains a sulfur atom that is derived from homocysteine but none of the carbon atoms of homocysteine. α-Ketobutyrate, which is the product of the transsulfuration reaction, is oxidatively decarboxylated to form propionyl-CoA.

21.3.5 Synthesis of Taurine

Taurine is synthesized from cysteine (Fig. 21-6). The first step in the pathway oxidizes the sulfhydryl group of cysteine to generate cysteinosulfinate. The second step is a decarboxylation reaction that produces hypotaurine. The sulfite group of hypotaurine is then oxidized to taurine.

21.3.6 Release of Inorganic Sulfate from Sulfur-Containing Amino Acids

Catabolism of cysteine results in the release of the sulfur atom as SO_4^{2-}. The initial step is the generation of cysteinosulfinate, as occurs in taurine synthesis. Cysteine

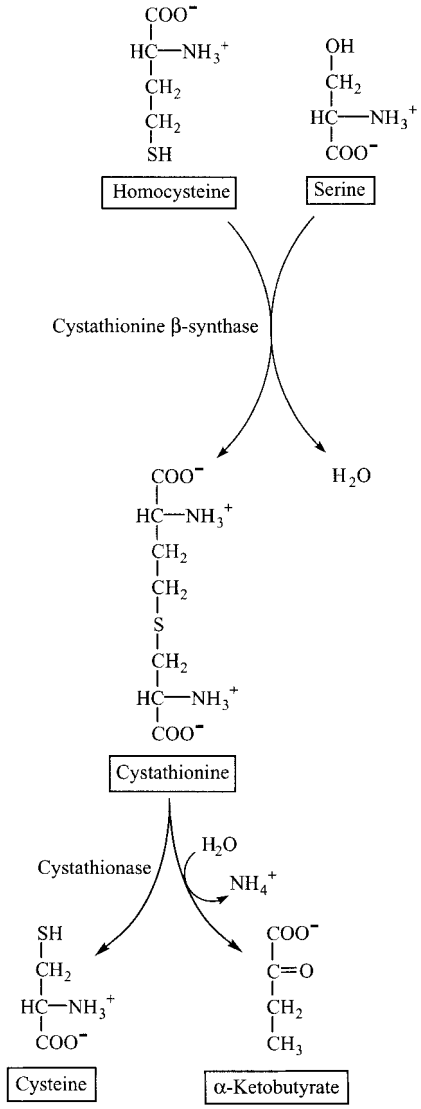

FIGURE 21-5 The transsulfuration pathway; the sulfur atom of homocysteine is used to synthesize cysteine.

aminotransferase then releases inorganic sulfite (Fig. 21-6):

$$\text{cysteinosulfinate} + \alpha\text{-ketoglutarate} \rightarrow HSO_3^- + \text{glutamate} + \text{pyruvate}$$

Sulfite oxidase then generates sulfate:

$$HSO_3^- + O_2 + H_2O \rightarrow SO_4^{2-} + H_2O_2 + H^+$$

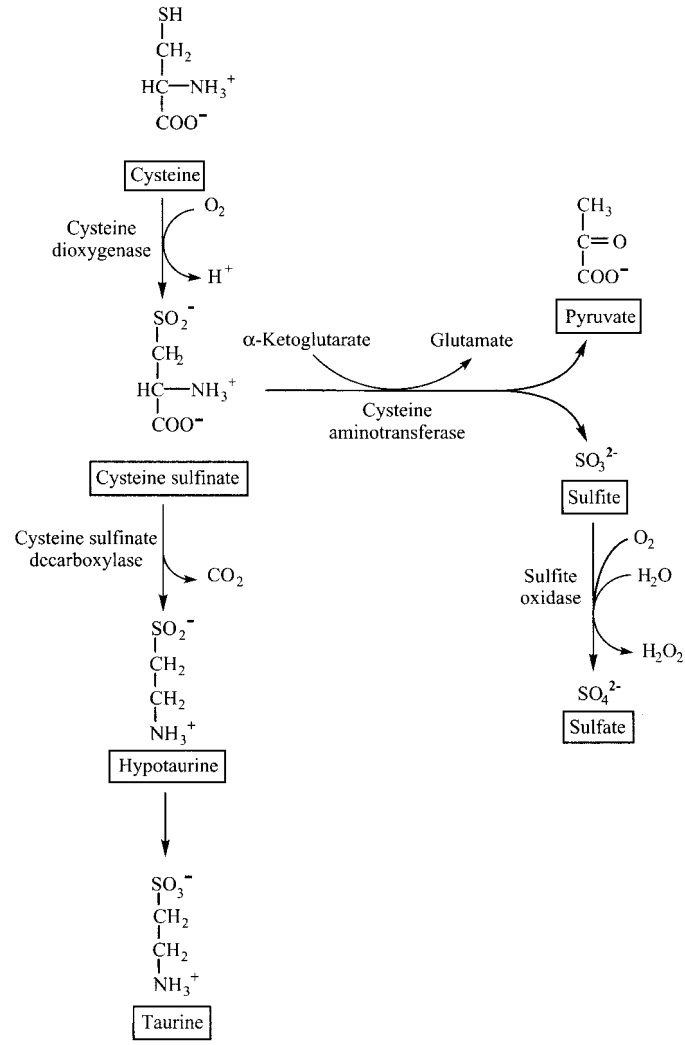

FIGURE 21-6 Metabolism of cysteine produces either taurine or inorganic sulfate.

21.3.7 Activation of Inorganic Sulfate

Inorganic sulfate can be activated at the expense of ATP to form 3′-phosphoadenosine 5′-phosphosulfate (PAPS), which serves as the sulfur donor for sulfation reactions. PAPS synthesis involves two successive ATP-dependent reactions that release PP_i and P_i, respectively:

$$SO_4^{2-} + \text{ATP} \rightarrow \text{adenosine phosphosulfate} + PP_i$$

$$\text{adenosine phosphosulfate} + \text{ATP} \rightarrow \text{phosphoadenosine phosphosulfate (PAPS)} + \text{ADP}$$

21.3.8 Synthesis of Polyamines

Polyamines have multiple positive charges that stabilize DNA during cell division and are therefore essential for cell survival (Fig. 21-3). Putrescine, the simplest of the polyamines, is produced by decarboxylation of ornithine. The larger, more positively charged polyamines, spermidine and spermine, are synthesized by means of the transfer of aminopropyl groups to putrescine. In this pathway, SAM is first decarboxylated to decarboxylated-SAM (*S*-adenosylmethylthiopropylamine). Transfer of an aminopropyl group from decarboxylated SAM to putrescine generates spermidine; transfer of a second aminopropyl group to spermidine generates spermine.

21.4 REGULATION OF SULFUR AMINO ACID METABOLISM

As described above, homocysteine is generated when *S*-adenosylmethionine serves as a methyl donor. There are two competing pathways that metabolize homocysteine: remethylation to regenerate methionine, and transsulfuration, which synthesizes cysteine and leads to the catabolism of the carbon skeleton of the homocysteine. Flux through these two completing pathways is determined by the concentration of *S*-adenosylmethionine. Since SAM is an activator of cystathionine β-synthase (Fig. 21-5), high concentrations of SAM tend to favor the transsulfuration pathway. In addition, SAM is an allosteric inhibitor of methylenetetrahydrofolate reductase, which synthesizes 5-methyltetrahydrofolate (N^5-methyl-F_4). N^5-methyl- F_4 donates its methyl group to cobalamin to form methylcobalamin, which in turn serves as the methyl donor for the remethylation of homocysteine (Fig. 21-4). Thus, high concentrations of SAM will inhibit the regeneration of methionine from homocysteine. The transsulfuration pathway is also regulated by end-product inhibition, whereby cysteine allosterically inhibits cystathionine β-synthase.

21.5 ABNORMAL FUNCTION

21.5.1 Deficiency of Cystathionine β-Synthase

Homocystinuria is an autosomal recessive genetic disease that results from a deficiency of cystathionine β-synthase (CBS). Patients with homocystinuria have marked elevations of both methionine and total homocysteine (tHcy = homocysteine + homocystine) in their blood. The clinical features of CBS deficiency include skeletal deformities, abnormalities of the ocular lens, and mental retardation. When untreated, patients with CBS deficiency also have a 50% chance of developing a myocardial infarction, stroke, or serious blood clot before the age of 30 years.

CBS is a pyridoxal phosphate–dependent enzyme, and many patients with CBS deficiency respond to pharmacological doses of pyridoxine (vitamin B_6). Other therapies have included restriction of dietary methionine, and supplementation with folate and vitamin B_{12} to enhance activity of the remethylation pathway. Patients with homocystinuria may also benefit from treatment with betaine, which is the alternate methyl donor for methyl cobalamin synthesis.

21.5.2 Hyperhomocysteinemia

Mild or moderate hyperhomocysteinemia (plasma tHcy concentration 15 to 100 μmol/L; normal < 12 μmol/L) is a multifactorial condition associated with a variety of different nutritional and genetic factors, impaired kidney function, or excessive alcohol intake. Vitamins required for homocysteine metabolism include pyridoxine, folate, and vitamin B_{12}. Genetic causes of hyperhomocysteinemia include polymorphisms in any of the enzymes involved in either the transsulfuration pathway or the remethylation pathway and related enzymes of folate metabolism [e.g., methylenetetrahydrofolate reductase (MTHFR)]. Regardless of etiology, even mild hyperhomocysteinemia confers an increased risk of adverse cardiovascular events. Although the mechanism by which homocysteine causes endothelial cell dysfunction is not fully understood, it is thought that homocysteine, which is a potent oxidizing agent, inactivates the vasoprotective agent nitric oxide. Alternatively, the impairment of sulfur amino acid metabolism and consequent decreased intracellular SAM/SAH ratio may result in hypomethylation of DNA and increased expression of particular genes.

21.5.3 Cirrhosis of the Liver

One of the features of cirrhosis of the liver is impaired synthesis of *S*-adenosylmethionine due to abnormally low activity of methionine adenosyltransferase. The enzyme is inactivated by a variety of reactive oxygen species which overwhelm the normal enzyme reactivation by reduced glutathione (GSH). As a result, intrahepatic concentrations of SAM are reduced under conditions of chronic oxidative stress such as those induced by alcohol or hepatitis C. The lack of SAM, in turn, exacerbates the liver injury. Pharmacological treatment with stable salts of SAM appears to be beneficial, especially for patients with less advanced liver disease.

CHAPTER 22

FOLATE AND VITAMIN B_{12} IN ONE-CARBON METABOLISM

22.1 FUNCTIONS OF ONE-CARBON METABOLISM

Many reactions in human metabolism involve the transfer of an activated one-carbon group from a donor molecule to an acceptor molecule. Some of these reactions function in catabolic pathways, for example in the breakdown of serine and histidine, whereas others occur in anabolic processes, such as in the pathway of purine synthesis or the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP).

One-carbon units can exist in various oxidation states. As discussed previously, biotin is the cofactor that carboxylases use to transfer CO_2, the most oxidized of the one-carbon units, to substrates such as pyruvate (to produce oxaloacetate). At the most reduced end of the oxidation–reduction spectrum, *S*-adenosylmethionine is the main donor of activated methyl groups for biosynthetic reactions. The body uses tetrahydrofolate (FH_4), the most reduced form of the vitamin folic acid, as the carrier of one-carbon groups in all the intermediate oxidation states of carbon and as the substrate for the oxidation or reduction of these one-carbon groups (Table 22-1). In addition, 5-methyl-FH_4 provides a source of methyl groups for the synthesis of methionine from homocysteine, thus providing a mechanism for replenishing the pool of methyl groups for methylation reactions that require *S*-adenosylmethionine. Transfer of a methyl group from methyl-FH_4 to homocysteine represents one of the two cofactor roles that vitamin B_{12} plays in humans; the other is as the cofactor in the reaction that converts methylmalonyl-CoA to succinyl-CoA.

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TABLE 22-1 One-Carbon Group Carried by Tetrahydrofolate

Oxidation Level	Group		Cofactor
Formic acid	Formyl	$-\overset{\overset{O}{\|}}{C}H-$	N^5-Formyl-FH_4, N^{10}-formyl-FH_4
	Methenyl	$-CH=$	N^5, N^{10}-Methenyl-FH_4
	Formimino	$-CH=NH$	N^5-Formimino-FH_4
Formaldehyde	Methylene	$-CH_2-$	N^5, N^{10}-Methylene-FH_4
Methanol	Methyl	$-CH_3$	N^5-Methyl-FH_4

FH_4, tetrahydrofolate.

22.2 LOCALIZATION OF REACTIONS INVOLVING ONE-CARBON TRANSFER

Although reactions involving one-carbon transfer occur in essentially all cells, they are especially prominent in the liver which is the major site of purine synthesis. Relatively high levels of enzymes that use tetrahydrofolate are also found in the brain, where one-carbon groups are used to maintain the pool of *S*-adenosylmethionine for the methylation reactions involved in both catecholamine synthesis and inactivation as well as to synthesize tetrahydrobiopterin, the cofactor for hydroxylation reactions of catecholamine and serotonin synthesis.

22.3 PHYSIOLOGICAL CONDITIONS IN WHICH ONE-CARBON METABOLISM IS ESPECIALLY ACTIVE

Because of the central role one-carbon metabolism plays in the synthesis of purines that are components of RNA and DNA and in the generation of thymidylate for DNA synthesis, it is most active during periods of rapid cellular growth, including embryogenesis and early postnatal development, and in rapidly dividing cells such as the intestinal epithelium and stem cells of both the erythropoietic and immune cell lineages.

22.4 REACTIONS OF ONE-CARBON METABOLISM

22.4.1 Absorption of Folate and Its Conversion to the Active Cofactor Form

Folic acid consists of three components: a pteridine ring, *para*-aminobenzoic acid (PABA), and glutamate (Fig. 22-1). Although humans can synthesize all of these components of the vitamin, they lack the enzyme required to join PABA to the pteridine ring. Natural forms of folic acid have a polyglutamate tail that contains an additional one to five glutamates joined in γ-peptide linkages. All but one of these glutamate residues is hydrolyzed sequentially in the small intestine by mucosal folate conjugase. Inside the enterocyte, folic acid is reduced to FH_4. The pathway

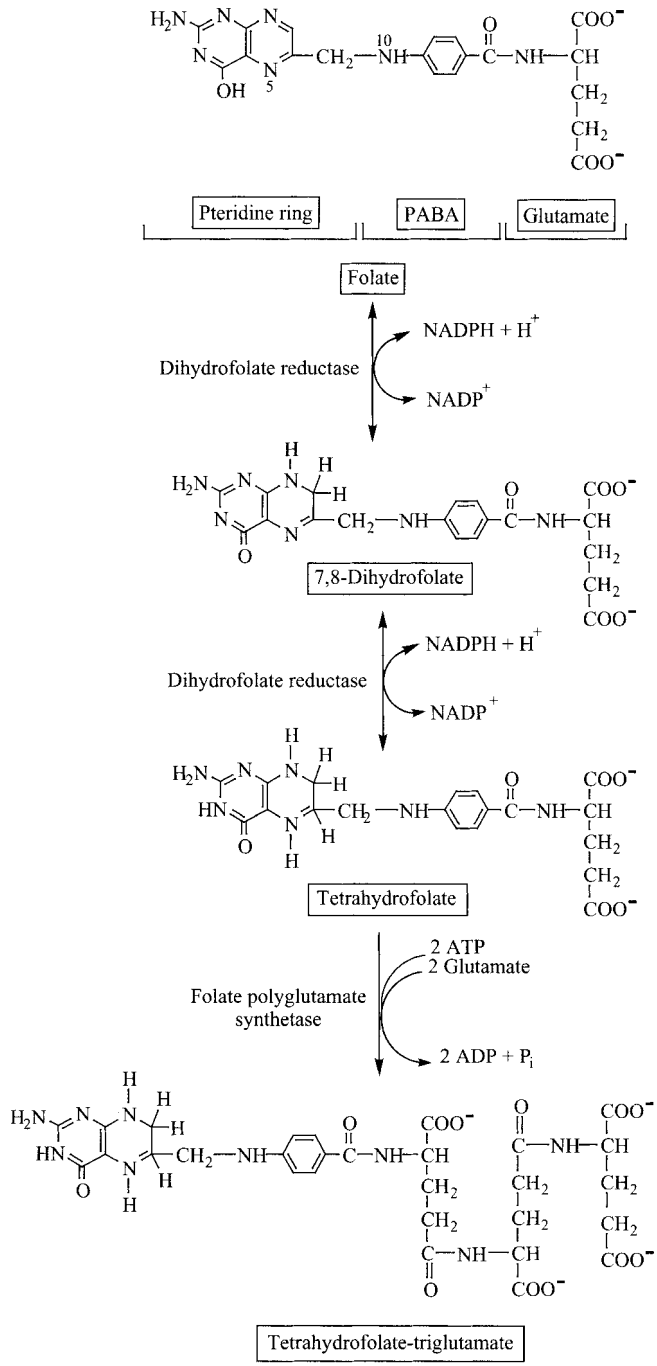

FIGURE 22-1 Structure of folate and its conversion to FH_4, the active form, and FH_4 polyglutamate, the form in which it is stored. PABA, *para*-aminobenzoic acid.

involves two successive reductions, both of which are catalyzed by dihydrofolate reductase, and both of which use NADPH as the reductant or hydrogen donor. After absorption from the intestine and transport of folate to the liver and other cells, the polyglutamate chain is restored by polyglutamate synthetase, trapping the active form of the cofactor within the cell. Subsequent release of the vitamin from hepatic stores into the blood requires hydrolysis of these additional glutamate residues by folate conjugase.

22.4.2 Oxidation States of the Folate One-Carbon Pool

FH_4 can carry one-carbon groups in several different oxidation states, including methyl ($-CH_3$), methylene ($-CH_2-$), and formyl ($-CHO$). Thus, there are several activated one-carbon carrying forms of FH_4, where the one-carbon group is covalently attached either to the N^5 or the N^{10} atom of FH_4, or forms a bridge between the N^5 and N^{10} atoms. Note that the reduction of N^5,N^{10}-methylene-FH_4 is irreversible:

$$N^5,N^{10}\text{-methylene-FH}_4 + \text{NADPH} + \text{H}^+ \rightarrow N^5\text{-methyl-FH}_4 + \text{NADP}^+$$

N^5-Methyl-FH_4 is the donor of methyl groups to vitamin B_{12}. Whenever transfer of a methyl group to vitamin B_{12} is blocked, FH_4 accumulates in the N^5-methyl-FH_4

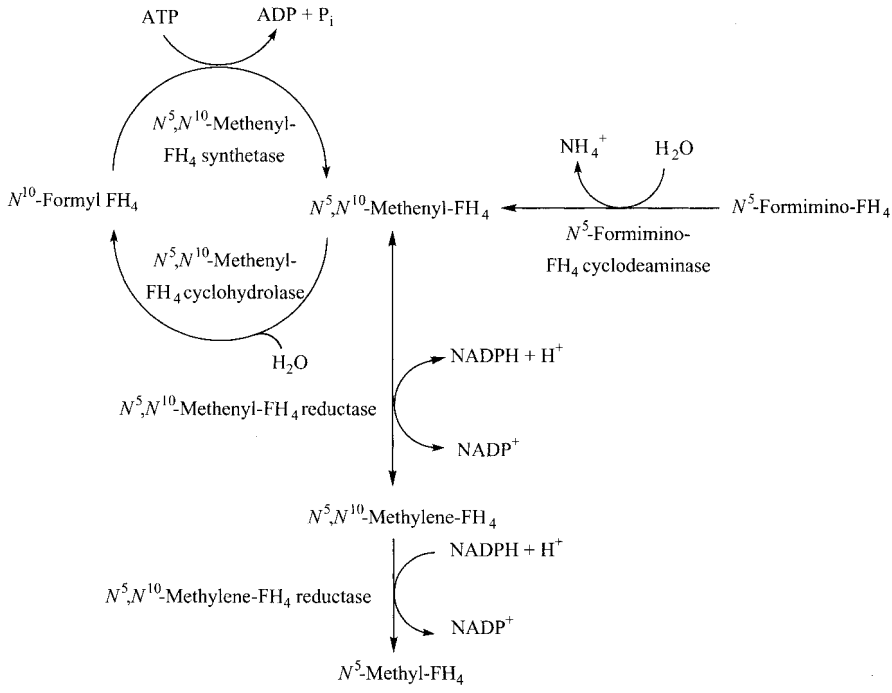

FIGURE 22-2 Interconversions of the one-carbon group attached to FH_4. The structures of the one-carbon units are shown in Table 22-1.

form and cannot be utilized for reactions requiring donation of one-carbon groups at other oxidation states. This phenomenon, referred to as the *methyltetrahydrofolate trap*, results in the depletion of the pool of FH_4 available for use in other pathways, such as purine synthesis.

22.4.3 Donors of One Carbon Groups to FH_4

22.4.3.1 Serine. The β-carbon atom of serine, which bears the hydroxyl group, is the major carbon source for the tetrahydrofolate one-carbon pool. Humans can synthesize serine from 3-phosphoglycerate, an intermediate in glycolysis, thereby providing a pathway for the utilization of carbohydrate-derived carbon atoms for biosynthetic reactions requiring one-carbon fragments (Fig. 22-3). Serine synthesis

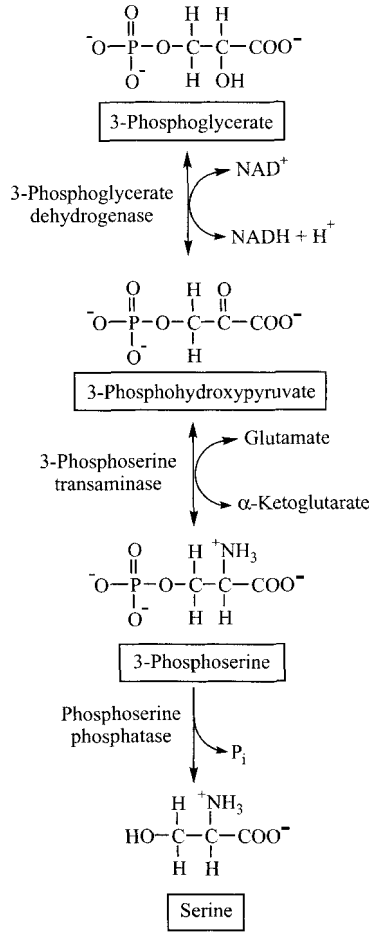

FIGURE 22-3 Synthesis of serine from the glycolytic intermediate 3-phosphoglycerate.

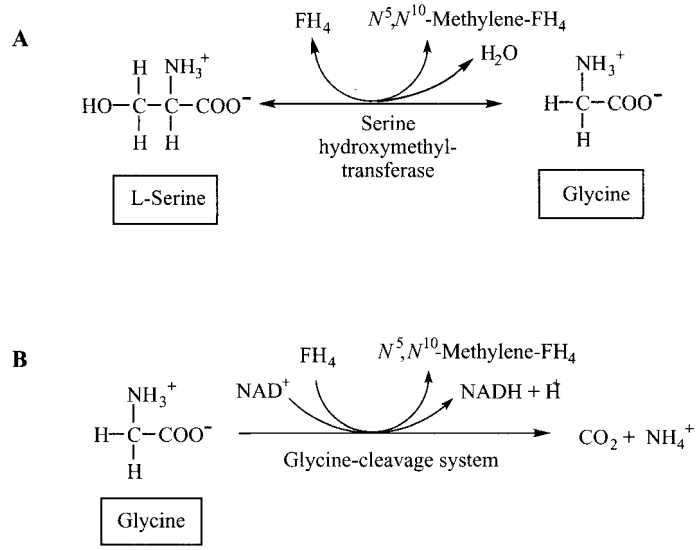

FIGURE 22-4 Reactions by which serine and glycine donate one-carbon units to tetrahydrofolate: (A) serine hydroxymethyltransferase; (B) the glycine-cleavage system.

involves the NAD^+-dependent reduction of 3-phosphoglycerate, transamination of 3-phosphohydroxypyruvate with glutamate serving as the amino group donor, and finally, hydrolysis of the phosphate from 3-phosphoserine.

The transfer of the carbon 3 of serine to tetrahydrofolate is catalyzed by serine hydroxymethyltransferase, which utilizes pyridoxal phosphate as a cofactor (Fig. 22-4A):

$$\text{serine} + FH_4 \rightleftharpoons N^5, N^{10}\text{-methylene-}FH_4 + \text{glycine}$$

22.4.3.2 Glycine. Glycine can donate a one-carbon unit to FH_4 in a reaction catalyzed by the glycine-cleavage system (Fig. 22-4B):

$$\text{glycine} + FH_4 + NAD^+ \rightleftharpoons N^5, N^{10}\text{-methylene-}FH_4 + CO_2 + NH_4^+ + NADH + H^+$$

The net effect of the two reactions shown in Figure 22-4 is to use two carbon atoms of serine to synthesize N^5, N^{10}-methylene-FH_4. Glycine can also be transaminated to form glyoxylic acid, which is then oxidized to oxalic acid (see Fig. 13-2). Excessive production of oxalic acid leads to the deposition of oxalate stones in the kidney.

22.4.3.3 Formate. Formic acid (HCOOH) is generated during the catabolism of tryptophan. It is also produced from ingested methanol by alcohol dehydrogenase

and aldehyde dehydrogenase. Formate can be incorporated into the one-carbon pool by formyltetrahydrofolate ligase:

$$\text{formate} + FH_4 + ATP \rightarrow N^{10}\text{-formyl-}FH_4 + ADP + P_i$$

22.4.3.4 Histidine. The pathway for the catabolism of histidine to *N*-formiminoglutamate (FIGLU) is shown in Figure 22-5. The formimino group of FIGLU is transferred to FH_4 by glutamate formiminotransferase:

$$N\text{-formiminoglutamate} + FH_4 \rightarrow N^5\text{-formimino-}FH_4 + \text{glutamate}$$

N^5,N^{10}-methenyl-FH_4 is then generated by deamination of N^5-formimino-FH_4 (Fig. 22-2):

$$N^5\text{-formimino-}FH_4 \rightarrow N^5,N^{10}\text{-methenyl-}FH_4 + NH_4^+$$

The complete pathway of histidine catabolism to *N*-formiminoglutamate is confined to the liver. Histidase, which catalyzes the first step of the pathway, is also present in skin, and urocanate (Fig. 22-5), the product of this reaction, is present in sweat.

22.4.4 Synthetic Reactions Utilizing One-Carbon Groups from Tetrahydrofolate Derivatives

22.4.4.1 Purines. A major role of FH_4 is to provide one-carbon units for synthesis of the ring structures of adenosine and guanine, the purine bases that are constituents of DNA and RNA. Synthesis of inosine monophosphate, the precursor of both AMP and GMP, involves donation of two separate formyl groups from N^{10}-formyl-FH_4 (see Chapter 23).

22.4.4.2 Deoxythymidylate (dTMP). The principal pyrimidine bases of RNA are uracil and cytosine. In DNA, however, the pyrimidine base thymine replaces uracil. Synthesis of the thymine base of deoxythymidylate is catalyzed by thymidylate synthase in a reaction that transfers a methyl group from N^5,N^{10}-methylene-FH_4 to deoxyuridylate (dUMP) (Fig. 22-6):

$$dUMP + N^5,N^{10}\text{-methylene-}FH_4 \rightarrow dTMP + FH_2$$

Note that in this reaction the methylene group is reduced to a methyl group and the FH_4 moiety is oxidized to FH_2. Dihydrofolate reductase regenerates FH_4:

$$FH_2 + NADPH + H^+ \rightarrow FH_4 + NADP^+$$

22.4.4.3 Serine. As indicated above, serine can donate a carbon atom to the one-carbon tetrahydrofolate pool, generating glycine (Fig. 22-4A). Since this reaction

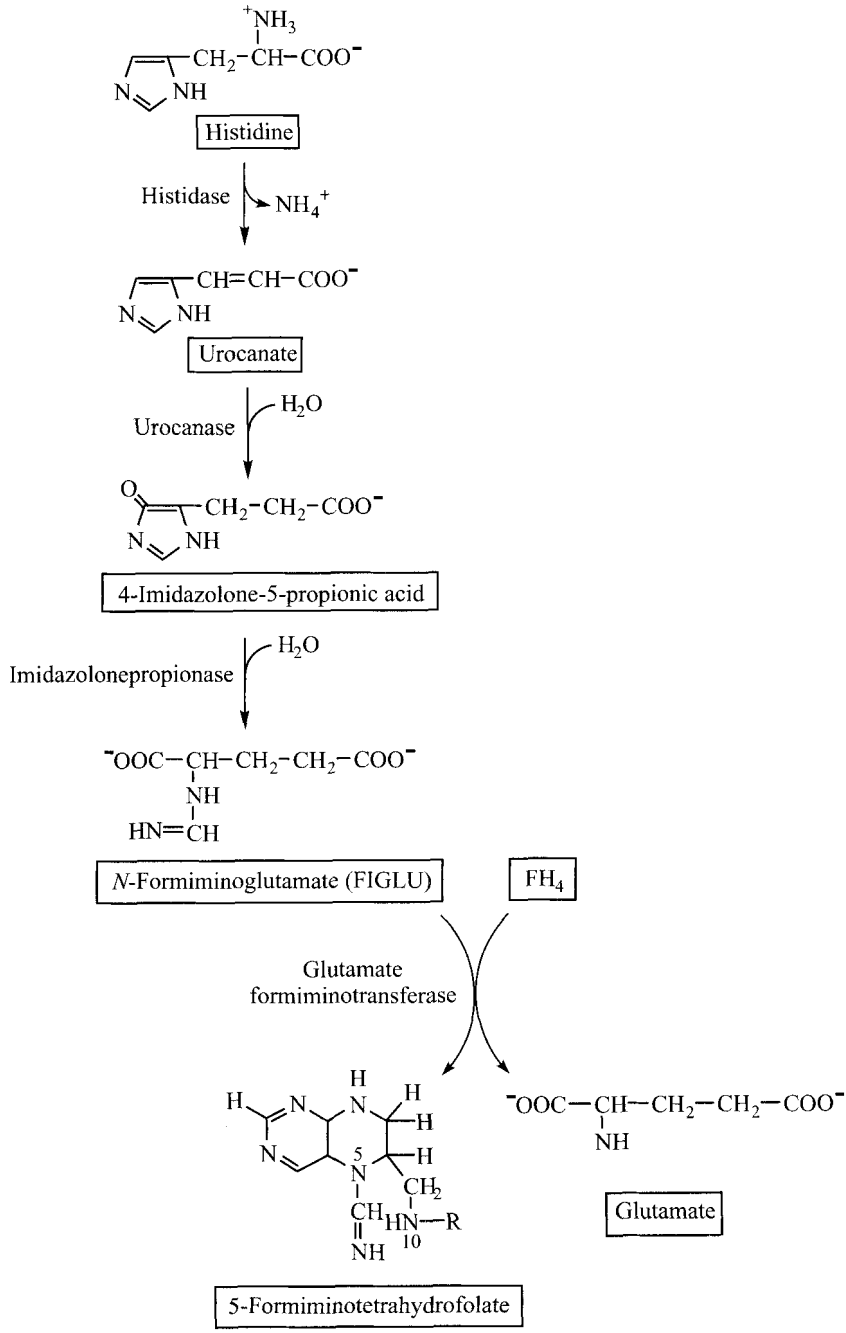

FIGURE 22-5 Metabolism of histidine results in the transfer of a formimino group to tetrahydrofolate. R, PABA-glutamate.

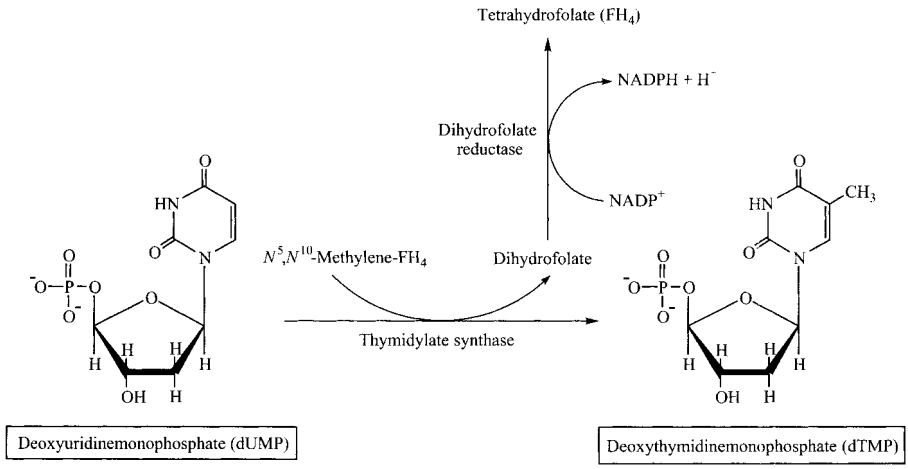

FIGURE 22-6 Synthesis of deoxythymidine monophosphate.

is reversible, when the level of N^5,N^{10}-methylene-FH_4 becomes high, it can also generate serine from glycine.

22.4.4.4 Methionine. Regeneration of methionine from homocysteine by methionine synthase (Fig. 22-7) requires methyl-B_{12} as a cofactor:

$$\text{homocysteine} + \text{methyl-B}_{12} \rightarrow \text{methionine} + \text{vitamin B}_{12}$$

Vitamin B_{12}, in turn, receives its methyl group from N^5-methyl-FH_4:

$$N^5\text{-methyltetrahydrofolate} + \text{vitamin B}_{12} \rightarrow \text{tetrahydrofolate} + \text{methyl-B}_{12}$$

This is the only reaction in the body that uses N^5-methyl-FH_4. Since the reduction of N^5,N^{10}-methylene-FH_4 to N^5-methyl-FH_4 is irreversible, lack of vitamin B_{12} to regenerate free tetrahydrofolate will "trap" the tetrahydrofolate as N^5-methyl-FH_4, thereby limiting the availability of tetrahydrofolate for other biosynthetic reactions.

22.4.5 Absorption of Vitamin B_{12}

Vitamin B_{12} (Fig. 22-8) is composed of a planar corin ring that is similar to the porphyrin ring structure of heme but contains cobalt instead of iron. In its active forms, the cobalt atom of vitamin B_{12} binds either a methyl or a deoxyadenosyl group. Most commercial vitamin B_{12} supplements have cyanide bound to the cobalt; they are rapidly converted in the body first to hydroxy-B_{12} and then ultimately to the active deoxyadenosyl-B_{12} or methyl-B_{12} coenzyme forms.

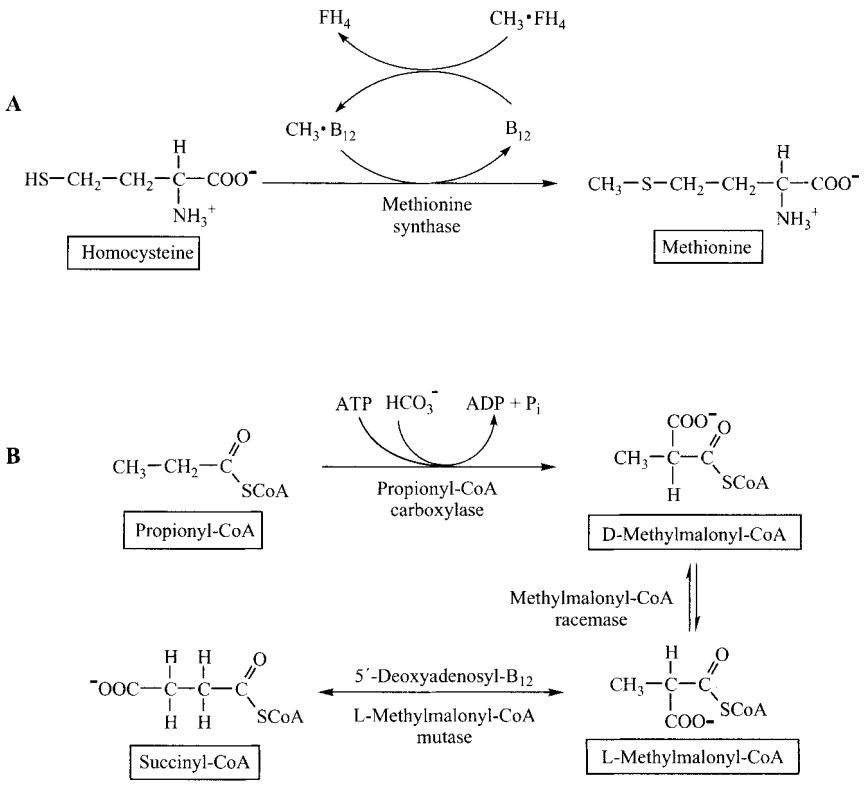

FIGURE 22-7 Two reactions catalyzed by vitamin B_{12}.

Utilization of dietary vitamin B_{12} is dependent on both gastric HCl and two specialized proteins, R proteins and intrinsic factor. Dietary vitamin B_{12} is covalently bound to polypeptides; release of vitamin B_{12} normally occurs in the stomach through the combined hydrolytic actions of HCl and pepsin. *R proteins* (also designated *haptocorrins* or *cobalophilins*) are present in both saliva and gastric juice. They bind the vitamin B_{12} prior to its release from the polypeptides, and remain associated with vitamin B_{12} until the R proteins are hydrolyzed in the small intestine.

Intrinsic factor (IF), a glycoprotein produced by the parietal cells of the stomach, is essential for the absorption of vitamin B_{12}. Intrinsic factor is so named because early studies demonstrated that both a dietary (extrinsic) factor and a protein produced by the normal stomach (intrinsic) were necessary for the prevention of pernicious anemia. As soon as vitamin B_{12} is released from the R proteins, it binds to intrinsic factor. The IF-B_{12} complex is then recognized by specific receptors, called *cubulins*, located primarily in the distal ileum. The major protein that transports vitamin B_{12} from the intestine to other tissues is transcobalamin II, which is a member of the R-protein family.

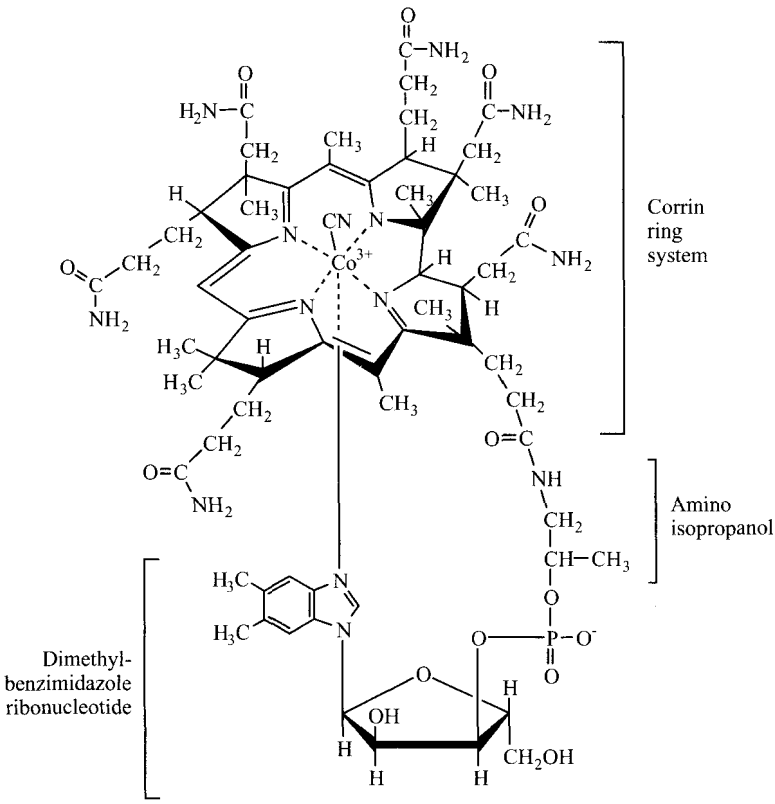

FIGURE 22-8 Structure of dietary vitamin B_{12}.

22.4.6 Reactions Utilizing Vitamin B_{12} as a Cofactor

Vitamin B_{12} is the precursor of two different cofactor forms that are involved in two very different metabolic reactions (Fig. 22-7). One, catalyzed by methionine synthase (a.k.a. homocysteine methyltransferase), uses methyl-B_{12} as a one-carbon donor. The other reaction is catalyzed by methylmalonyl-CoA mutase, which utilizes 5′-deoxyadenosyl-B_{12} as a cofactor and involves the transfer of a one-carbon unit within the molecule rather than between reactants.

22.4.6.1 Resynthesis of Methionine from Homocysteine. As described above, vitamin B_{12} is the cofactor through which the methyl group of N^5-methyl-FH_4 is transferred to homocysteine to regenerate methionine. Vitamin B_{12} is active in its reduced form, Cob(I). Over time a fraction of the Cob(I) oxidizes spontaneously to Cob(II). The catalytic activity of methionine synthase is regenerated by methionine synthase reductase, which uses *S*-adenosylmethionine to catalyze the reductive

methylation of vitamin B_{12}:

$$\text{Cob(II)} + \textit{S}\text{-adenosylmethionine} \rightarrow \text{methyl-Cob(I)} + \textit{S}\text{-adenosylhomocysteine}$$

In most cells, N^5-methyl-FH_4 is the only methyl donor for the synthesis of methyl-B_{12}. Liver cells, however, have a second form of homocysteine methyltransferase which can use betaine (trimethylglycine) as the methyl donor:

$$\text{betaine} + \text{homocysteine} \rightarrow \text{methionine} + \text{dimethylglycine}$$

Betaine is derived from choline when the latter is oxidized by choline oxidase. The betaine pathway is normally of minor importance in humans because we have a low level of choline oxidase. Betaine supplements, however, are effective in promoting methionine regeneration in patients with hyperhomocysteinemia.

22.4.6.2 Methylmalonyl-CoA Mutase. Deoxyadenosyl-B_{12} is a cofactor for methylmalonyl-CoA mutase, which catalyzes the reaction (Fig. 22-7B)

$$\text{methylmalonyl-CoA} \rightleftharpoons \text{succinyl-CoA}$$

This reaction is a key component of the pathway by which the carbon skeleton of propionyl-CoA is metabolized. Propionyl-CoA is generated when odd-chain fatty acids are oxidized and during the catabolism of the carbon skeletons of valine, isoleucine, and cysteine. The pathway by which propionyl-CoA is converted to succinyl-CoA is shown in Figure 22-7B. Subsequent metabolism of succinyl-CoA by means of the TCA cycle generates oxaloacetate, which can be used to synthesize glucose.

22.5 REGULATION OF ONE-CARBON METABOLISM

22.5.1 Regulation of N^5,N^{10}-Methylenetetrahydrofolate Reductase

As described in Chapter 21, *S*-adenosylmethionine (SAM) is an allosteric inhibitor of the reduction of N^5,N^{10}-methylene-FH_4 to N^5-methyl-FH_4. High concentrations of SAM inhibit the irreversible conversion of the folate one-carbon pool to a form that can only be used to regenerate methionine.

22.5.2 Serine Hydroxymethyltransferase

Because of the reversibility of the serine hydroxymethyltransferase (SHMT) reaction, it can either generate or consume N^5,N^{10}-methylene-FH_4. There are two SHMT isozymes, one cytosolic and the other mitochondrial. The cytosolic isozyme preferentially supplies one-carbon units for thymidylate synthesis. The cytosolic isozyme also binds 5-methyl-FH_4, thereby limiting the activity of methionine synthase. Thus,

the level of expression of the cytosolic SHMT gene appears to modulate competition between nucleotide synthesis and *S*-adenosylmethionine synthesis for the one-carbon units carried on FH_4.

22.6 ABNORMAL FUNCTIONING OF THE PATHWAYS OF ONE-CARBON METABOLISM

22.6.1 Folate Deficiency

A dietary deficiency of folate impairs one-carbon metabolism and preferentially impacts rapidly dividing cells, including the stem cells that generate erythrocytes, enterocytes, and cells of the immune system. The typical clinical presentation of folate deficiency is megaloblastic anemia. Lack of adequate nucleic acid synthesis results in decreased red cell number and release into the circulation of normochromic red blood cells, which are larger than normal (megaloblasts) due to impaired cell division. Persons with folate deficiency also often have decreased white cell counts. One measure of folate insufficiency is increased urinary excretion of *N*-formiminoglutamate (FIGLU) because of the absence of sufficient FH_4 for removal of the formimino group of FIGLU and formation of glutamine.

Inadequate folate levels during the first weeks of pregnancy increase the risk for congenital neural tube defects such as spina bifida. The fortification of grains with folate in both the United States and Canada is credited with a 15 to 30% decrease in the incidence of neural tube defects in recent years. Women who are able to become pregnant are advised to obtain 400 μg of folic acid daily from fortified foods, supplements, or both; the synthetic form of folate is preferred because it is more bioavailable than folate in foods.

Epidemiological studies indicate that lack of adequate dietary folate is also associated with increased incidence of other pathological conditions, including cardiovascular disease and colon cancer. As discussed in Chapter 21, hyperhomocysteinemia is a risk factor for cardiovascular disease. Lack of sufficient folate is one of the factors that can contribute to hyperhomocysteinemia; a deficiency of vitamin B_{12} (cobalamin) or vitamin B_6 (pyridoxine) can also result in hyperhomocysteinemia due to impaired remethylation of homocysteine or transsulfuration of homocysteine to generate cysteine, respectively. The increased cancer risk associated with folate deficiency appears to be the result of decreased methylation of DNA and to increased DNA damage secondary to incorporation of deoxyuridylate into DNA in the absence of an adequate supply of thymidylate for DNA synthesis.

22.6.2 Genetic Polymorphisms and Increased Risk of Neural Tube Defects

Mutations affecting many of the genes related to one-carbon metabolism have been described. For example, there are rare cases of methylenetetrahydrofolate reductase (MTHFR) deficiency which are characterized by severe hyperhomocysteinemia and

homocystinuria. There are also more common genetic polymorphisms of MTHFR that are associated with only partial loss of enzymatic activity. The most prevalent of these is C677T, where there is an alanine-to-valine substitution in MTHFR, resulting in a thermolabile enzyme. The 10 to 20% of the population in the United States who are homozygous for the 677TT genotype have approximately 33% of normal enzyme activity and exhibit only mild to moderate hyperhomocysteinemia. This particular polymorphism is associated with an increased risk of neural tube defects, which can be prevented by supplementation with larger amounts of folate than is required by people with the CC or CT genotype.

22.6.3 Use of Folate Analogs for Chemotherapy

Rapidly dividing cells require high levels of DNA and RNA synthesis. For this reason, drugs that reduce the availability of FH_4 for one-carbon metabolism have proven useful chemotherapeutic agents for treating cancers. Methotrexate and aminopterin are two structurally similar analogs of FH_2 that are potent competitive inhibitors of dihydrofolate reductase. Dihydrofolate reductase inhibitors also impair the functioning of normal, rapidly dividing cells such as the stem cells in bone marrow, hair follicles, and enterocytes, thus producing a variety of adverse side effects.

22.6.4 Pernicious Anemia

Pernicious anemia is a severe megaloblastic anemia that results from inadequate tissue levels of vitamin B_{12}. The underlying problem is a lack of intrinsic factor production by the stomach. Pernicious anemia is due principally to an autoimmune gastritis in which the blood contains antibodies against intrinsic factor and other proteins of the parietal cells. These antibodies damage the patient's mucosa and abolish the secretion of both intrinsic factor and HCl.

The hematological presentation of pernicious anemia is indistinguishable from that of folic acid deficiency. Vitamin B_{12} deficiency results in an inability to transfer the methyl group from 5-methyl-FH_4 to homocysteine to form methionine. Since the methyl group of 5-methyl-FH_4 cannot be oxidized to 5′,10′-methylene-FH_4 or other one-carbon folate derivatives, the FH_4 pool becomes "trapped" as 5-methyl-FH_4 in vitamin B_{12}–deficient persons. This, in turn, diminishes the availability of FH_4 for nucleotide synthesis, resulting in megaloblastic anemia.

With time, pernicious anemia can result in progressive neurological degeneration, which in its later stages may present with tingling or numbness of the extremities and diminished reflexes. Neurological damage may be due to insufficient 5′-deoxyadenosyl-B_{12} to support the methylmalonyl-CoA mutase reaction, resulting in a buildup of methylmalonic acid. When present at elevated concentrations, methylmalonyl-CoA can substitute for malonyl-CoA in fatty acid synthesis, leading to the synthesis of branched-chain fatty acids, which are incorporated into phospholipids of the myelin sheath. Alternatively, the neurological symptoms may be due to inadequate amounts of *S*-adenosylmethionine in neural tissues.

Urinary excretion of methylmalonic acid and propionic acid provides a laboratory means for distinguishing vitamin B_{12} deficiency from folic acid deficiency. Vitamin B_{12} deficiency can also be detected by abnormally low serum vitamin B_{12} levels or by elevated blood levels of methylmalonic acid. For many years the Schilling test was used to distinguish dietary vitamin B_{12} deficiency from intrinsic factor deficiency. In this test, the patient is given an oral dose of radioactive cobalt-labeled vitamin B_{12}, followed by an intramuscular injection of nonradiolabeled vitamin B_{12}. The injected vitamin B_{12} saturates the vitamin B_{12}–binding sites in tissues and promotes urinary excretion of absorbed radioactive vitamin B_{12}; excretion of 10% or more of the labeled vitamin B_{12} in 24 hours indicates normal absorption, whereas less than 5% excretion indicates malabsorption.

Since the underlying deficit in pernicious anemia is malabsorption of vitamin B_{12}, treatment involves intramuscular vitamin B_{12} injections rather than oral vitamin therapy. The hematological symptoms of pernicious anemia can also be ameliorated with folate supplements, which replenish the FH_4 pool. Therapeutic doses of folate are, however, ineffective in preventing the ongoing and eventually irreversible neurological degeneration. Thus, folic acid supplements may mask the underlying vitamin B_{12} deficiency and, in some cases, even exacerbate it by promoting utilization of vitamin B_{12} by cells in the bone marrow rather than in neural tissues.

22.6.5 Other Causes of Vitamin B_{12} Deficiency

The term *pernicious anemia* is usually reserved for the pathology that results from a lack of gastric intrinsic factor. There are, however, other conditions that can result in poor vitamin B_{12} status, as described below.

22.6.5.1 Dietary Deficiency of B_{12}. Nutritional deficiency of vitamin B_{12} is relatively rare but can occur in people who consume vegan diets. Plants do not contain vitamin B_{12} unless they are contaminated by bacteria. For people who consume a normal diet with ample intake of animal products, the large stores of vitamin B_{12} in the liver provide protection for up to 5 to 7 years of reduced vitamin B_{12} intake or absorption.

22.6.5.2 Insufficient Gastric Acid. The proteolytic activity of pepsin is required to release food-bound vitamin B_{12}. Many forms of gastritis, including those resulting from *Helicobacter pylori* infection and AIDS, impair both pepsinogen secretion and the availability of acid for the conversion of pepsinogen to pepsin. Hypochlorhydria (decreased stomach acid) can also result from prolonged use of proton-pump inhibitors.

22.6.5.3 Absent or Diseased Ileal Mucosa. As described earlier, the vitamin B_{12}–intrinsic factor complex is absorbed by a receptor-mediated process in the terminal ileum. Ilial resection or mucosal disease such as Crohn's disease will impair absorption of the vitamin.

22.6.5.4 Impaired Translocation of Vitamin B_{12}. Transcobalamin II (TCII), the protein that transports newly absorbed vitamin B_{12}, is synthesized in both the ileum and the liver. Genetic defects resulting in lack of functional TCII produce biochemical and hematological signs of vitamin B_{12} deficiency in early infancy.

CHAPTER 23

PURINES AND PYRIMIDINES

23.1 FUNCTIONS OF PURINES AND PYRIMIDINES

23.1.1 Purines and Pyrimidines Play a Key Structural and Informational Role in DNA and RNA

Both DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) contain purine and pyrimidine bases attached to sugar phosphates. The backbones of DNA and RNA are composed of sugar phosphate polymers (deoxyribose phosphate and ribose phosphate, respectively), while the purine and pyrimidine bases provide the specific information of the genetic code.

23.1.1.1 Structures of Purines. Purines (adenine and guanine) contain two fused heterocyclic, nitrogen-containing rings (Fig. 23-1). They are components of both DNA and RNA.

23.1.1.2 Structures of Pyrimidines. Pyrimidines are six-membered, heterocyclic, nitrogen-containing carbon structures. The pyrimidine bases cytosine and thymine are found in DNA. RNA also contains cytosine but has uracil in lieu of thymine (Fig. 23-2).

23.1.1.3 Nucleosides and Nucleotides. When purine or pyrimidine bases are attached to pentoses (ribose or deoxyribose), they are called *nucleosides* (Fig. 23-3), and when they are attached to ribose or deoxyribose that has one or more

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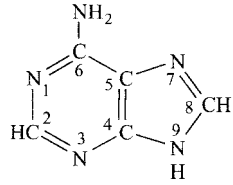
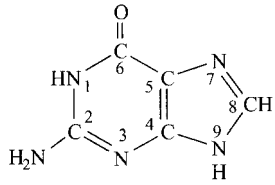


Adenine



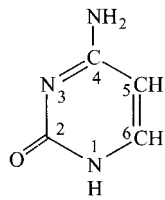
Guanine

FIGURE 23-1 Purine bases.

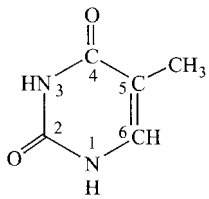
phosphates attached to the 5′-hydroxyl group of the pentoses, they are called *nucleotides*. Thus, the nucleoside adenosine is formed by attachment of adenine to ribose, and deoxyadenosine is formed by attachment of adenine to deoxyribose. Adenine linked to ribose 5-phosphate is adenosine 5′-phosphate (AMP), and adenine linked to deoxyribose 5′-phosphate is deoxyadenosine 5′-phosphate (dAMP). The adenine nucleotides are sometimes called *adenylates*.

23.1.2 Energy Metabolism

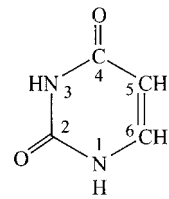
Purine nucleoside triphosphates are at the heart of energy metabolism. ATP is the high-energy phosphoanhydride product of both glycolysis and oxidative phosphorylation. ATP is also the main source of energy for metabolic work, including biosynthesis, active ion transport, muscle contraction, and detoxification of xenobiotics.



Cytosine



Thymine



Uracil

FIGURE 23-2 Pyrimidine bases.

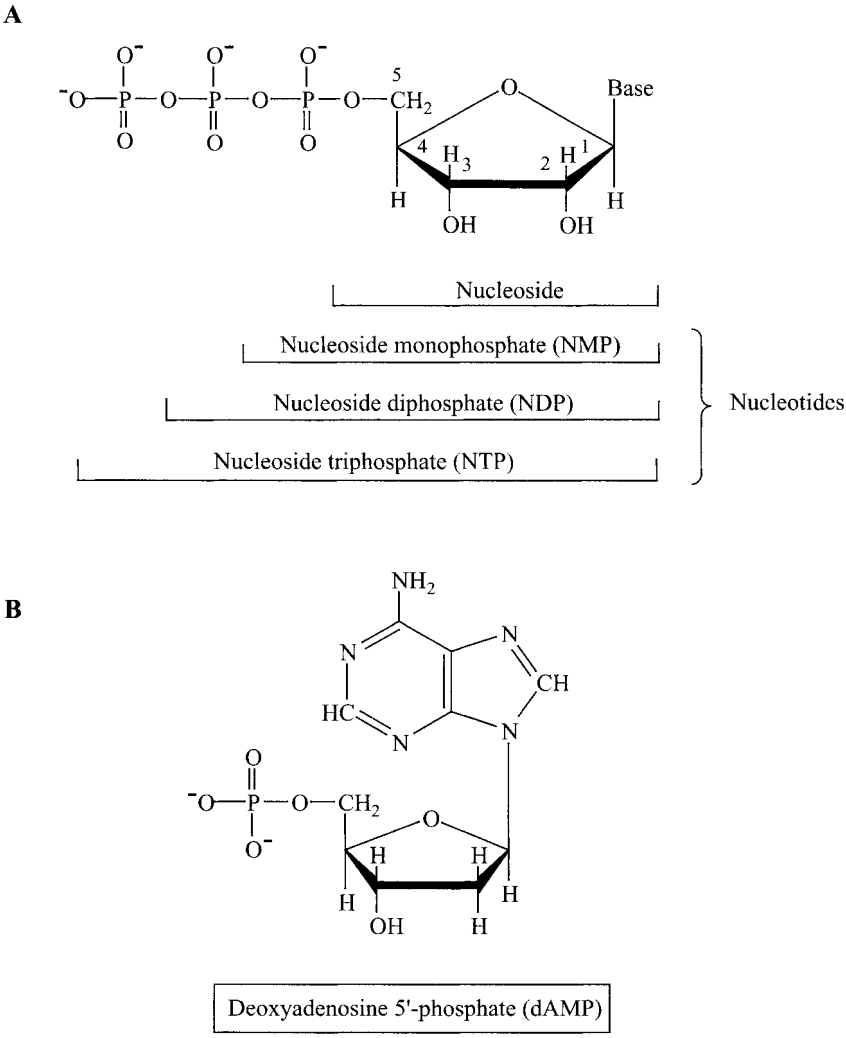


FIGURE 23-3 Structures of nucleoside and nucleotides: (A) structures of a generic ribonucleoside and related phosphorylated ribonucleotides; (B) structure of the deoxynucleotide, deoxyadenosine 5′-phosphate.

Other ribonucleoside triphosphates also participate directly in metabolic pathways. GTP, which is produced directly in the tricarboxylic acid cycle, provides energy for protein synthesis and for certain biosynthetic reactions, such as that catalyzed by phosphoenolpyruvate carboxykinase during gluconeogenesis. Uridine nucleotide-activated monosaccharides such as UDP-glucose and UDP-glucuronic acid are involved in the synthesis of polysaccharides, lactose, and the oligosaccharide chains of glycoproteins, and in glucuronidation reactions involved in detoxification processes

(e.g., bilirubin conjugation; Chapter 24). Several CTP-activated substrates, including CDP-choline, CDP-ethanolamine, and CDP-diacylglycerol, are intermediates in glycerophospholipid synthesis.

The GTP, CTP, and UTP required for energy metabolism are generated from ATP by reactions catalyzed by nucleoside diphosphate kinase: for example,

$$\text{GDP} + \text{ATP} \rightleftharpoons \text{GTP} + \text{ADP}$$

23.1.3 Components of Cofactors

Adenosine monophosphate (AMP) is a core element in the structure of the major enzymatic cofactors of oxidation–reduction reactions, including NAD^+/NADH, $NADP^+$/NADPH, and FAD/$FADH_2$. FAD/$FADH_2$ and FMN/$FMNH_2$ are also key components of several of the complexes of the mitochondrial electron transport chain. Adenine nucleotides are components of coenzyme A, which is involved in the synthesis of bile acids and the catabolism of branched-chain amino acids as well as providing the activated form of acetate and fatty acids for both biosynthetic and catabolic reactions. In addition, *S*-adenosylmethionine (SAM) is the activated form of methionine, which functions as a methyl donor in the synthesis of epinephrine, phosphatidylcholine, and other methyl group–containing compounds.

23.1.4 Activation of G Proteins

Guanine nucleotides bind to and regulate the activity of G proteins, which are important components of intracellular signal-transduction cascades. The heterotrimeric G proteins are activated by G protein–coupled receptors, such as the receptors for glucagon, epinephrine, and various prostaglandins. The smaller monomeric G proteins, such as Ras, are components of the MAP kinase (mitogen-activated protein kinase) cascade, which is linked to receptor tyrosine kinases such as the epidermal growth factor receptor. Both classes of G proteins become active when the GDP bound to them is exchanged for a GTP molecule. Hydrolysis of the bound GTP to GDP (and P_i) returns the G protein to its inactive state.

23.1.5 Activation of Protein Kinases

The cyclic purine nucleotides cAMP and cGMP are second messengers that regulate numerous metabolic processes. cAMP-dependent protein kinase A (PKA) is the major mediator by which glucagon and epinephrine regulate multiple aspects of energy metabolism, including gluconeogenesis, glycogen mobilization, and lipolysis in adipocytes. cGMP activates protein kinase G and thus provides the mediator by which nitric oxide (NO) triggers muscle relaxation.

AMP-activated protein kinase (AMPK) plays an important role in energy homeostasis. Whereas cAMP and cGMP are generated in response to hormonal and autocoid signals, increases in cellular AMP occur when the cell becomes depleted

of ATP. AMPK-mediated protein phosphorylation inhibits pathways that require ATP, such as fatty acid and cholesterol synthesis, and activate pathways such as fatty acid oxidation that generate ATP. The activation of AMPK also exerts long-term effects on gene expression and protein synthesis.

23.1.6 Extracellular Signaling Molecules

Adenosine, ATP, and diadenosine polyphosphates (two adenosines coupled via ester bonds to three to six phosphates, Ap_nA) are neurotransmitters that activate certain classes of G protein–coupled receptors. ATP also activates cation-permeable ligand-gated ion channels. Although these various receptors are all called purinergic receptors, some are also activated by UTP, a pyrimidine triphosphate.

23.2 LOCALIZATION OF PURINE AND PYRIMIDINE METABOLISM

Pyrimidine synthesis occurs in a variety of tissues, including spleen, thymus, testes, and intestinal enterocytes. By contrast, de novo synthesis of purines is active primarily in liver. Nonhepatic tissues generally rely on preformed purines salvaged from intracellular turnover and purines synthesized by the liver. The *purine salvage pathway* is particularly important in the brain, where it serves to regenerate adenine nucleotides from the neurotransmitter adenosine.

The end product of purine catabolism in humans is uric acid, a relatively insoluble substance that is excreted in the urine. The final steps of uric acid synthesis occur only in the liver and intestine.

23.3 PATHWAYS OF NUCLEOTIDE METABOLISM

Purine and pyrimidine bases are mostly synthesized in the body, with dietary sources contributing less than 1% to the body's needs. The reactions involved in the de novo synthesis of purines and pyrimidines take place in the cytosol and mitochondria, and the enzymes of each pathway tend to function as components of multienzyme complexes. The two pathways differ primarily in that the purine base is constructed sequentially on phosphoribosyl-1-pyrophosphate (PRPP) (Fig. 23-4), whereas the pyrimidines are synthesized as free bases which are then attached to PRPP.

23.3.1 Synthesis of the Sugar Moieties

The two pentoses found in nucleotides are ribose and deoxyribose. Ribose, generated as ribose 5-phosphate via the pentose phosphate pathway, is activated to 5-phosphoribosyl 1-pyrophosphate (PRPP), which is used to synthesize ribonucleotides. Deoxyribose is synthesized by oxidizing the ribose moiety of ribonucleotides.

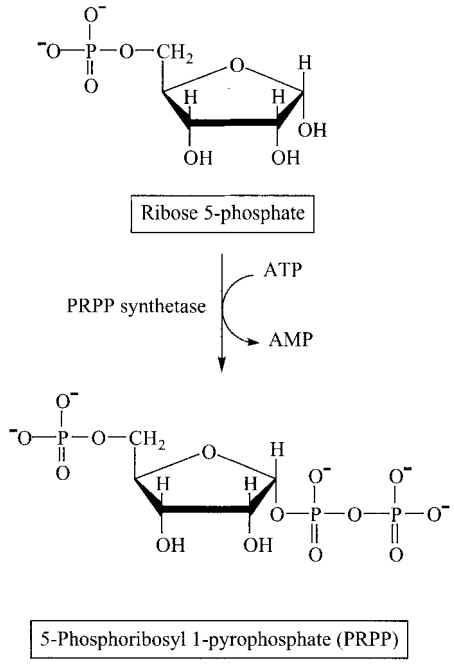


FIGURE 23-4 Synthesis of 5-phosphoribosyl 1-pyrophosphate.

23.3.1.1 Synthesis of 5-Phosphoribosyl 1-Pyrophosphate. PRPP synthetase, also called ribose phosphate pyrophosphokinase, catalyzes the reaction that attaches a pyrophosphate moiety to carbon-1 of the ribose molecule (Fig. 23-4):

$$\text{ribose 5-phosphate} + \text{ATP} \rightarrow \text{5-phosphoribosyl 1-pyrophosphate} + \text{PP}_i$$

23.3.1.2 Synthesis of Deoxyribose. The typical cell contains 5 to 10 times as much total RNA (mRNAs, rRNAs, and tRNAs) as DNA. Therefore, the bulk of nucleotide biosynthesis has as its purpose the production of ribonucleoside triphosphates (NTPs). However, because proliferating cells must replicate their genomes, the production of deoxyNTPs (dNTPs) is also necessary for the synthesis of DNA. Nucleoside diphosphates are the substrate for the reduction of carbon 2 of ribose to deoxyribose; the reaction is catalyzed by ribonucleotide reductase (Fig. 23-5):

$$\text{NDP} + \text{NADPH} + \text{H}^+ \rightarrow \text{deoxy-NDP} + \text{NADP}^+$$

The subsequent phosphorylation of dNDPs to dNTPs is catalyzed by nucleoside diphosphate kinases that use ATP as the phosphate donor:

$$\text{deoxy-NDP} + \text{ATP} \rightleftharpoons \text{deoxy-NTP} + \text{ADP}$$

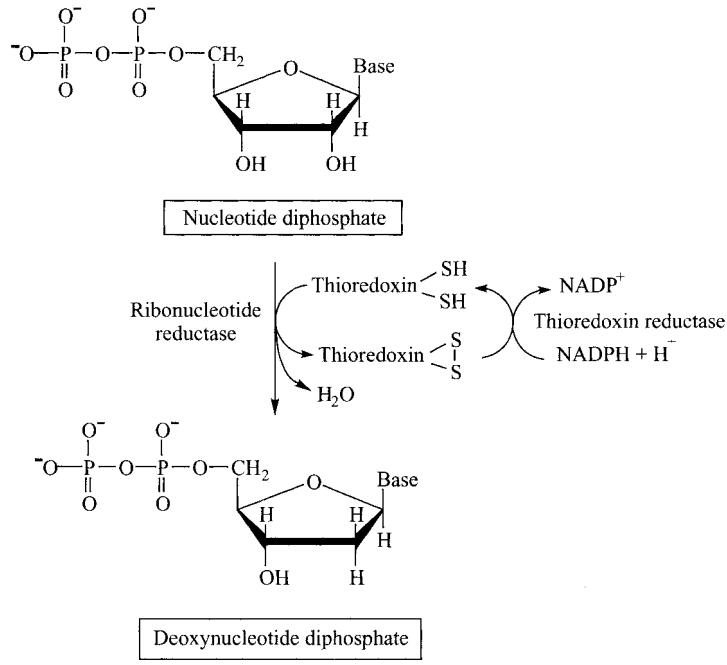


FIGURE 23-5 Role of ribonucleotide reductase in the generation of deoxynucleotides. The reaction results in the oxidation of thioredoxin (shown) or glutaredoxin (not shown).

Ribonucleotide reductase is a multifunctional enzyme complex that contains redox-active thiol groups. In the process of reduction of NDPs, the thiol groups become oxidized. They are then reduced by either one of two low-molecular weight proteins, thioredoxin or glutaredoxin. Oxidized thioredoxin, in turn, is reduced by the flavoprotein thioredoxin reductase (Fig. 23-5), while oxidized glutaredoxin is reduced by the combined action of glutathione and glutathione reductase. In both cases, NADPH is the ultimate reductant.

23.3.2 De Novo Synthesis of Purines

23.3.2.1 Synthesis of Inosine Monophosphate. Starting with PRPP, 10 steps are required for the de novo synthesis of inosine monophosphate (IMP), which is the common precursor for both adenosine monophosphate (AMP) and guanosine monophosphate (GMP) (Fig. 23-6). The multienzyme complex that carries out de novo purine synthesis is called the *purinosome*. The names of the enzymes and intermediates in the de novo purine pathway are cumbersome and complex; therefore, except for the steps that are regulated or the ones that are implicated in a particular human disease, there is little need for a medical student to focus on all the reactions.

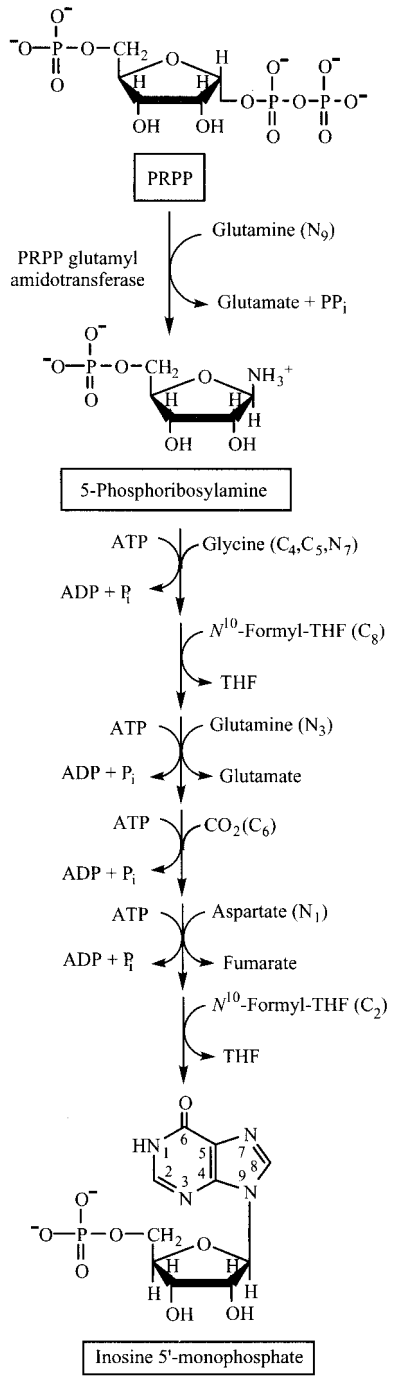


FIGURE 23-6 Synthesis of inosine 5′-monophosphate. The subscripts after C and N indicate the location of the atom in the purine ring.

However, it is worth noting several important features of the de novo synthesis of IMP from PRPP:

- Four of the 10 enzymes utilize ATP, thereby underscoring the fact that de novo purine synthesis is a costly process. Additional ATP is required for the synthesis of PRPP from ribose 5-phosphate.
- Two of the four nitrogen atoms in the ring structure of inosine are derived from glutamine and one each are derived from glycine and aspartate (Fig. 23-6).
- Two enzymes utilize N^{10}-formyl-FH_4 to donate carbon atoms to the purine rings. These reactions underscore the dependency of nucleic acid synthesis on adequate dietary folate. Inadequate folate impairs cell division, as exemplified by the megaloblastic anemia associated with folate deficiency. Inhibitors of dihydrofolate reductase such as methotrexate and aminopterin are useful cancer chemotherapeutic agents.

23.3.2.2 Synthesis of AMP and GMP from IMP. Synthesis of both the adenine and guanine ring structures from IMP involves addition of an amino group to the ring structure (Fig. 23-7). The amino group of adenine is derived from aspartate, whereas glutamine is the source of the amino group of guanine. Both of these reactions require energy: GTP is required for the conversion of IMP to AMP, and ATP is consumed in the conversion of IMP to GMP.

23.3.3 Catabolism of Purines

The catabolism of purines is an especially important process in humans, in part at least, because the pathway ends in uric acid, the accumulation of which causes gout. Unlike most other mammals, humans lack the enzyme urate oxidase, which converts uric acid to allantoin, a more soluble end product than urate.

GMP catabolism is initiated by a 5′-nucleotidase, which converts GMP to guanosine:

$$\text{GMP} + \text{H}_2\text{O} \rightarrow \text{guanosine} + \text{P}_\text{i}$$

The release of ribose from the purine base is catalyzed by purine nucleoside phosphorylase:

$$\text{guanosine} + \text{P}_\text{i} \rightarrow \text{guanine} + \text{ribose 1-phosphate}$$

Guanine is then deaminated to xanthine (Fig. 23-8):

$$\text{guanine} \rightarrow \text{xanthine} + \text{NH}_4^+$$

There are two alternative pathways for catabolism of AMP (Fig. 23-8), both of which involve deamination of the adenosine moiety to inosine and hydrolysis of

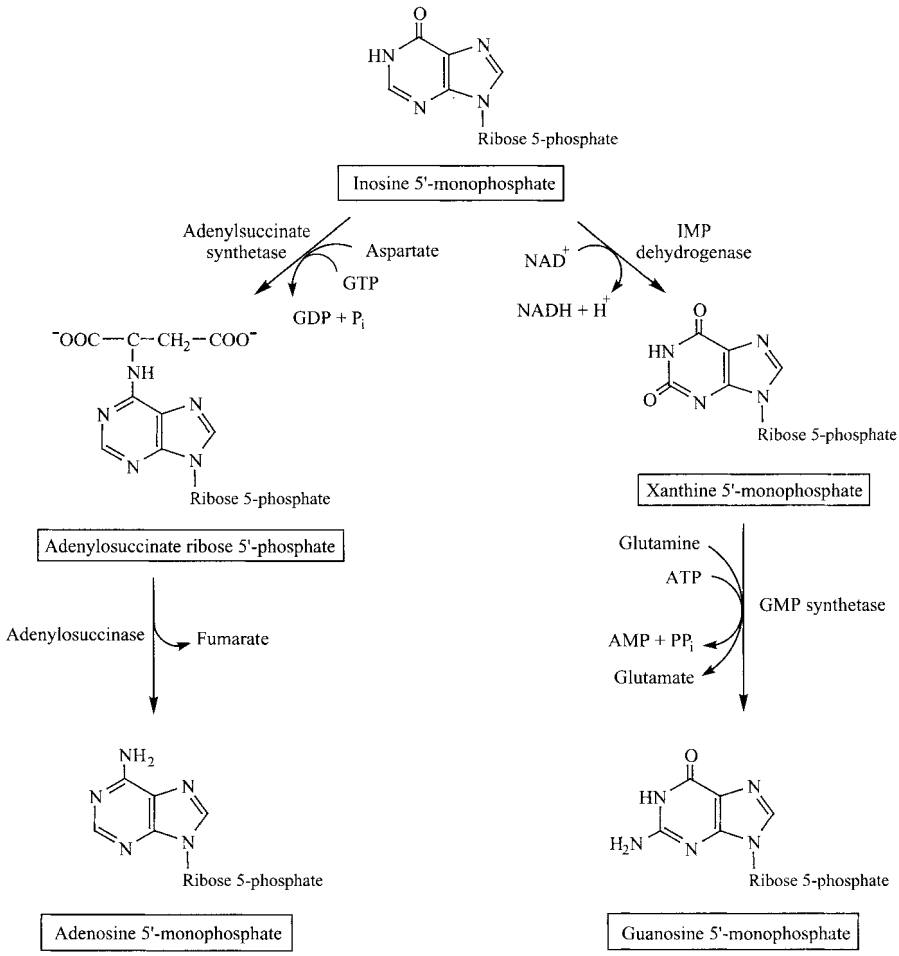


FIGURE 23-7 Synthesis of adenosine 5′-monophosphate and guanosine 5′-monophosphate from inosine 5′-monophosphate.

the nucleoside monophosphate to a nucleoside. The major route of AMP catabolism involves generation of inosine monophosphate (IMP) by AMP deaminase (a.k.a. adenosine monophosphate deaminase), followed by removal of the phosphate group by 5′-nucleotidase. The AMP deaminase reaction is also part of the purine nucleotide cycle discussed below and shown in Figure 23-9. In the alternative pathway of AMP catabolism, 5′-nucleotidase converts AMP to adenosine, which is then deaminated by adenosine deaminase. Purine nucleoside phosphorylase then catalyzes release of the ribose moiety:

$$\text{inosine} + P_i \rightarrow \text{hypoxanthine} + \text{ribose 5-phosphate}$$

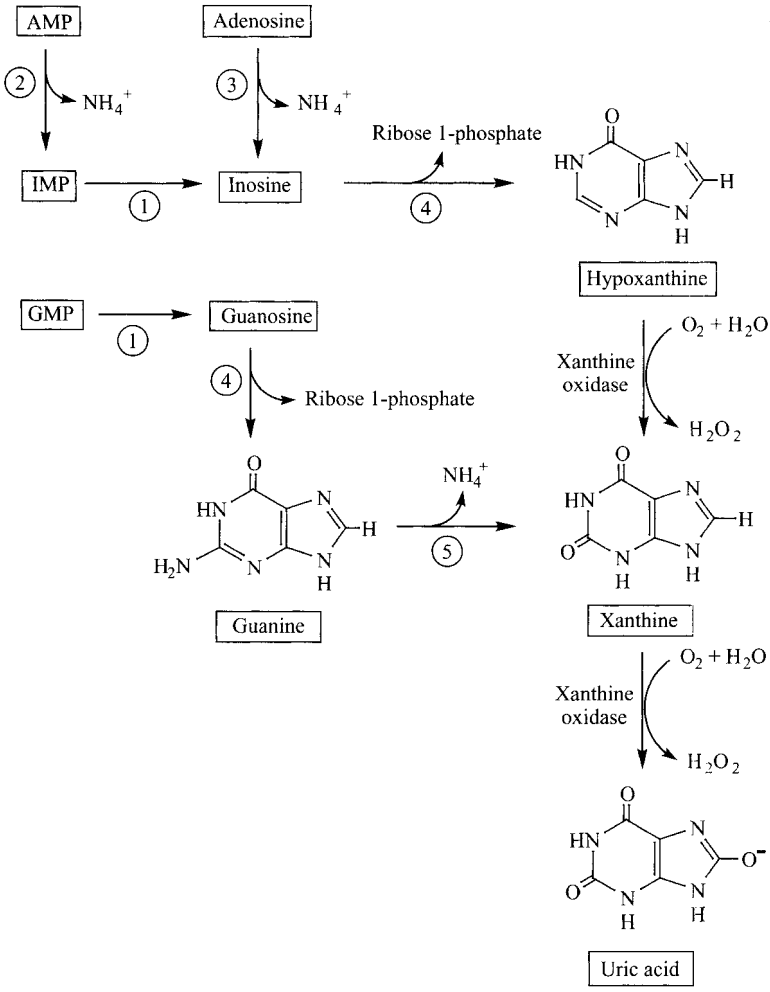


FIGURE 23-8 Catabolism of purine nucleotides: ①, 5′-nuclease; ②, AMP deaminase; ③, adenosine deaminase; ④, purine nucleoside phosphorylase; ⑤, guanine deaminase.

The catabolism of the deoxynucleotides dAMP and dGMP follow the same pathways as those that catabolize AMP and GMP, respectively, with the release of deoxyribose rather than ribose.

The final enzyme in the pathway of purine catabolism is xanthine oxidase, a molybdenum-containing flavoenzyme that uses molecular oxygen and produces hydrogen peroxide. Xanthine oxidase oxidizes both hypoxanthine and xanthine (Fig. 23-8):

$$\text{hypoxanthine} + O_2 \rightarrow \text{xanthine} + H_2O_2$$

$$\text{xanthine} + O_2 \rightarrow \text{uric acid} + H_2O_2$$

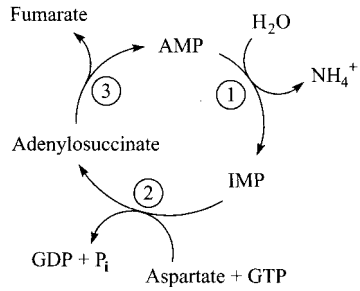


FIGURE 23-9 Purine nucleotide cycle: ①, AMP deaminase; ②, adenylosuccinate synthase; ③, adenylosuccinase. AMP, adenosine 5′-monophosphate; IMP, inosine 5-monophosphate.

23.3.4 Salvage of Purines

As described above, de novo purine synthesis involves the stepwise assembly of the purine base on PRPP. When free purines are released during nucleotide catabolism, they can be salvaged by reattachment of the bases to PRPP. The key enzyme in the salvage of purines is hypoxanthine-guanine phosphoribosyltransferase (HGPRTase), which utilizes both guanine and hypoxanthine, the product of adenine deamination:

$$\text{guanine} + \text{PRPP} \rightarrow \text{guanosine monophosphate} + PP_i$$

$$\text{hypoxanthine} + \text{PRPP} \rightarrow \text{inosine monophosphate} + PP_i$$

The IMP generated by HGPRTase is then converted to AMP by enzymes of the de novo pathway.

A second phosphoribosyl transferase, adenine phosphoribosyltransferase, uses adenine as a substrate:

$$\text{adenine} + \text{PRPP} \rightarrow \text{adenosine monophosphate} + PP_i$$

However, since the major pathway for the catabolism of adenosine generates inosine and then hypoxanthine, salvage of adenine is of only minor physiological importance. Unlike guanosine, adenosine can also be phosphorylated directly back to the level of a nucleotide by adenosine kinase:

$$\text{adenosine} + \text{ATP} \rightleftharpoons \text{AMP} + \text{ADP}$$

23.3.5 Purine Nucleotide Cycle

During exercise, there is increased catabolism of AMP to IMP in skeletal muscle. Multiple bouts of sprint exercise result in the loss of adenine nucleotides from muscle and subsequent urinary excretion of increased amounts of purine metabolites,

including inosine, hypoxanthine, and uric acid. However, when oxidative metabolism is not stressed, most of the IMP is converted back to AMP and then to ADP and ATP (Fig. 23-9). This process, called the *purine nucleotide cycle*, serves several interrelated functions, discussed below.

23.3.5.1 Generation of ATP. During high-intensity exercise, cytosolic ATP is rapidly converted to ADP. Additional ATP can be generated directly from ADP by myokinase (adenylate kinase):

$$2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$$

The reversible myokinase reaction is driven to the right by the action of AMP deaminase, which removes AMP:

$$\text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3$$

AMP deaminase is expressed at high levels in skeletal muscle and is associated with myofibrils.

23.3.5.2 Generation of Ammonium Ions for Glutamine Synthesis. Aspartate is the nitrogen donor in the pathway that generates AMP from IMP:

$$\text{IMP} + \text{aspartate} + \text{GTP} \rightarrow \text{adenylosuccinate} + \text{GDP} + \text{P}_i$$

$$\text{adenylosuccinate} \rightarrow \text{AMP} + \text{fumarate}$$

Fumarate can then be hydrated to malate by fumarase and transported into the mitochondrion, where it is converted to oxaloacetate and transaminated to regenerate aspartate. Some of the fumarate generated in the purine nucleotide cycle is metabolized and thus represents a source of energy for muscle.

Aspartate provides the amino group for the regeneration of AMP, and thus for subsequent release of ammonium ions in the AMP deaminase reaction. The ammonium ions are utilized for the synthesis of glutamine from glutamate. Although the immediate nitrogen donor for the transamination of oxaloacetate to aspartate is glutamate, most of the amino groups are ultimately derived from the catabolism of branched-chain amino acids.

23.3.6 De Novo Synthesis of Pyrimidines

The pathway for pyrimidine synthesis differs from the de novo purine synthesis pathway in that the pyrimidine ring is assembled first and then combined with PRPP to form the initial pyrimidine nucleotide, UMP (Fig. 23-10).

23.3.6.1 Synthesis of Uracil-Containing Nucleotides. The uracil synthesis pathway (Fig. 23-10) starts with the synthesis of carbamoyl phosphate from glutamic

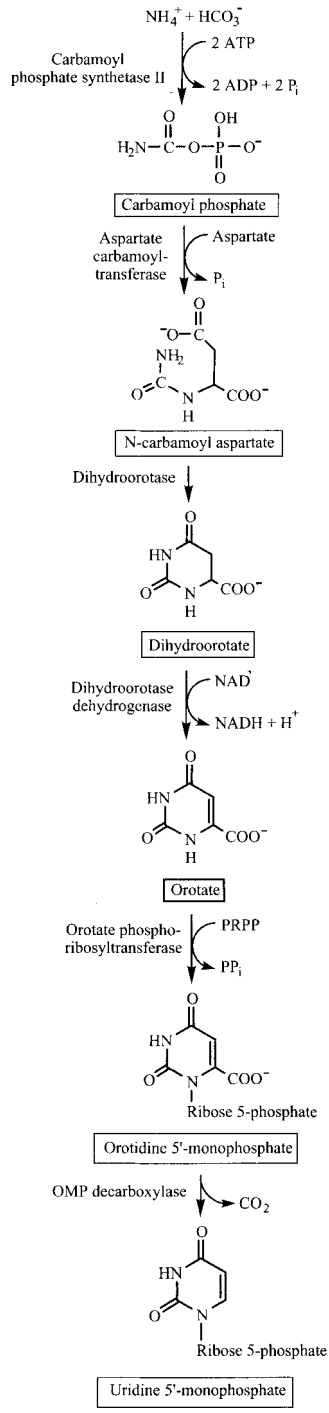


FIGURE 23-10 Synthesis of uridine 5′-monophosphate.

acid and CO_2 (HCO_3^-), catalyzed by carbamoyl phosphate synthetase II (CPS II):

$$\text{glutamine} + CO_2 + \text{ATP} \rightarrow \text{carbamoyl phosphate} + \text{ADP} + \text{glutamic acid}$$

This enzyme is distinct from carbamoyl phosphate synthetase I (CPS I), which is localized to mitochondria and catalyzes the first step in urea synthesis. CPS II utilizes glutamine rather than free ammonia as the nitrogen sources, and unlike CPS I is not activated by *N*-acetylglutamate.

The next reaction in pyrimidine synthesis is both the committed step and the regulated step, and it is catalyzed by aspartate transcarbamoylase:

$$\text{aspartate} + \text{carbamoyl phosphate} \rightarrow N\text{-carbamoyl aspartate} + P_i$$

Next, dehydration of *N*-carbamoyl aspartate by dihydroorotase forms the ring structure dihydroorotic acid (Fig. 23-10). An NAD^+-linked dehydrogenase introduces a double bond into the ring, producing orotate. Orotate is then transferred to PRPP by orotate phosphoribosyltransferase:

$$\text{orotate} + \text{PRPP} \rightarrow \text{orotidine 5-monophosphate} + PP_i$$

Once the initial pyrimidine nucleotide has been synthesized, orotidylate decarboxylase removes the carboxyl group from the ring to form uridylic acid (UMP), the primary product of the de novo pyrimidine pathway. Two kinase reactions then convert UMP to UTP:

$$\text{UMP} + \text{ATP} \rightarrow \text{UDP} + \text{ADP}$$

$$\text{UDP} + \text{ATP} \rightarrow \text{UTP} + \text{ADP}$$

23.3.6.2 Synthesis of Cytosine-Containing Nucleotides. Cytidylate synthetase (CTP synthetase) catalyzes the conversion of UTP to CTP (Fig. 23-11), with glutamine as the nitrogen donor:

$$\text{uridine 5}'\text{-triphosphate} + \text{glutamine} + \text{ATP} \rightarrow \text{cytidine 5}'\text{-triphosphate} + \text{ADP} + P_i$$

23.3.6.3 Synthesis of Deoxythymidylate. As noted earlier, DNA contains thymine (methyluracil) in place of uracil. The methylation of uracil is catalyzed by thymidylate synthase, which utilizes deoxyuridylate (dUMP) as its substrate (Fig. 23-12):

$$\text{deoxyuridine 5}'\text{-monophosphate} + N^5, N^{10}\text{–methylene} - FH_4 \rightarrow$$
$$\text{deoxythymidine 5}'\text{-monophosphate} + 7, 8\text{-}FH_2$$

N^5,N^{10}-methylene-FH_4 is regenerated by the sequential action of two enzymes: dihydrofolate reductase and serine hydroxymethyltransferase. Because thymidylate

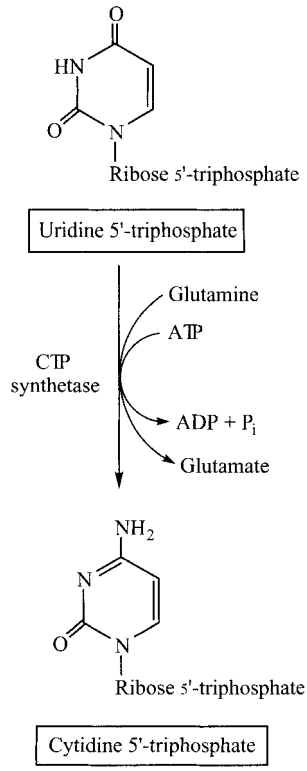


FIGURE 23-11 Synthesis of cytidine 5′-triphosphate from uridine 5′-triphosphate.

synthetase is strongly dependent on an adequate supply of the components of the folate one-carbon pool, cancer chemotherapeutic agents (e.g., methotrexate) that interfere with steps in purine nucleotide synthesis also inhibit the synthesis of dTMP.

23.3.7 Catabolism of Pyrimidines

The ribonucleotides and deoxyribonucleotides are first dephosphorylated to the level of nucleosides by nonspecific phosphatases. Cytidine and deoxycytidine are then deaminated by pyrimidine nucleoside deaminase to uridine and deoxyuridine, respectively:

$$\text{cytidine} + H_2O \rightarrow \text{uridine} + NH_4^+$$

5′-Nucleosidases (nucleoside phosphorylases) then catalyze the phosphorolytic cleavage of uridine, deoxyuridine, and deoxythymidine to generate the free nitrogen bases (uracil and thymine) and ribose 1-phosphate or deoxyribose 1-phosphate:

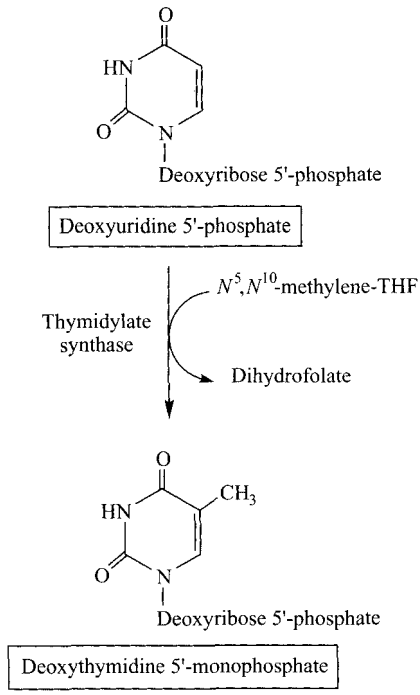


FIGURE 23-12 Synthesis of thymidylate (deoxythymidine 5′-phosphate) from deoxyuridine 5′-phosphate.

for example,

$$\text{uridine} + P_i \rightarrow \text{uracil} + \text{ribose 1-phosphate}$$

In contrast to purines, pyrimidines can undergo ring cleavage. Catabolism of both uracil and thymine involves the same three-enzyme sequence, which releases ammonia and carbon dioxide and generates a β-amino acid from the rest of the ring (Fig. 23-13). β-Aminoisobutyrate, the main end product of thymine metabolism, is mostly excreted in the urine. The end product of uracil metabolism is β-alanine, some of which is incorporated into carnosine (histidine-β-alanine) and anserine (methyl histidine-β-alanine), two dipeptides found in brain and muscle. Excess β-alanine is excreted in the urine.

23.3.8 Salvage of Pyrimidines

Salvage of pyrimidine bases is only a minor pathway in humans. However, the pyrimidine nucleosides can be salvaged by nucleoside kinases:

$$\text{nucleoside} + \text{ATP} \rightarrow \text{nucleotide} + \text{ADP}$$

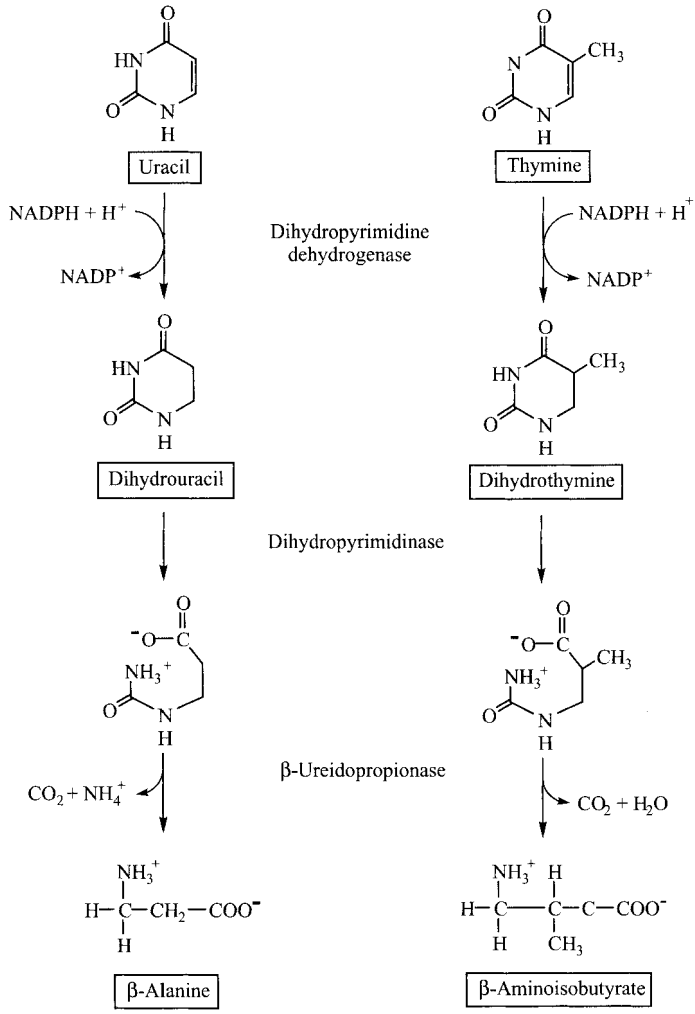


FIGURE 23-13 Catabolism of the pyrimidine bases uracil and thymine.

23.4 REGULATION OF PURINE AND PYRIMIDINE METABOLISM

23.4.1 Purine Synthesis

As is the case with many metabolic pathways, the first committed step in de novo purine synthesis is the regulated step. Glutamine PRPP amidotransferase, which catalyzes the first step in the process of IMP synthesis (Fig. 23-6), is inhibited allosterically by the end products of the pathway, AMP and GMP. A second level of control is exerted at the level of PRPP synthesis, where PRPP synthetase is inhibited by both ADP and GDP.

23.4.2 Balance Between the Generation of Adenine and Guanine Nucleotides

Two different reciprocal regulatory mechanisms act to maintain the balance between synthesis of AMP and GMP (see Fig. 23-7). First, adenylosuccinate synthase and IMP dehydrogenase, which catalyze the initial steps in the conversion of IMP to AMP and GMP, respectively, are inhibited by AMP and GMP, respectively. Second, the synthesis of AMP requires GTP, while that of GMP requires ATP.

23.4.3 Pyrimidine Synthesis

The key control point in pyrimidine synthesis is the reaction catalyzed by carbamoyl phosphate synthetase II. The enzyme is inhibited by UTP, and activated by PRPP. Carbamoyl phosphate synthetase II is also regulated by posttranslational phosphorylation. Phosphorylation of CPS II by MAP kinase results in an enzyme that is more sensitive to activation by PRPP and is not inhibited by UTP, thus increasing carbamoylphosphate synthetase II activity as cells approach the S-phase of the cell cycle.

23.5 ABNORMAL PURINE AND PYRIMIDINE METABOLISM

23.5.1 Gout

Gout occurs when a high concentration of uric acid in the blood (hyperuricemia) results in deposition of monosodium urate crystals in tissues and joints, causing pain and inflammation. Uric acid is poorly soluble in plasma, especially at lower temperatures; thus the hallmark of gout is deposition of tophi or urate crystals under the skin of the ear, fingers, and toes. Gout can be caused by either underexcretion or overproduction of uric acid. Causes of underexcretion of purines include lactic acidosis and drugs such as thiazide diuretics.

Hyperuricemia is one of the hallmarks of tumor lysis syndrome, a cluster of metabolic complications of cancer chemotherapy. The catabolism of purines from large numbers of lysed cancer cells results in increased production of uric acid. One of the genetic causes of increased uric acid synthesis is impaired salvage of purine bases as occurs in people who have a partial deficiency of HRPTase (see below). Gout is also associated with von Gierke disease, the glycogen storage disease that results from a deficiency in glucose 6-phosphatase activity. The increased availability of glucose 6-phosphate increases the rate of flux through the pentose phosphate pathway, yielding an elevation in the level of ribose 5-phosphate, which results in increased synthesis of PRPP and excess purine biosynthesis.

The drug allopurinol, which is used to treat gout, is oxidized by xanthine oxidase to oxypurinol, which is a potent inhibitor of xanthine oxidase. When xanthine oxidase is inhibited, hypoxanthine and xanthine accumulate and the concentration of uric acid is reduced. Hypoxanthine and xanthine are more water-soluble than uric acid, thereby facilitating the urinary excretion of purine degradation products and reducing the likelihood of urate crystal deposition.

23.5.2 Lesch–Nyhan Disease

Lesch–Nyhan disease is a rare, X-linked disorder caused by a genetic defect in hypoxanthine-guanine phosphoribosyltransferase (HGPRTase), the regulated enzyme in the purine salvage pathway that catalyzes the conversion of the purine bases hypoxanthine and guanine to their respective nucleotides, IMP and GMP. Lacking HGPRTase activity, PRPP levels increase and purines are synthesized in excess by the de novo pathway, resulting in high plasma and urine concentrations of urate. Affected children have severe neurologic deficits, retarded motor development, muscle weakness, and self-injurious behavior; the most characteristic feature is loss of tissue from biting themselves. Although the mechanism is not fully understood, it appears that abnormalities in purine metabolism impair dopaminergic function in the basal ganglia. Allopurinol is effective in controlling the hyperuricemia and goutlike symptoms of Lesch–Nyhan disease, but is ineffective in modifying the neurological or behavioral manifestations of the disease. A partial deficiency of HGPRTase (residual activity $> 8\%$) results in gout, renal stones, and uric acid nephropathy without the neurological problems associated with Lesch–Nyhan disease.

23.5.3 Adenosine Deaminase Deficiency

Adenosine deaminase (ADA) deaminates both adenosine to inosine (Fig. 23-8) and deoxyadenosine to deoxyinosine. Deficiency of ADA causes severe combined immunodeficiency disease. People with ADA deficiency have 100-fold increases in the concentration of dATP, which is a potent inhibitor of ribonucleotide reductase. The resulting deficiency in the other deoxynucleotide triphosphates inhibits DNA synthesis and impairs the immune response of both B and T lymphocytes. The disease has been treated successfully by bone marrow transplantation.

Immunodeficiency can also be caused by a deficiency of purine nucleoside phosphorylase, the enzyme that catalyzes the phosphorolytic cleavage of inosine to hypoxanthine. Unlike ADA deficiency, however, purine nucleoside phosphorylase deficiency affects only the T cells; B-cell function remains normal.

23.5.4 Orotic Aciduria

Orotic aciduria is an inborn error of metabolism characterized by large quantities of orotic acid in the urine and by megaloblastic anemia, which is unresponsive to vitamin B_{12} and folic acid. The metabolic defect is in uridine-5-monophosphate synthase, which contains two enzyme activities in a single protein: orotate phosphoribosyltransferase and orotidylate decarboxylase. The disease is treated effectively with high doses of oral uridine, which leads to increased UMP production; the UMP then inhibits carbamoyl phosphate synthetase II, thereby decreasing synthesis of orotic acid.

23.5.5 Cholera and Whooping Cough

Cholera is an acute, life-threatening diarrheal disease caused by *Vibrio cholera* bacteria that are transmitted in water contaminated by human wastes. The bacterium

produces a toxin that catalyzes a covalent modification that attaches an ADP-ribose moiety derived from NADH to a specific arginine of the $G_{\alpha s}$ subunit of G proteins.

$$G_{\alpha} + NAD^{+} \rightarrow \text{ADP-ribose-}G_{\alpha} + \text{niacin}$$

ADP ribosylation stabilizes the $G_{\alpha s}$ in the active form by preventing hydrolysis of bound GTP. As a result, intestinal epithelial cells produce excess cAMP, which stimulates the secretion of excess sodium into the intestinal lumen. Water follows the sodium, resulting in diarrhea and dehydration. Treatment requires aggressive rehydration therapy.

The bacterium *Bordetella pertussis*, which causes pertussis or whooping cough, also produces a toxin that catalyzes ADP-ribosylation. In this case, the target is a different Gα subunit called $G_{\alpha i}$, and the result is the stabilization of the inactive GDP-bound form of the G protein.

NAD^{+} is also a substrate for transfer of ADP-ribose in a number of physiological reactions. Poly(ADP-ribose) polymerases, a family of cell-signaling enzymes in the nucleus of eukaryotic cells, are involved in poly(ADP-ribosylation) of DNA-binding proteins and use poly(ADP)ribose to repair DNA single-strand breaks. ADP-ribose cyclases generate cyclic AMP-ribose, which is an intracellular messenger involved in Ca^{2+} signaling.

23.5.6 Cancer Chemotherapy

Since tumor cells have a much greater demand for nucleotides for DNA and RNA synthesis, certain enzymes in the nucleotide biosynthetic pathways are major targets of cancer chemotherapy. For example, azaserine and acivicin are analogs of glutamine that inhibit glutamine-PRPP amidotransferases involved in nucleotide synthesis. One of the earliest chemotherapeutic agents, fluorouracil, inhibits thymidylate synthase. Hydroxyurea, which is used as a component of some cancer protocols, inhibits ribonucleotide reductase. Methotrexate and aminopterin inhibit dihydrofolate reductase, thus impairing synthesis of purines as well as thymidylate.

CHAPTER 24

HEME AND IRON

24.1 FUNCTIONS OF HEMOGLOBIN AND OTHER IRON-CONTAINING PROTEINS

24.1.1 Hemoglobin and Myoglobin

Heme (Fe^{2+}-protoporphyrin IX) is the prosthetic group of hemoglobin and myoglobin. Hemoglobin is the molecule in red blood cells that transports oxygen from the lungs to peripheral tissues. Myoglobin is an intracellular protein that extracts oxygen from the red blood cells and stores it until the oxygen is needed in various oxidation–reduction reactions and respiration. Hemoglobin and myoglobin both contain heme, a porphyrin or tetrapyrrole that consists of four pyrrole rings joined by methylene bridges (Fig. 24-1). All of the double bonds in heme are conjugated; that is, double bonds and single bonds alternate throughout the structure.

Porphyrins differ in the side chains attached to the tetrapyrrole ring; in the case of heme, one methyl and one vinyl group ($-CH=CH_2$) are located on each of the A and B rings, and methyl and propionic acid groups are attached to rings C and D. The iron in the center of the porphyrin ring is in the ferrous (Fe^{2+}) state and is the site where molecular oxygen binds. The iron atom also binds noncovalently to a particular histidine in the hemoglobin and myoglobin proteins. Myoglobin contains one polypeptide chain and one protoporphyrin IX ring. Hemoglobin is a tetrameric molecule with four polypeptide chains, two α and two β chains, each with a bound heme molecule.

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

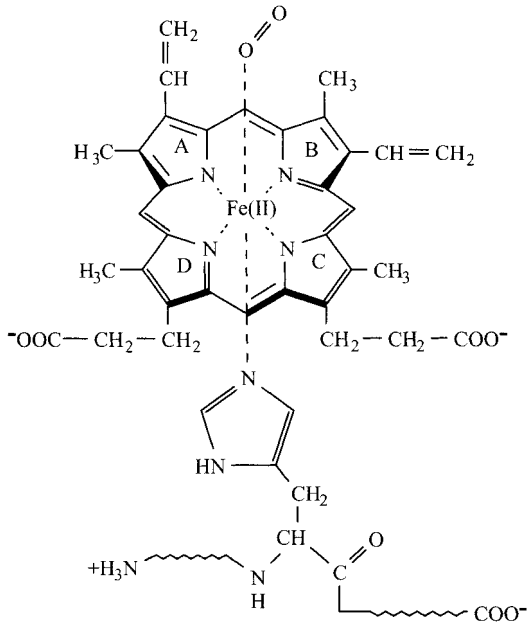


FIGURE 24-1 Structure of oxyheme in oxyhemoglobin. The sawtooth represents the globin chain.

24.1.2 Cytochromes

Heme is also the prosthetic group for a large class of hemoproteins called *cytochromes*. Whereas the iron in hemoglobin and myoglobin remains in the ferrous (Fe^{2+}) state as molecular oxygen is bound successively to and then released from the molecule, the iron in cytochromes accepts and donates electrons by undergoing reversible interconversion between the ferrous (Fe^{2+}, reduced) and ferric (Fe^{+3}, oxidized) forms. Heme-containing enzymes include prostaglandin H synthase (a.k.a. cyclooxygenase), and catalase, which converts hydrogen peroxide to water and O_2. Heme is also a component of members of the superfamily of cytochrome P450–containing enzymes that catalyze the oxidation of many molecules, including steroids and xenobiotics. Cytochromes differ from each other in the nature of the side chains attached to the porphyrin ring. Furthermore, in some cytochromes, such as cytochrome c_3, the porphyrin ring is covalently bound to the protein through the side chains of two cysteine residues of the protein via thioether bonds with vinyl groups of protoporphyrin IV.

24.1.3 Nonheme Iron

There are also some proteins in which nonheme iron participates in enzyme-catalyzed oxidation–reduction reactions. These include the iron–sulfur (Fe–S) clusters in proteins of the electron-transport system and ribonucleotide reductase, which converts

ribonucleotides to deoxynucleotides. Iron also plays a central role in the generation of the hydroxyl radical ($OH^{\bullet}$) in the (nonenzymatic) Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

24.2 LOCALIZATION OF HEME AND IRON METABOLISM

24.2.1 Synthesis of Heme

Heme is synthesized de novo in all cells. Most of heme synthesis takes place in the liver and in erythroid cells within the bone marrow. A total of eight enzymes are required for heme synthesis. The first and last three steps take place in the mitochondrion, whereas the second through fifth steps take place in the cytosol.

24.2.2 Heme Catabolism

When hemoproteins turn over, the heme is degraded rather than salvaged. The iron atom is reutilized, while the porphyrin ring is oxidized and cleaved to produce the breakdown product, bilirubin. The liver converts hydrophobic bilirubin to the more water-soluble bilirubin diglucuronide, which is secreted into the bile and ultimately excreted in the feces.

Most of the bilirubin arising from the degradation of hemoglobin is produced in splenic phagocytes. This means that bilirubin must be transported from nonhepatic phagocytic cells to the liver. It is critical that bilirubin is transported in the blood bound to albumin; when the binding capacity of albumin is exceeded, the unbound bilirubin can be toxic.

24.2.3 Localization of Iron in the Body

The average man and woman contain about 3500 and 2600 mg of iron, respectively (Fig. 24-2). The hemoglobin of red blood cells and the myoglobin of muscle cells account for approximately 2500 and 100 mg of this iron, respectively. Approximately 0.8% of a person's red blood cells are broken down each day by the reticuloendothelial (macrophage) system, which results in the release of 20 mg of iron into the blood. Ninety-five percent of this iron is recycled and reutilized by the bone marrow to synthesize new red blood cells, which replace those that were broken down.

The body is also capable of storing iron, mainly in the liver. Ferritin is a ubiquitous iron-binding protein that is found mostly in the cytosol. Ferritin is composed of two subunits, designated H and L. The apoferritin shell, which is formed by assembly of 24 of these dimers, can accommodate up to 4500 atoms of iron in its core. The ratio of H to L subunits is tissue-dependent: Liver and spleen contain mostly L-subunit ferritin, whereas the H subunit predominates in kidney and heart. In a healthy adult,

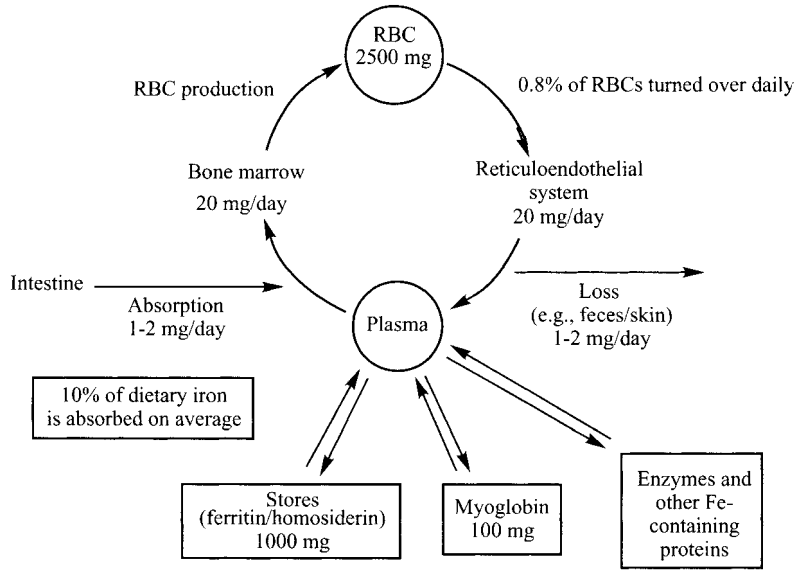


FIGURE 24-2 Disposition of iron in the body. RBC, red blood cells.

approximately 1000 mg of iron is found in ferritin. As ferrous iron enters the pores of the apoferritin shell, it is oxidized to the Fe^{3+} state and deposited as hydrated ferric oxide crystals.

Iron is transported between cells and tissues in the circulation in the ferric form, most of which is complexed to a protein named *transferrin* that is synthesized and secreted by hepatocytes. Dietary iron, absorbed in the proximal small intestine, is also transported on transferrin. Transferrin is normally about one-third saturated with ferric iron. The Fe^{3+}–transferrin complex binds to transferrin receptors on the plasma membrane of iron-utilizing cells and is internalized by receptor-mediated endocytosis (Fig. 24-3). A proton pump in the endosome acidifies the endosome, resulting in the release of iron from transferrin, which is then transported out of the endosome to the cytosol, where it becomes available for heme synthesis or storage as ferritin. The transferrin : transferin receptor complex is recycled to the plasma membrane, whereupon transferrin dissociates from its receptor.

Small amounts of ferritin are also present in plasma, reflecting slow release of this iron protein from storage sites (mostly liver) during normal cellular turnover. Since the plasma ferritin concentration is directly proportional to the intracellular stores of ferritin, determination of the plasma ferritin level in the clinical laboratory provides a convenient way of assessing a person's iron status.

About 1 mg of iron is lost each day through exfoliation of skin and intestinal cells. This iron loss is made up for by the absorption of an equivalent amount of iron from the intestine.

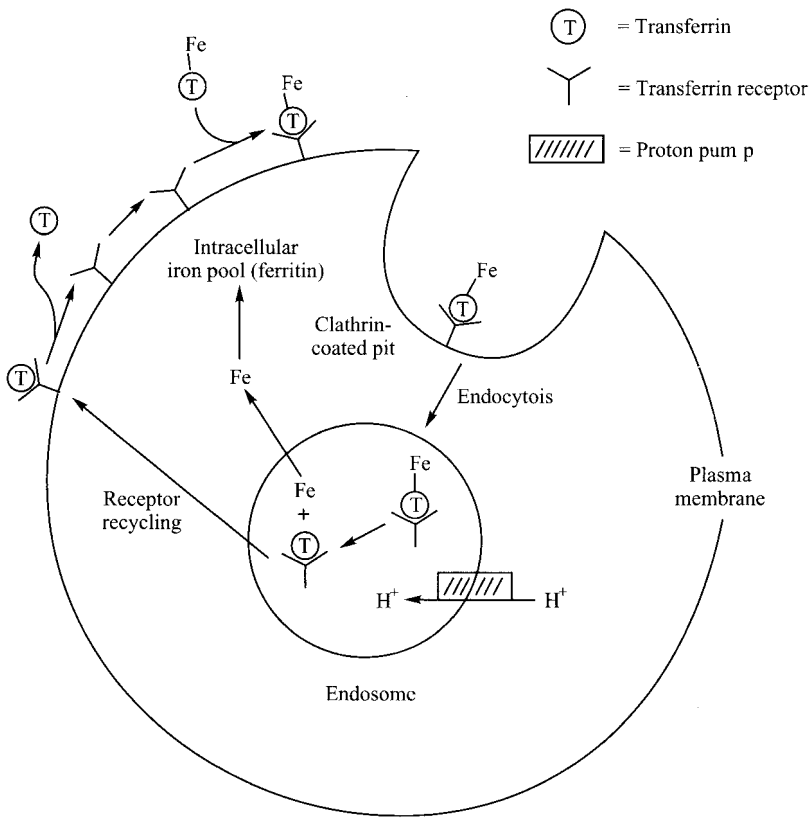


FIGURE 24-3 Pathway of iron uptake by the liver and peripheral tissues.

24.3 CONDITIONS WHERE HEME METABOLISM IS ESPECIALLY ACTIVE

Heme synthesis in erythroid cells is required for erythropoiesis and is therefore most active during periods of growth or pregnancy and when the body is compensating for increased blood loss or destruction of existing red blood cells. Heme synthesis in the liver is most active when there is induction of enzymes of the cytochrome P450 family which participate in the detoxification of drugs and xenobiotics.

24.4 PATHWAYS OF HEME AND IRON METABOLISM

24.4.1 Heme Synthesis

The overall pathway of heme synthesis is summarized in Figure 24-4. The first step is catalyzed by δ-aminolevulinic acid (ALA) synthase and involves the condensation

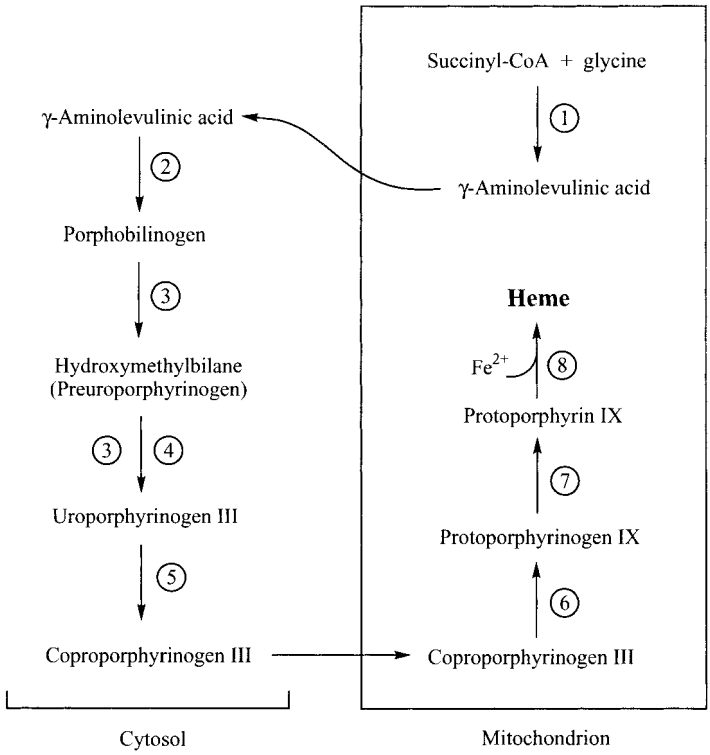


FIGURE 24-4 Synthesis of heme: ①, δ-aminolevulinic acid synthase; ②, porphobilinogen synthase; ③, uroporphrinogen synthase; ④, uroporphyrinogen III cosynthase; ⑤, uroporphyrinogen decarboxylase; ⑥, coproporphyrinogen oxidase; ⑦, protoporphyrinogen oxidase; ⑧, ferrocheletase.

of glycine with succinyl-CoA (Fig. 24-5):

$$\text{glycine} + \text{succinyl-CoA} \rightarrow \delta\text{-aminolevulinic acid} + CO_2 + \text{CoASH}$$

This reaction is irreversible and represents the committed step in the pathway. ALA synthase is a mitochondrial enzyme and the succinyl-CoA for the reaction is provided by the TCA cycle.

The next step, the condensation of two molecules of δ-aminolevulinic acid to form a pyrrole ring, is catalyzed by ALA dehydratase (also called *porphobilinogen synthase*) (Fig. 24-5):

$$2\ \delta\text{-aminolevulinic acid} \rightarrow \text{porphobilinogen} + 2H_2O$$

Porphobilinogen deaminase then generates hydoxymethylbilane by the sequential condensation of four porphobilinogen molecules with the concomitant release of ammonia at each step.

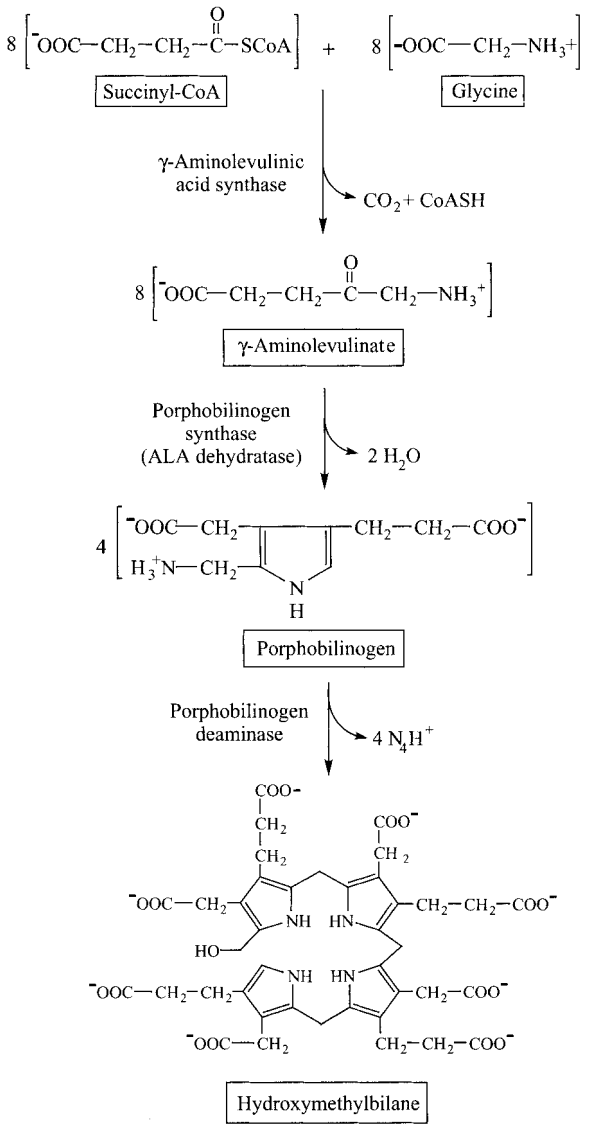


FIGURE 24-5 Synthesis of hydroxymethylbilane.

Next, hydoxymethylbilane is converted to uroporphyrinogen III by the combined action of uroporphyrinogen synthase and uroporphyrinogen III cosynthase (Fig. 24-6). Uroporphyrinogen synthase catalyzes formation of the porphyrinogen ring. The function of uroporphyrinogen III cosynthase is to direct the fourth pyrrole ring into the inverse orientation relative to that of the other three pyrrole rings. Uroporphyrinogen III decarboxylase then converts uroporphyrinogen III to coproporphyrinogen III, which is then transported from the cytosol into the mitochondrion.

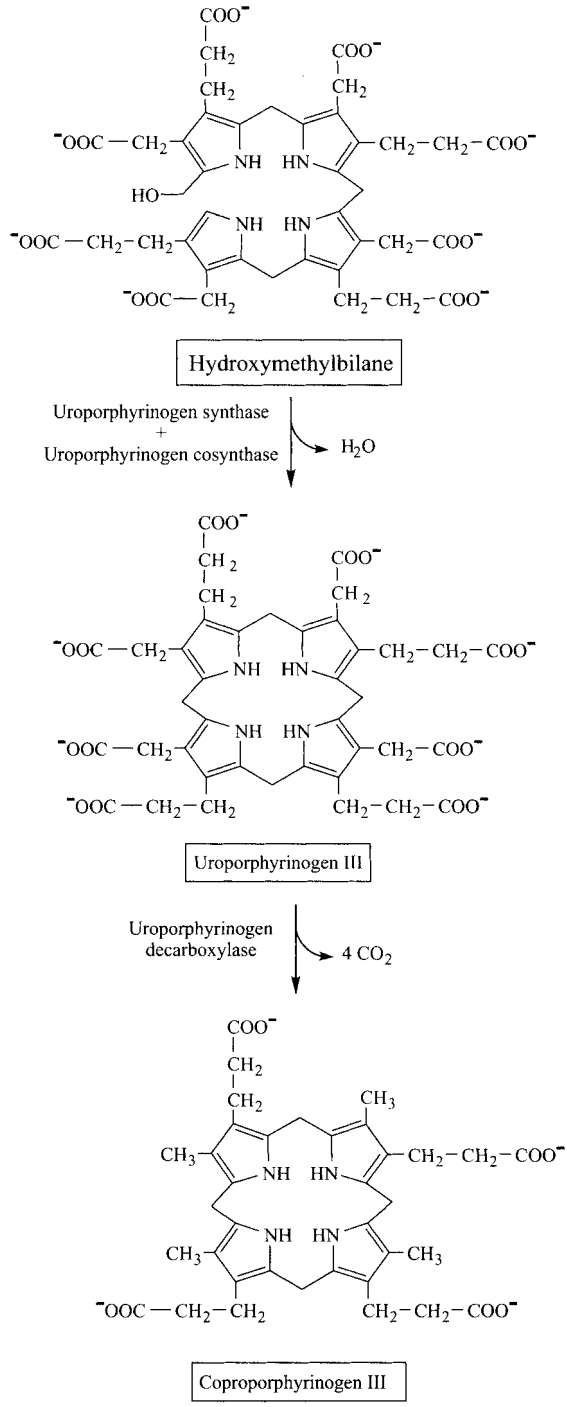


FIGURE 24-6 Synthesis of coproporphyrinogen III from hydroxymethylbilane.

Inside the mitochondrion, the propyl side chains of rings A and B of coproporphyrinogen III are oxidized to vinyl groups by coproporphyrinogen III oxidase, thereby generating protoporphyrinogen IX (Fig. 24-7). The methylene rings connecting the pyrrole rings of colorless protoporphyrinogen IX are then oxidized by protoporphyrinogen oxidase to generate protoporphyrin IX. This reaction generates a molecule that has resonance of double bonds around the entire great ring and the characteristic red color of heme. Finally, ferrochelatase (heme synthetase) catalyzes the insertion of ferrous iron (Fe^{2+}) into protoporphyrin IX to form heme.

24.4.1.1 Reduction of Methemoglobin. The iron of heme is normally in the ferrous state. Hemoglobin cannot bind oxygen when the iron of hemoglobin is in the ferric state (a.k.a. *methemoglobin*). Furthermore, methemoglobin is a potent oxidizing agent that can damage red blood cells and shorten their lifespan. Red blood cells contain an enzyme called *methemoglobin reductase* (a.k.a. *diaphorase*), which uses NADH or NADPH to reduce the iron atom of methemoglobin from the ferric to the ferrous state:

$$\text{methemoglobin} + \text{NAD(P)H} + \text{H}^+ \rightarrow \text{hemoglobin} + \text{NAD(P)}^+$$

24.4.2 Heme Catabolism and Bilirubin Excretion

Bilirubin is the breakdown product of heme. About 75% of bilirubin is derived from hemoglobin that has been ingested by phagocytic cells during the process of destroying senescent red cells in the spleen and liver. Bilirubin is also derived from the turnover of other heme-containing proteins (e.g., myoglobin, catalase, cytochromes).

Some free hemoglobin is released into the circulation when senescent red cells are destroyed. This hemoglobin is complexed to haptoglobins, a family of plasma glycoproteins synthesized by the liver. Haptoglobin : hemoglobin complexes are removed from the circulation by splenic phagocytes and Kupffer cells in the liver. Any free heme that dissociates from hemoglobin in the circulation is complexed immediately by hemopexin, another liver-synthesized plasma glycoprotein, and transported to the liver. Binding of hemoglobin and heme to haptoglobins and hemopexin, respectively, serves both to prevent loss of iron through filtration by the kidney and to protect against oxidative stress. Both the haptoglobins and hemopexin are acute-phase proteins whose synthesis is increased in response to infection and which, by removing hemoglobin and heme from the plasma, prevent iron-utilizing bacteria from benefitting from the iron released by hemolysis.

24.4.3 Catabolism of Heme

The conversion of heme to bilirubin (Fig. 24-8) can be visualized in a bruise that is initially reddish purple (heme) and with time turns yellow-green (biliverdin) and then red-orange (bilirubin). The initial reaction that cleaves the porphyrin ring is catalyzed by heme oxygenase, producing biliverdin IX and carbon monoxide, and concurrently

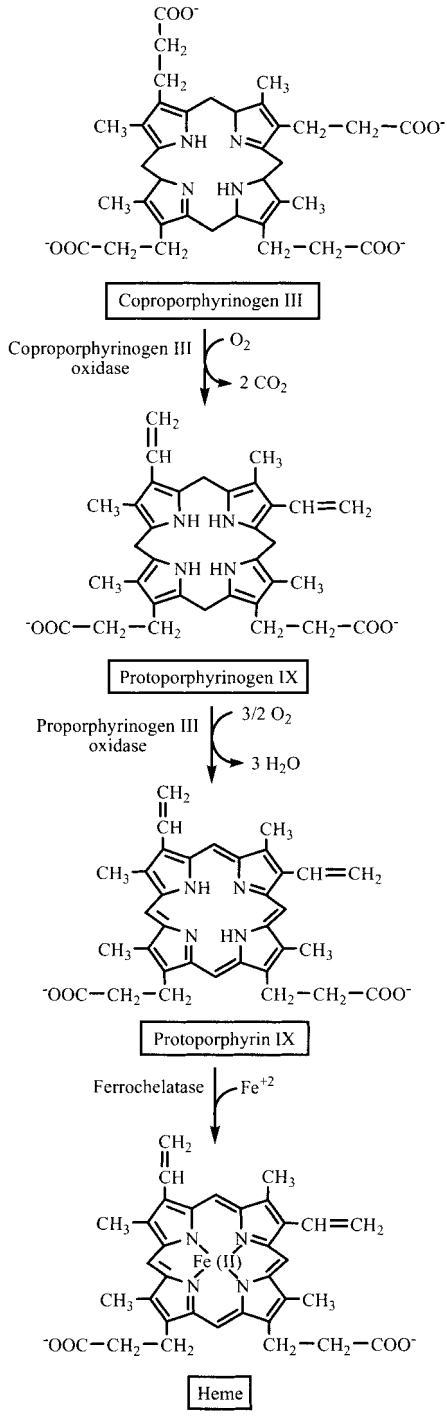


FIGURE 24-7 Synthesis of heme from coproporphyrinogen III.

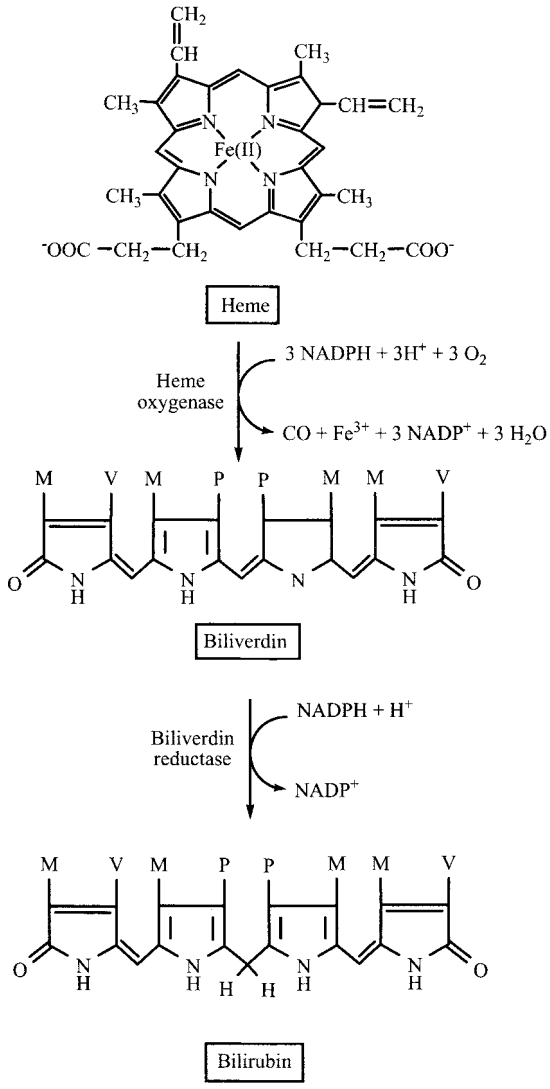


FIGURE 24-8 Metabolism of heme to bilirubin. M, methyl; P, propionyl, V, vinyl.

releasing the oxidized Fe^{3+} ion:

$$\text{heme} + 3O_2 + 3\text{NADPH} + 3H^+ \rightarrow \text{biliverdin} + CO + Fe^{3+} + 3\text{NADP}^+ + 3H_2O$$

Note that heme oxygenase is a mixed-function oxidase that utilizes both molecular oxygen and NADPH; this reaction is the only known endogenous source of carbon monoxide. The substrate for heme oxygenase is Fe^{2+} heme; any hemin (Fe^{3+} heme) that is produced during the phagocytic process must first be reduced to the Fe^{2+} form.

Biliverdin reductase then reduces the central methene bridge of biliverdin, producing bilirubin (Fig. 24-8):

$$\text{bilirubin} + \text{NADPH} + \text{H}^+ \rightarrow \text{biliverdin} + \text{NADP}^+$$

24.4.3.1 Conjugation of Bilirubin. Although all cells contain heme oxygenase and can convert heme generated during turnover of hemoproteins to bilirubin, only the liver is capable of converting bilirubin to the more water-soluble bilirubin diglucuronide. This reaction, catalyzed by UDP-glucuronyl transferase, involves successive transfers of two glucuronic acid residues from UDP-glucuronic acid to form ester linkages with the propionic acid side chains of bilirubin (Fig. 24-9):

$$\text{bilirubin} + \text{UDP-glucuronate} \rightarrow \text{bilirubin glucuronide} + \text{UDP}$$

$$\text{bilirubin glucuronide} + \text{UDP-glucuronate} \rightarrow \text{bilirubin diglucuronide} + \text{UDP}$$

Conjugation with glucuronic acid is also the mechanism the liver uses to increase the solubility of steroids and certain drugs prior to their excretion. UDP-glucuronic acid is synthesized by the oxidation of UDP-glucose by UDP-glucose dehydrogenase (Fig. 24-10):

$$\text{UDP-glucose} + 2\text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{UDP-glucuronic acid} + 2\text{NADH} + 2\text{H}^+$$

Direct and Indirect Bilirubin. Clinically, conjugated bilirubin or bilirubin diglucuronide is often called *direct-acting bilirubin* and unconjugated bilirubin is called *indirect-acting bilirubin*. This nomenclature is related to the colorimetric reaction, called the van den Bergh reaction, which is commonly used to quantify the two

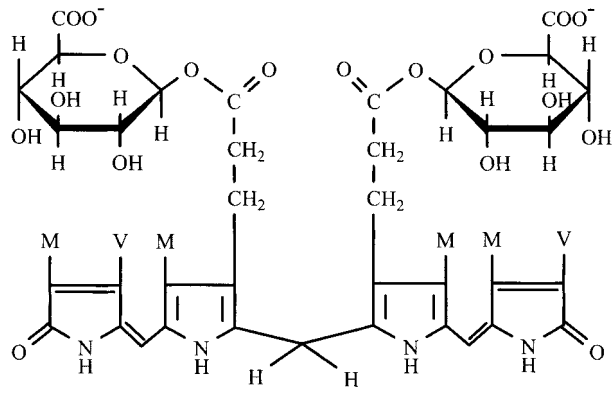


FIGURE 24-9 Structure of bilirubin diglucuronide. M, methyl; P, propionyl; V, vinyl.

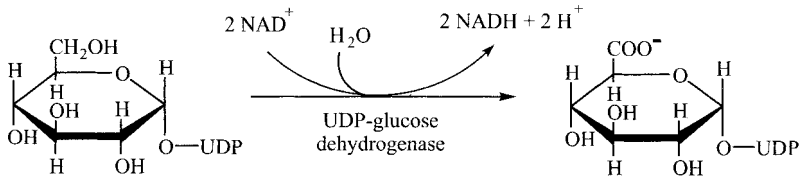


FIGURE 24-10 Synthesis of UDP-glucuronic acid.

forms of bilirubin, which is important for the differential diagnosis of the causes of hyperbilirubinemia. In this assay, conjugated bilirubin reacts readily with an azo dye. Unconjugated bilirubin, on the other hand, is much more lipophilic and tightly bound to serum albumin; it must be released with alcohol before the dye-coupling reaction can occur. The van den Bergh assay first quantifies conjugated bilirubin; then, with the addition of alcohol, the test quantifies total plasma bilirubin. The quantity of unconjugated bilirubin is determined by subtraction, and unconjugated bilirubin is therefore designated *indirect bilirubin.*

24.4.3.2 Metabolism of Excreted Bilirubin Diglucuronide. Conjugated bilirubin is released into the biliary system and delivered into the small intestine when the gallbladder contracts. In the lower intestine and colon, bacterial β-glucuronidases remove glucuronic acid to form unconjugated bilirubin. Further metabolism of bilirubin by bacteria reduces bilirubin to a colorless tetrapyrrolic compound called *urobilinogen.* A small amount of urobilinogen is absorbed and enters into the enterohepatic circulation; a minor fraction of this urobilinogen is ultimately excreted by the kidney, partly as the oxidized, colored compound urobilin, which imparts the characteristic yellow color of urine. Most of the urobilinogen formed in the gut is further metabolized by the enteric bacteria to stercobilinogen and excreted mainly in its oxidized form, stercobilin, which imparts the characteristic color of stool.

24.4.4 Iron Utilization

24.4.4.1 Oxidation and Reduction of Iron. Much of the metabolism of iron is involved with its interconversion between the reduced (Fe^{2+}) and oxidized (Fe^{3+}) forms. Hemoglobin, the major iron-containing molecule of the body, contains ferrous iron, while both plasma transport of iron by transferrin and intracellular storage as ferritin (cytosolic) and frataxin (mitochondrial) require ferric iron. Many enzymes catalyze the oxidation or reduction of molecular iron, including the following three.

Ceruloplasmin. Ceruloplasmin is a blue-colored, copper-containing glycoprotein synthesized by and secreted from the liver. This protein, also known as *plasma ferroxidase*, oxidizes Fe^{2+} to Fe^{3+}. It functions to provide ferric iron for transport by transferrin. The dependency on a copper-containing enzyme for iron transport accounts for why a copper deficiency results in anemia.

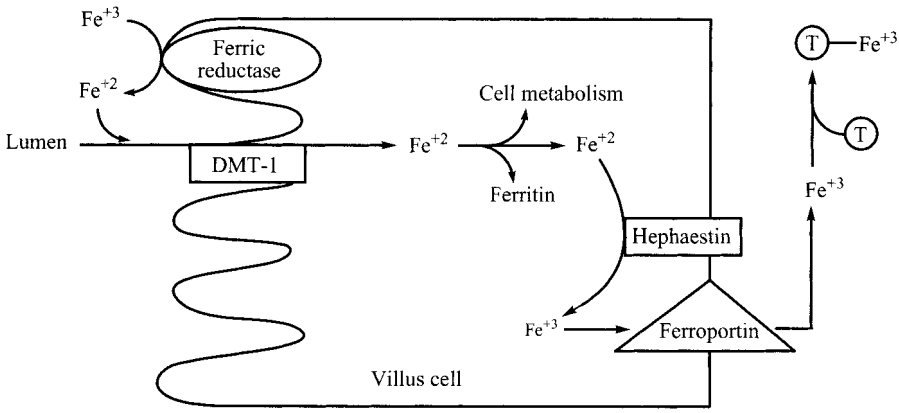


FIGURE 24-11 Pathway of iron uptake by enterocytes. DMT-1, divalent metal transporter-1. Ⓣ, transferrin.

Hephaestin. Hephaestin is a membrane-bound protein which, like ceruloplasmin, is a copper-dependent ferroxidase (Fig. 24-11). It is located on the basal–lateral surface of intestinal enterocytes and acts to provide ferric iron to ferroportin, the basolateral transporter that transfers Fe^{3+} from the enterocyte to plasma transferrin.

Ferric Reductase. This enzyme, localized on the luminal surface of duodenal enterocytes, catalyzes the conversion of Fe^{3+} to Fe^{2+}, permitting absorption of dietary nonheme iron.

24.4.4.2 Absorption of Dietary Iron. Normally, about 10% of dietary iron is absorbed into the blood. The efficiency of iron absorption in the small intestine is influenced by a number of factors, including the nature of the dietary source of iron, a person's iron status, and the availability of reducing agents (ascorbic acid, cysteine) and an appropriate pH. In general, heme iron is absorbed more efficiently than nonheme iron.

Absorption of Heme Iron. In North America and Europe, about one-third of dietary iron is heme iron, which enters the enterocyte as the intact metalloporphyrin by a mechanism involving heme carrier protein 1 (HCP1). Inside the enterocyte heme oxygenase cleaves the porphyrin ring and releases the free Fe^{2+} (Fig. 24-11).

Absorption of Nonheme Iron. For dietary nonheme iron to be absorbed, it must first be freed from proteins and other ligands during digestion. The dissociation of iron from iron-binding ligands is facilitated by the acid pH of the stomach, which aids the reduction of ferric iron to ferrous iron; ascorbic acid (vitamin C) enhances this process. Enterocytes absorb nonheme iron in the ferrous form; any iron that has been oxidized to Fe^{3+} in the alkaline intestinal lumen is first reduced to Fe^{2+} by a brush-border ferric reductase (Fig. 24-11). Ferrous iron is then transported into the

enterocyte by means of a divalent metal iron transporter called *DMT1*, which also transports copper, zinc, manganese, and lead.

Fate of Iron Within Enterocytes. Iron, whether derived from heme or nonheme sources, has two potential fates within the enterocyte. Some of the iron is reoxidized to Fe^{3+} by hephaestin and exported by ferroportin for transport on transferrin. The remainder is bound to ferritin and trapped within the enterocyte. This ferritin-sequestered iron is ultimately excreted when the enterocytes reach the end of their life cycle and are sloughed into the intestinal lumen.

Delivery of Iron to Cells. The circulating iron : transferrin complex binds to transferrin receptors on the plasma membrane of hepatocytes, cells of the reticuloendothelial system and other cells, and is internalized by receptor-mediated endocytosis.

24.5 REGULATION OF HEME AND IRON METABOLISM

24.5.1 Regulation of Heme Synthesis

ALA synthase catalyzes the regulated step of heme synthesis. Both the synthesis and activity of the enzyme are inhibited by heme and by hemin (which contains ferric iron). There are two isoforms of ALA synthase: ALAS1 in nonerythroid cells and ALAS2 in erythroid cells and their expression is regulated differently. Essentially all of the heme made by erythroid cells is committed to hemoglobin synthesis. Hypoxia and erythropoietin increase heme synthesis in erythroid cells. ALAS2 mRNA contains an iron-responsive element (IRE) in its 5′-untranslated region (UTR) and is responsive to the intracellular availability of iron. Heme synthesis is also coordinated with globin-chain protein synthesis.

By contrast, most of the heme synthesized in hepatocytes is incorporated into cytochromes of the electron-transport chain and P450-type cytochromes involved in the detoxification of xenobiotics. The expression of ALAS1 in hepatocytes is increased in response to many of the drugs and toxins that are metabolized in the liver (e.g., carbon tetrachloride, phenobarbital, acetaminophen, some oral contraceptives).

24.5.2 Iron Homeostasis

Since there are no mechanisms to eliminate excess iron from the body, iron homeostasis is regulated primarily by controlling the uptake of dietary iron by the intestine and release of iron from stores in the liver. Furthermore, all cells regulate their intracellular iron concentrations, a process necessary for preventing toxicity associated with iron-catalyzed free-radical reactions.

Iron metabolism is regulated by two iron-binding proteins, IRP-1 and IRP-2 (Fig. 24-12). IRP-1 is actually a cytosolic form of the iron-containing protein aconitase, the enzyme normally thought of in the context of the tricarboxylic acid cycle and energy metabolism, while IRP-2 lacks aconitase activity. Both IRP-1 and IRP-2

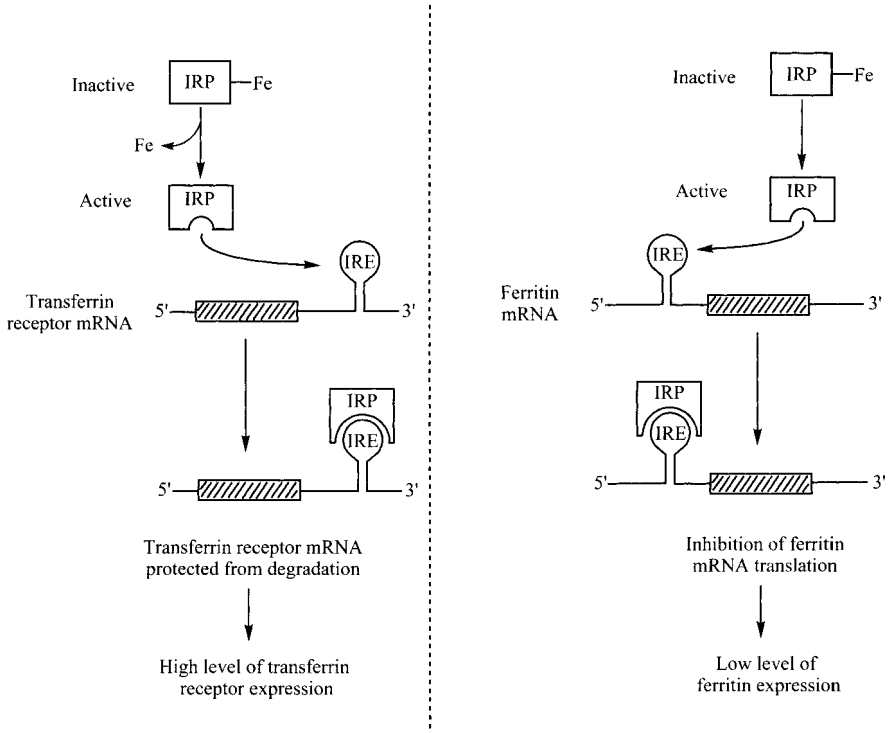


FIGURE 24-12 Regulation of iron stores in hepatocytes when there is iron sufficiency.

act by binding to iron-responsive elements in mRNAs and play similar roles in post-transcriptional gene regulation.

24.5.2.1 How Do Hepatocytes Regulate Their Iron Stores? The synthesis of transferrin and ferritin from their respective mRNA molecules is regulated by the iron-binding proteins IRP-1 and IRP-2. When these proteins bind iron, they are inactive (Fig. 24-12). However, in the iron-depleted state, the apo-IRPs bind to stem–loop structures: in particular, mRNA molecules. Binding of IRP to iron-responsive elements (IRE) in the 5′-untranslated region of ferritin mRNA inhibits initiation of translation, thus decreasing ferritin synthesis. By contrast, the mRNA for the transferrin receptor has IRE elements in the 3′-untranslated region; binding of apo-IRP to these IREs stabilizes the mRNA molecule and protects it from degradation. The result of these two regulatory processes is such that when iron stores are low, hepatocytes synthesize transferrin receptors but not ferritin, thereby enabling iron to be taken up from the blood and made available for heme synthesis (Fig. 24-12). Conversely, when the intracellular iron concentration is high, the two regulatory processes act to inhibit iron uptake (by down-regulating transferrin receptor expression) and increase storage of excess iron in the form of ferritin.

24.5.2.2 How Does the Intestine Regulate the Uptake of Dietary Iron into the Body? As described earlier, uptake of iron into the enterocyte requires the activity of the transporter DMT1. Furthermore, absorbed iron can either be transferred to transferrin in the blood by ferroportin or sequestered in ferritin and eventually excreted. Developing enterocytes in the crypt of the intestinal villus are programmed by the IRP-IRE mechanism to synthesize the appropriate amounts of DMT1, ferritin, and ferroportin. This programming provides a direct response to the levels of dietary iron, since when mucosal cells have accumulated iron, additional uptake into the body is down-regulated.

Furthermore, when iron stores are abundant, hepatocytes synthesize a protein named *hepcidin* and secrete it into the blood. Hepcidin travels to the enterocytes, where it forms a complex with ferroportin. The ferroportin : hepcidin complex is internalized and degraded; the end result of this process is to decrease release of iron from enterocytes into the blood. Hepcidin also binds to ferroportin in the plasma membrane of macrophages, thereby decreasing the release of iron from macrophages that have catabolized hemoglobin contained in senescent red blood cells. An additional, although not yet well-characterized mechanism for regulation of intestinal iron metabolism involves a soluble regulatory molecule produced by erythroid cells in response to anemia.

24.6 DISEASES INVOLVING HEME AND IRON METABOLISM

24.6.1 Iron Deficiency Anemia

Iron deficiency can result from inadequate intake of iron or loss of iron resulting from hemorrhage (e.g., gastrointestinal-tract bleeding) or excessive menstrual blood loss. Globally, inadequate dietary intake of iron remains the major cause of iron deficiency, especially where the diet is largely cereal-based and contains little meat. Iron-deficiency anemia is a major cause of pregnancy-related mortality in developing countries. Infants beyond 1 year of age who are fed solely breast milk or cow's milk without iron supplementation are also at risk of anemia. Although certain foods contain phytates, oxalates, and tannins that chelate nonheme iron, thereby rendering the iron less available for absorption, malabsorption of iron is uncommon in the absence of disease of the small intestine (e.g., celiac disease, enteritis, parasitic infection). Persistent achlorohydria (decreased HCl secretion by the stomach) may result in iron deficiency because low pH increases the solubility of iron and promotes the release of protein- and mucin-bound iron in the gastrointestinal tract.

Hemorrhage is the leading cause of excess loss of body iron. Indeed, in men and postmenopausal women, the differential diagnosis of iron deficiency starts with gastrointestinal cancer. Younger women tend to have lower body stores of iron than men and lower plasma ferritin levels; this difference is due to menstrual blood loss. Excessive iron loss during menstruation, or the iron requirements of frequent pregnancies, can also result in iron deficiency and anemia.

In iron-deficiency anemia, red blood cells are small (microcytic) and pale (hypochromic). Prior to development of frank anemia, the most sensitive clinical indicator of emerging iron deficiency is a plasma ferritin concentration that falls below the reference range. When iron stores become so low as to compromise erythropoiesis, there is an increase in the serum concentration of transferrin (increased iron binding capacity) and a decrease in transferrin saturation (less than the normal 30% of the iron-binding sites of transferrin occupied by iron atoms). Iron deficiency is commonly treated by supplementing the diet with ferrous salts (e.g., ferrous sulfate) and juices containing ascorbic acid and citric acid which enhance iron absorption.

24.6.2 Hereditary Hemochromatosis

Hereditary hemochromatosis (HH), a relatively common autosomal recessive disease in Caucasians, is characterized by progressive accumulation of excess iron in cells of the liver, heart, pancreas, and other organs. Menstrual blood loss explains why women are usually less severely affected than males. The clinical hallmarks of HH include cirrhosis, diabetes mellitus, cardiac failure, and bronzing of the skin. In people with HH, the total serum iron concentration and the ferritin level are increased, and the transferrin saturation is usually >50% and often >90%.

Classic hemochromatosis is the result of mutations in the gene for a protein called *HFE*. Normally, HFE binds to the transferrin receptor and reduces the affinity of the receptor for transferrin. The HH mutations cause loss of function of HFE and increased absorption of iron by the liver. This, in turn, down-regulates hepcidin synthesis and ultimately increases uptake of iron from the intestine into the blood. Interestingly, a genetic deficiency of hepcidin results in a juvenile form of hemochromatosis that is associated with a severe form of iron overload.

The only therapy recommended for HH is routine phlebotomy, with the removal of one unit (0.5 L) of whole blood (approximately 250 mg of iron) weekly or biweekly until iron stores return to normal levels. This therapy is quite effective if initiated prior to end-organ damage.

24.6.3 Hemosiderosis

Hemosiderin is an intracellular iron-storage complex that contains denatured ferritin (Fig. 24-2). Hemosiderin accumulates in macrophage following the increased phagocytosis of red blood cells associated with hemorrhage. Accumulation of hemosiderin in liver is associated with conditions of iron overload, as can occur in people who receive frequent blood-transfusion therapy for sickle cell anemia or thalassemia, as well as in patients with hemochromatosis.

24.6.4 Lead Poisoning

Lead inhibits both ALA dehydratase and ferrochelatase, thereby reducing heme synthesis and resulting in microcytic, hypochromic anemia. Plasma ALA and erythrocyte protoporphyrin concentrations are increased in people with lead poisoning.

Furthermore, since heme is the prosthetic group of many enzymes and proteins, including the cytochromes of the mitochondrial electron-transport chain, lead poisoning can also have detrimental effects on energy metabolism. Lead is especially toxic to the nervous system, probably due to accumulation of δ-aminolevulinic acid as well as to impaired energy metabolism.

24.6.5 Porphyrias

Porphyrias are a family of genetic diseases caused by deficiencies of the various enzymes in the pathway of heme synthesis, with resulting accumulation of intermediate metabolites. Depending on the particular gene affected, porphyrias can affect heme synthesis in all cells or be primarily either hepatic or erythropoietic. Most porphyrias are associated with nervous system pathology. Accumulation of porphyrinogens in the skin can lead to photosensitivity.

24.6.5.1 Acute Intermittent Porphyria. Acute intermittent porphyria is caused by a defect in *porphobilinogen deaminase* (also called *hydroxymethylbilane synthase*). It is associated with elevated δ-aminolevulinic acid and PBG levels and results in severe neurological symptoms. People with acute intermittent porphyria have periodic crises that are usually precipitated by drugs such as barbiturates and sulfonamides that induce the synthesis of heme-containing cytochrome P450 enzymes.

24.6.6 Jaundice

Jaundice (also known as *icterus*) is a condition of impaired heme catabolism. It is characterized by a yellow color of the skin and sclerae of the eyes that is the result of an elevated plasma concentration of bilirubin. Bilirubin toxicity or kernicterus occurs when the plasma level of bilirubin is high enough to result in transfer of excess bilirubin to membrane lipids, particularly in the brain. Jaundice can be a symptom of many different clinical problems.

24.6.6.1 Prehepatic Jaundice. In hemolytic anemias, the excess breakdown of red blood cells results in the production of abnormally large quantities of bilirubin, which may overload the liver's capacity to conjugate bilirubin. As a result, the plasma concentration of unconjugated bilirubin rises. Unconjugated bilirubin may also spill over into bile and increase the risk of developing pigmented gallstones (calcium bilirubinate).

Genetic causes of hemolytic anemia include sickle cell anemia (especially during sickling crises), thalassemia, and hereditary spherocytosis, which is a defect in a red cell membrane protein that results in premature breakdown of the red cells. Inborn errors of metabolism that cause hemolytic anemia include inadequate activities of pyruvate kinase (required for the generation of ATP) and glucose 6-phosphate dehydrogenase (required to generate NADPH and thus maintain adequate levels of reduced glutathione). Hemolytic anemia can also result from infections (particularly malaria), certain drugs (e.g., primaquine), autoimmune reactions, and poisons (e.g., paraquat).

Low plasma levels of haptoglobin are diagnostic for hemolytic anemia. Excess hemoglobin released into the blood binds to haptoglobin and the resulting hemoglobin–haptoglobin complex is cleared by the reticuloendothelial system, thus removing haptoglobin from the circulation. An older diagnostic approach relies on finding increased urinary and fecal levels of urobilinogen, reflecting increased secretion of conjugated bilirubin by the liver and subsequent bacterial metabolism of that bilirubin in the intestine.

24.6.6.2 Hepatic Jaundice. Impaired liver function is one of the major causes of jaundice. Hepatitis and cirrhosis impair the ability of hepatic UDP-glucuronyl transferase to conjugate bilirubin. The secretion of conjugated bilirubin into the bile is also compromised. As a result, both unconjugated and conjugated bilirubin accumulate in the blood, while fecal and urinary urobilinogen levels are decreased.

Hepatic jaundice can also result from deficiency of one or more of the enzymes involved in the metabolism and excretion of bilirubin. Three of these diseases are related to defects in the activity of UDP-glucuronyl transferase (also called UGT1A1) and result in elevated levels of unconjugated bilirubin. The most common of these diseases is Gilbert syndrome, which is generally benign, with affected persons exhibiting mild elevations in unconjugated bilirubin. By contrast, Crigler–Najjar (CN) syndrome type I patients have essentially no UGT1A1 activity and are unable to conjugate any bilirubin. CN type II patients produce a mutated protein that retains residual glucuronyl transferase activity.

24.6.6.3 Posthepatic Jaundice. Obstruction of the common bile duct due to a stone or (less commonly) a tumor results in posthepatic jaundice. The backup of conjugated bilirubin in the liver results in abnormal spillage of conjugated bilirubin into the blood and its excretion in the urine, thereby imparting a dark color. By contrast, lack of biliary excretion results in pale stools that lack normal pigmentation. Prolonged obstruction of the biliary system can lead to liver damage and result in increased plasma levels of both unconjugated and conjugated bilirubin.

Dubin–Johnson syndrome and Rotor syndrome are two rare but benign genetic diseases associated with elevated levels of conjugated bilirubin. They are caused by reduced function of the ATP-dependent pump that transports conjugated bilirubin across the bile canaliculus from hepatocytes into bile.

24.6.6.4 Neonatal Jaundice. Jaundice is common in infants, but it is usually benign and self-limiting. Hyperbilirubinemia often occurs because of a combination of two factors: breakdown of fetal hemoglobin as it is replaced by adult hemoglobin, and limited hepatic conjugation of bilirubin.

Premature infants often develop more severe jaundice, due primarily to hepatic immaturity. It is important that the gene for UDP-glucuronyl transferase not be activated early in fetal development, when it would be undesirable to produce conjugated bilirubin and secrete it into the biliary system; instead, bilirubin produced by the fetus is transferred to the maternal circulation. The activity of UDP-glucuronyl transferase normally increases just before birth and continues to increase after birth. In premature

infants, the activity of UDP-glucuronyl transferase is often low, leading to impaired conjugation and secretion of bilirubin and elevated plasma levels of unconjugated bilirubin.

Neonatal jaundice can also result from hemolytic diseases of the newborn, usually associated with blood-group (i.e., ABO, Rh) incompatibilities between mother and child. If during pregnancy a small amount of fetal blood enters the maternal circulation, the mother may produce antibodies against fetal blood cells. If maternal antibodies then pass through the placenta to the fetus, they will produce hemolysis in the fetus or neonate.

Phototherapy continues to be the standard treatment of neonatal hyperbilirubinemia. The effectiveness of this form of therapy is based on the ability of photons to convert bilirubin IXα into photoisomers that are more water-soluble. Because of their increased solubility, these bilirubin photoproducts can also be excreted by way of the liver without requiring glucuronic acid conjugation. Blue light (approximately 450 nm), longer-wavelength green light, and more commonly, fluorescent white light have been used for phototherapy.

CHAPTER 25

INTEGRATION OF METABOLISM

25.1 BALANCE BETWEEN ENERGY PRODUCTION AND UTILIZATION IN HUMAN METABOLISM

The major role of metabolism is to capture chemical energy from foodstuffs as ATP and utilize that ATP for a variety of essential functions, including synthesis of cellular components, active transport of ions and solute, and muscle work. Humans can generate ATP by oxidizing carbohydrates, fatty acids, and amino acids. At the simplest level, energy homeostasis involves a balance between dietary fuel intake and energy expenditure so that the body is neither fuel-depleted (starvation) nor storing excess triacylglycerol (obesity). Since humans do not eat continuously, dietary fuels in excess of immediate needs are therefore processed and stored for subsequent use. Consequently, specific metabolic pathways must be regulated and the activities of different organs coordinated to satisfy the needs of the body.

25.1.1 How Much Energy Can One Get From Different Metabolic Fuels?

The chemical energy in foods is measured in kilocalories (kcal), sometimes denoted Calories (1 Calorie = 1 kcal). Since 1 kcal is defined as the energy required to raise the temperature of 1 kilogram of water by 1 degree Celsius, one can determine the energy content of different chemicals by measuring their heat of combustion in vitro in a calorimeter or by determining physiologically how much heat a person

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generates when a particular fuel is being oxidized. The values used by nutritionists and nutritional biochemists are:

Carbohydrates	4 kcal/g
Triacylglycerol	9 kcal/g
Protein	4 kcal/g
Ethanol	7 kcal/g

For carbohydrates, triacylglycerol (fat), and ethanol, the physiological fuel values are approximately equal to their respective heats of combustion. The actual heat of combustion of protein is close to 5.4 kcal/g; the lower physiological value reflects the energy cost of excreting nitrogen as urea. (The synthesis of urea from ammonia, CO_2, and the amino group of aspartate requires four high-energy phosphate bonds.)

25.1.2 What Are the Fuel Stores of a Normal Person?

25.1.2.1 Carbohydrates. In the fed state, the reference standard 70-kg male has about 300 g of glycogen stored in his muscles and 100 g in his liver, with only minor quantities in adipose tissue and the brain.

25.1.2.2 Triacylglycerols. Since triacylglycerols (TAG) have a higher energy content than carbohydrates (9 kcal/g vs. 4 kcal/g) and are stored without hydration, they provide a much more compact form of energy storage than glycogen. Normal body stores of TAG total approximately 15 kg, or 135,000 kcal, compared to only 1600 kcal for glycogen. Although nearly all of this fat is stored in adipocytes, skeletal muscle and liver each contain about 50 g of triacylglycerol, and trained endurance athletes have even greater amounts of intramuscular triacylglycerol. Unlike the storage of carbohydrate as glycogen, the body has a virtually unlimited capacity to store TAG. An imbalance between energy intake and energy expenditure underlies the current epidemic in obesity.

25.1.2.3 Protein. Although there are no stores of proteins as such in the body, some of the normal cellular proteins are mobilized when amino acids are required for other needs, such as synthesis of new protein and providing carbon skeletons for gluconeogenesis. Most of the mobilizable proteins are found in skeletal muscle (6 kg) and in liver (0.1 kg). In cases of starvation and severe negative nitrogen balance, heart muscle proteins may also be degraded.

25.1.3 The Respiratory Quotient Can Be Used to Assess Which Fuels Are Being Utilized at a Particular Time

The nature and quantities of fuels being utilized at a particular time by an organism can be estimated using "indirect calorimetry," which measures oxygen consumption and carbon dioxide production rather than heat generation during a defined interval. The overall equations for the complete oxidation of glucose and a typical triacylglycerol

molecule, in this case triolein, are used to determine the *respiratory quotient* (RQ = CO_2/O_2) for each reaction:

	$RQ = CO_2/O_2$
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$	1.0
$C_{57}H_{104}O_6 + 80O_2 \rightarrow 57CO_2 + 52H_2O$	0.71

25.1.3.1 What Can We Learn from RQ Data? A fasted person at rest has an RQ of approximately 0.75. Based on the equations above, the RQ value indicates that this person is primarily oxidizing fat. By contrast, when the same person begins running rapidly, say on a treadmill, the RQ value will rise to nearly 1.0, indicating that he or she is utilizing mostly carbohydrates (i.e., glycogen, blood glucose). An RQ of 0.85 indicates that a person is utilizing a mixture of carbohydrates and fats.

25.1.3.2 Don't These Calculations Ignore Amino Acid Oxidation? Many studies use first approximations to estimate the proportions of the various fuels being oxidized and ignore the contribution of protein as a fuel, primarily because considering protein oxidization complicates the picture. Consider the oxidation of alanine:

	RQ
$C_3H_7O_2N + 6O_2 \rightarrow (NH_2)_2CO + 5CO_2 + 5H_2O$	0.83

The only way to accurately estimate the extent to which amino acids are oxidized is to measure urinary excretion of urea (usually over a 24-hour period). By subtraction, one then corrects for the amounts of O_2 consumed and the CO_2 produced during generation of the measured quantity of urea. The remaining O_2 and CO_2 are then use to calculate a "nonprotein" RQ value that can be used to determine the contributions of carbohydrate and fat to total metabolism.

For normal persons consuming a typical American diet, protein usually provides 12 to 20% of metabolic fuel. Thus, an RQ of 0.83 indicates that a person is obtaining approximately half of his or her calories from carbohydrate and half from fat rather than 100% from protein.

25.2 WHAT ARE THE MAJOR PHYSIOLOGICAL CONDITIONS THAT AFFECT FUEL UTILIZATION?

25.2.1 Fasting or Basal State

The *basal metabolic rate* (BMR) is the minimum energy expenditure required for involuntary work of the body (e.g., pumping of the heart, maintenance of ion gradients, protein turnover). BMR is measured in the morning, while the subject is in a prone position and has fasted for at least 12 hours. The measurement is made at

an ambient temperature, where shivering thermogenesis and sweating is minimized. For convenience, the *resting energy expenditure* (REE) is usually measured instead of BMR; measurement of REE requires a less stringent fast (2 to 4 hours) and gives slightly higher values. One can approximate the BMR for a given person as 1 kcal/kg per hour for men and 0.9 kcal/kg per hour for women, or 1680 and 1200 kcal per day, respectively, for a 70-kg (154-lb) man and a 56-kg (124-lb) woman. The gender difference in BMR is due to the relatively greater adipose stores and lower muscle mass of women than men. Indeed, the BMR correlates primarily with lean body mass and can be increased by exercise, which promotes accrual of muscle.

25.2.2 Fed State

The resting metabolic rate is higher when measured in a person who has recently eaten a meal. The difference, sometimes referred to as the *thermic effect* of food, reflects the extra energy required for the digestion, transport, and storage of dietary fuels, including the active transport of solutes into cells and the activation of molecules (i.e., glucose to glucose 6-phosphate, fatty acids to acyl-CoAs). The thermic effect of food increases energy expenditure over BMR by 10 to 15%, depending on the person and the diet, with protein-rich foods requiring the greatest amount of energy to process and dietary TAG the least.

25.2.3 Physical Activity

Voluntary movement, including normal daily activities, fidgeting, and purposeful exercise, increases energy expenditure. Physical activity is the most variable component of a person's daily energy expenditure, and represents 20 to 40% of the total for the average person. Physical activity is also the only component of total energy expenditure that is easily altered. Energy expenditure during exercise is affected by the nature of the activity itself (running vs. walking); the intensity, duration, and efficiency of the activity; and the person's body mass.

25.3 ROLES OF DIFFERENT ORGANS IN THE INTEGRATION OF METABOLISM

The metabolism of certain cells changes little between physiological states. Red blood cells, for example, depend exclusively on glycolysis in both fasted and fed states. Similarly, the brain is dependent primarily on glucose as a fuel and does not begin to use significant amounts of ketones until day 3 or 4 of a fast. By contrast, most other cells and organs alter their pattern of fuel utilization, fuel storage, and fuel export to meet the current needs of those cells and of the body as a whole in various physiological states (Table 25-1).

TABLE 25-1 Major Organs Involved in Integration of Fuel Metabolism

Organ	Major Fuel Store	Fuel Exported	When Exported
Adipocytes	TAG	Free fatty acids and glycerol	Fasting, moderate-intensity exercise
Liver	Glycogen	Glucose	Fasting, exercise
		Ketones	Fasting
		VLDL-TAG	Fed state
Skeletal muscle	Glycogen	Lactate	Intense exercise
	Protein[a]	Alanine, glutamine	Fasting

[a]Mobilizable structural proteins.
VLDL, very low-density lipoprotein; TAG, triacylglycerol.

25.3.1 Liver

The liver plays a major role in all aspects of energy metabolism. When glucose is plentiful, the liver utilizes glucose as fuel, stores glycogen, and metabolizes excess glucose to acetyl-CoA. The acetyl-CoA, in turn, is used to synthesize fatty acids and ultimately TAG, which is exported from the liver in the form of VLDL. By contrast, when glucose is required by other cells, the liver switches to utilizing fatty acids to generate energy, mobilizes glycogen stores to maintain plasma glucose levels, and begins synthesizing both glucose and ketones. Utilization of the carbon skeletons of amino acids such as alanine and glutamine for gluconeogenesis is accompanied by conversion of their amino groups to urea.

25.3.2 Adipose Depot

Triacylglycerols are the major fuel stores of the body, and adipocytes are the major site of triacylglycerol storage. In response to hormonal (e.g., glucagon, hydrocortisone) and neuroendocrine (epinephrine) stimulation, free fatty acids are released when needed: for example, during fasting or to meet the increased energy demands of exercise, stress, and trauma. The glycerol generated when TAG is hydrolyzed is available to the liver for gluconeogenesis. By contrast, in the fed state, the body directs dietary fatty acids and glucose into triacylglycerol stores. Lipoprotein lipase in adipose capillaries hydrolyzes the TAG of VLDL: the fatty acids thus released are taken up by adipocytes, incorporated into TAG and stored. In the fed state, adipocytes also oxidize glucose, both to provide the glycerol backbone of TAG and to generate acetyl-CoA for a modest amount of fatty acid synthesis.

25.3.3 Skeletal Muscle

25.3.3.1 Muscle in the Fed State. When glucose (and insulin) levels rise, muscle cells take up glucose via GLUT4 transporters and store that glucose as glycogen. Eating a meal and the subsequent rise in circulating insulin levels also stimulate uptake of amino acids into muscle and promote protein synthesis.

25.3.3.2 Muscle in the Fasted State. During an overnight (or longer) fast, skeletal muscle plays a major role in providing fuel to other organs, including the brain. Since muscle lacks glucose 6-phosphatase, muscle glycogen cannot be used to maintain plasma glucose levels. There is, however, considerable catabolism of muscle proteins during a fast. The carbon skeletons of branched-chain amino acids are primarily utilized as fuel by muscle, whereas alanine and glutamine are exported to support gluconeogenesis in liver and kidney, respectively. In the fasted state, muscles also use plasma free fatty acids and ketones to satisfy their fuel needs.

25.3.3.3 Exercising Muscle. Physical activity requires muscles to markedly increase the rate of ATP production. The mixture of fuels used by the muscle is dependent on both the intensity and duration of the exercise.

Sprinting. The immediate source of energy for muscles during a rapid sprint is ATP itself, along with the modest intramuscular stores of creatine phosphate, which can sustain a 6-second sprint. Muscle glycogen is also used by a sprinter, and under intense activity, muscle exports lactate into the circulation.

Walking and Similar Moderate Exercise. Fatty acids are the preferred substrates for exercise up to about 50% of $VO_{2\,max}$ (the maximum amount of oxygen the body can use).

Moderate-Intensity Exercise. As the rate of sustained exercise increases from 65% to 85% of $VO_{2\,max}$, the relative contribution of carbohydrate to total metabolism increases, with the ratio of ATP generated from carbohydrate and fat oxidation being in the range 40:60 to 60:40. As muscle glycogen is depleted, there is greater reliance on a mixture of bloodborne fatty acids and bloodborne glucose, with a concomitant drop in RQ from > 0.9 to as low as 0.75. Under these conditions, muscle fatigue may occur if the workload intensity is not decreased. It should be noted that it is only the glycogen stored in the exercising muscles that is depleted; the amount of glycogen in less active muscles (i.e., the arm muscles of a bicyclist) does not decrease.

Adaptations with Athletic Training. Highly fit persons (e.g., triathletes) are able to exercise at greater workloads and sustain their activity for long intervals. Physically fit persons have greater intramuscular stores of both glycogen and TAG than those of relatively inactive persons. They also have an increased $VO_{2\,max}$ values which results in the same level of exercise (i.e., speed of running) occurring at a lower VO_2, thus permitting a greater reliance on fatty acids than glucose to satisfy their energy needs.

25.3.3.4 Heart Muscle. Although the heart is never at rest, its metabolism is similar to that of the skeletal muscles of a person at rest, in that when the body is at rest the heart preferentially utilizes free fatty acids as fuel. Cardiac glycogen stores are mobilized for the greater cardiac work that exercise demands.

25.4 INTEGRATION OF ORGAN METABOLISM IN DIFFERENT PHYSIOLOGICAL STATES

Coordination of the metabolic activities of different organs serves to support glucose homeostasis and provide a steady supply of glucose to meet the needs of the brain and erythrocytes, both of which are constantly dependent on glucose as fuel. Integrated metabolism also serves to store fuel efficiently in times of plenty in order to provide for periods of fuel scarcity or times of high energy utilization.

25.4.1 Fasting State

Figure 25-1 shows the role of different organs in the coordinated metabolism of the basal metabolic state, when a person is at rest after an overnight fast. During the night, glucagon stimulates glycogenolysis and the glycogen stores in the liver become depleted. By morning, the major source of plasma glucose is hepatic (and

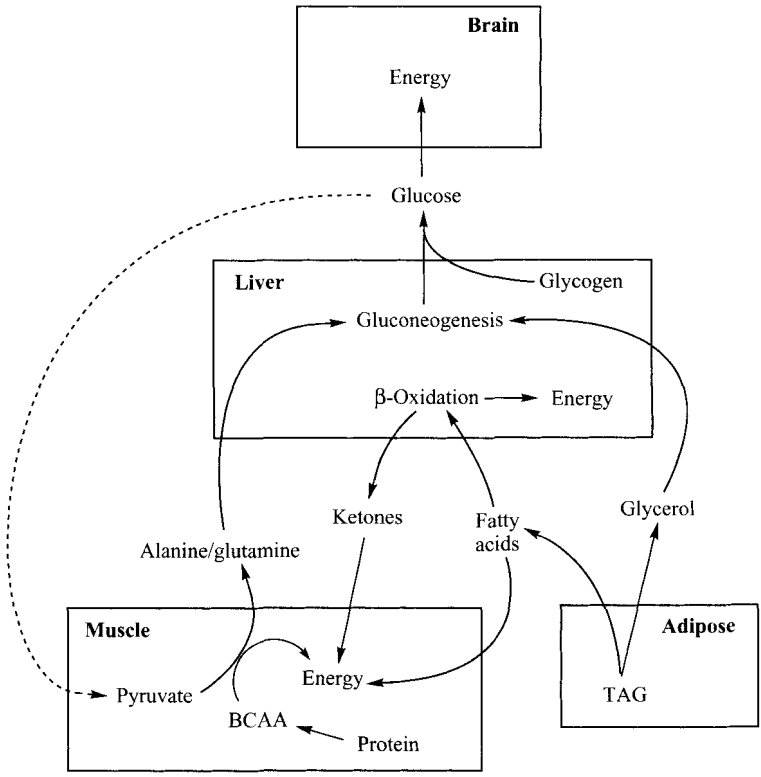

FIGURE 25-1 Major metabolic pathways in the fasted state. The dashed line represents utilization of glucose as a component of the alanine cycle. BCAA, branched-chain amino acids; TAG, triacylglycerols.

to some extent, renal) gluconeogenesis. Substrates for gluconeogenesis are provided by adipocytes (glycerol), muscle (alanine and glutamine), and erythrocytes (lactate). The increase in the rate of protein catabolism in muscle that is associated with gluconeogenesis is accompanied by increased hepatic synthesis of urea. β-Oxidation of fatty acids provides the large amounts of ATP required for both gluconeogenesis and ureagenesis. As a result of diversion of oxaloacetate from the TCA cycle to gluconeogenesis, the liver uses most of the acetyl-CoA from β-oxidation to synthesize ketones.

During a fast, free fatty acids mobilized from TAG in adipocytes are the major fuel supply for organs other than the brain and erythrocytes. As the fast continues, skeletal muscle cells oxidize a mixture of free fatty acids released from adipocytes, ketones produced by the liver, and branched-chain amino acids generated through catabolism of muscle proteins. Muscle cells also require a certain amount of glucose to generate the carbon skeleton of alanine, which is the means by which they export the amino groups released during the catabolism of the branched-chain amino acids.

25.4.1.1 Why Doesn't the Brain Oxidize Fatty Acids? Erythrocytes lack mitochondria and therefore cannot utilize either β-oxidation or the TCA cycle, both of which are mitochondrial processes. By contrast, although neural cells do have mitochondria, they do not use free fatty acids as an energy source during fasting because free fatty acids and other lipophilic substances do not readily pass through the blood–brain barrier. Thus, the mechanism for protecting the brain from a variety of deleterious substances renders the brain strongly dependent on a constant supply of glucose fuel.

25.4.1.2 What Happens During Prolonged Fasting? The changes in fuel utilization that occur with long-term fasting are referred to collectively as the *adaptation to starvation* (Fig. 25-2). Circulating levels of ketones rise markedly during the first few weeks of a prolonged fast and the brain begins to use ketones as well as glucose as fuel; after 2 to 3 weeks of fasting, ketones can satisfy as much as two-thirds of the energy requirement of the brain. Although the brain still does not use free fatty acids directly, neural oxidation of ketones represents, in essence, the brain's ability to utilize some of the energy originally stored in fatty acids. Increased ketone availability to the brain is facilitated by muscle, which stops oxidizing ketones and turns almost entirely to free fatty acids for energy. As this transition occurs, there is less demand for glucose by the brain and a concomitant decrease in the rate of catabolism of muscle proteins to provide gluconeogenic substrate for the liver and kidney. At the same time, relatively more of the amino acid–derived nitrogen excreted in the urine will be in the form of ammonium ions rather than urea. The ammonium ions buffer urinary acetoacetic acid and β-hydroxybutyric acid. Renal production of ammonium ions is directly coupled to an increase in renal use of the carbon skeleton of glutamine for gluconeogenesis.

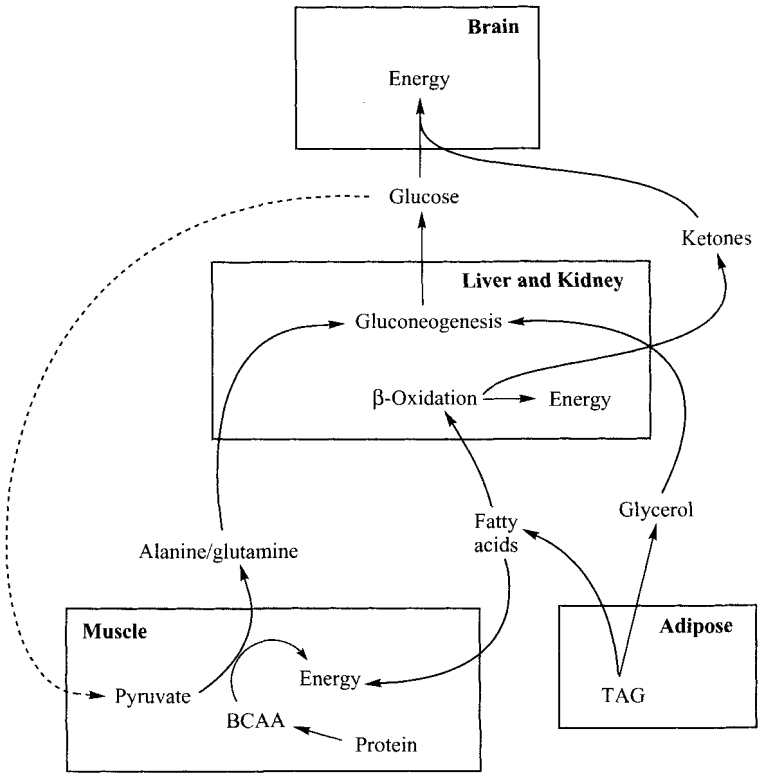

FIGURE 25-2 Metabolic pathways during prolonged starvation. The dashed line represents utilization of glucose as a component of the alanine cycle. BCAA, branched-chain amino acids, TAG, triacylglycerols.

25.4.2 Metabolism in the Fed State

The changes in metabolism in various organs that occur after ingestion of a mixed meal (carbohydrate, fat, and protein) reflect the assimilation of these nutrients and their processing for both immediate utilization and storage (Fig. 25-3).

25.4.2.1 Liver. When the plasma glucose concentration is high, the liver extracts glucose from the blood. Some of that glucose is used for glycogen synthesis; the remainder is oxidized to acetyl-CoA and used primarily for fatty acid synthesis. The resulting long-chain fatty acids are secreted from the liver as VLDL triacylglycerol.

In the fed state, the liver utilizes amino acids primarily for protein synthesis. However, with high protein intakes the excess amino acids are catabolized, with their carbon skeletons being converted to fatty acids and their amino groups utilized for urea synthesis.

25.4.2.2 Adipocytes. In the fed state, the adipose depot synthesizes and stores TAG. Free fatty acids are obtained from the exogenous TAG of chylomicrons and the

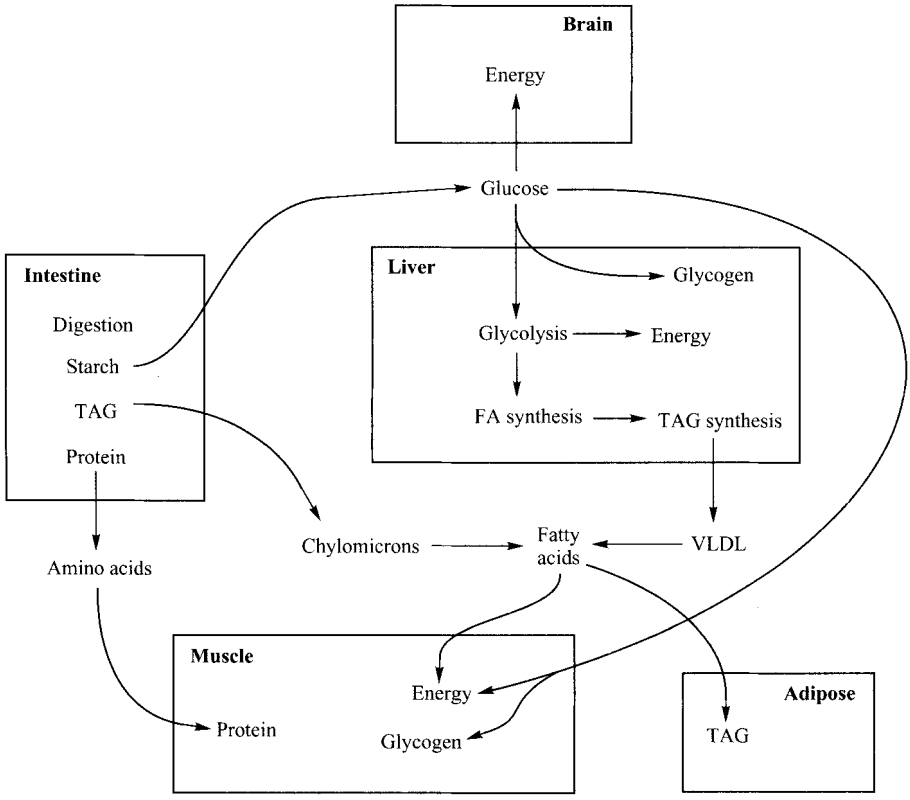

FIGURE 25-3 Major metabolic pathways in the fed state. Substantial amino acid uptake and protein synthesis also occurs in the liver (not shown). FA, fatty acid; TAG, triacylglycerols.

endogenous TAG of VLDL. Glucose is utilized to synthesize the glycerol backbone of TAG as well as the synthesis of long-chain fatty acids.

25.4.2.3 Muscle. When circulating levels of glucose and insulin are increased, muscle extracts glucose from the blood and uses it to synthesize glycogen. Under normal conditions, the synthesis of muscle glycogen functions merely to replenish glycogen stores. However, if carbohydrates are consumed after muscle glycogen has been depleted by strenuous exercise, resynthesis of glycogen may result in even higher glycogen levels than were present prior to the exercise. Athletes commonly refer to this phenomenon as *glycogen loading*.

25.4.3 Metabolism During Moderate Exercise

As discussed above, skeletal muscle can utilize a variety of fuels during exercise. Particularly during short bouts of intense exercise (the 100-meter dash), muscle cells derive energy from creatine phosphate and glycogen stores within the muscle itself.

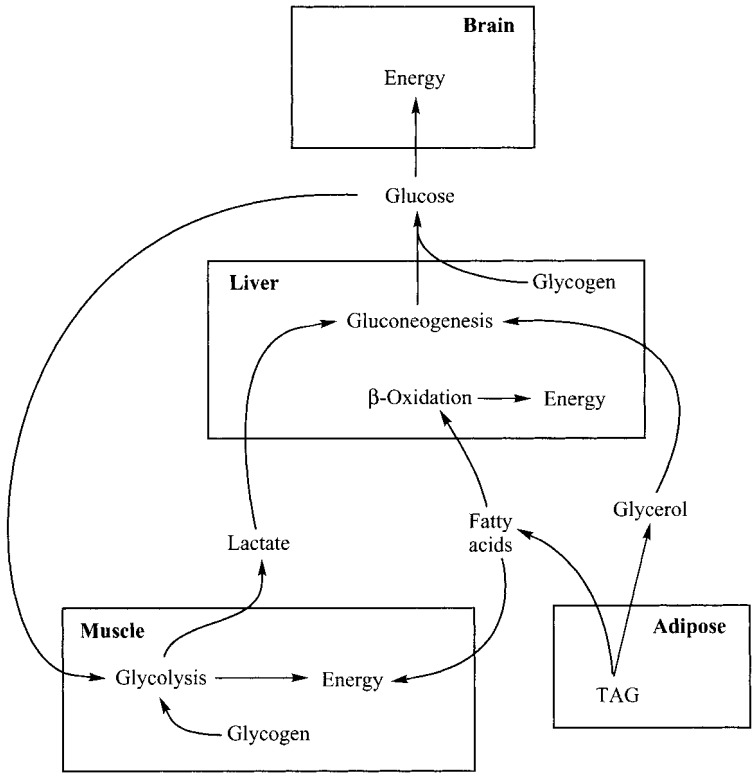

FIGURE 25-4 Major metabolic pathways during exercise. The extent of lactate generation will vary with the intensity of the exercise. TAG, triacylglycerols.

During strenuous exercise, much of the glycolysis that occurs in muscle is anaerobic; the resulting lactate is exported from the muscle and taken up by the liver, where it can either be oxidized further or used as a substrate for gluconeogenesis.

By contrast, moderate exercise relies on circulating free fatty acids and glucose as well as stores within the muscle (Fig. 25-4). TAG stored in adipocytes are the primary source of those free fatty acids, while liver glycogen provides plasma glucose. After 60 to 90 minutes of vigorous running, liver glycogen becomes depleted and hepatic gluconeogenesis is required to maintain plasma glucose levels. Initially, gluconeogenesis utilizes gluconeogenic precursors generated within the liver and glycerol derived from the breakdown of adipose TAG. However, as the exercise period lengthens, there is increased breakdown of muscle proteins to provide alanine and glutamine for gluconeogenesis, with the carbon skeletons of branched-chain amino acids providing an additional fuel source for the muscle. The increased utilization of amino acids for gluconeogenesis is reflected in an increase in hepatic urea synthesis and excretion of urea, primarily in sweat. As expected, people with low carbohydrate stores will excrete more urea during exercise than will those who are carbohydrate replete when they begin exercising.

25.4.3.1 Burning Glucose Versus Fat: Which Is Better for Weight Loss?

It is common for people wishing to lose weight to seek an exercise regime that burns fat rather than glucose. It turns out, however, that the crucial issue in exercise is total caloric expenditure. Most types of exercise promote utilization of a mixture of fuels, although more moderate exercise (brisk walking as opposed to running) utilizes relatively more fatty acid than glucose. To the extent that one utilizes free fatty acids during exercise, one decreases fat stores proportionally. Oxidation of glucose during moderate-duration exercise tends to deplete muscle and liver glycogen stores. When the next meal is consumed, the glycogen stores of the muscles are replenished, and less of the dietary carbohydrate is converted into fat, thereby resulting in lower adipose triacylglycerol stores than if the person had not exercised.

25.5 REGULATION OF METABOLISM

The regulatory affects of hormones on individual metabolic pathways are discussed in other chapters. Nevertheless, it is useful at this point to consider how hormones coordinate the multiorgan integration of metabolism. In general, insulin is anabolic and acts to stimulate the metabolic responses to the fed state, while a number of other hormones (often referred to as *insulin counterregulatory hormones*) oppose the actions of insulin and coordinate fuel mobilization and energy production during fasting or exercise.

25.5.1 Insulin

Insulin acts on many different tissues and has several effects in each, all of which are consistent with the anabolic needs of the body in the fed state. Thus, insulin stimulates translocation of GLUT4 transporters to the plasma membrane and uptake of glucose into both adipocytes and muscle cells. Insulin increases secretion of lipoprotein lipase by adipocytes, thereby increasing the release of free fatty acids from both chylomicrons and VLDL. Within adipocytes, insulin stimulates glycolysis, a modest amount of fatty acid synthesis, and triacylglycerol synthesis. Concurrently, insulin promotes both glycogen synthesis and glycolysis in muscle cells as well as uptake of amino acids into muscle and protein synthesis therein. Not all tissues are regulated by insulin. In particular, the uptake of glucose into neural cells and subsequent glycolysis are glucose-independent.

Although transport of glucose into hepatocytes is insulin-independent, insulin does stimulate the activity of key regulatory enzymes in the pathways that use glucose (glycogen synthesis, glycolysis) while inhibiting pathways that generate glucose (glycogenolysis, gluconeogenesis). Insulin increases the activity of acetyl-CoA carboxylase, thus stimulating hepatic fatty acid synthesis and subsequent VLDL synthesis. In addition, increased acetyl-CoA carboxylase activity generates malonyl-CoA, which prevents concurrent β-oxidation of fatty acids by inhibiting carnitine palmitoyl transferase (CPT-1). Insulin also stimulates synthesis of the sterol response element–binding proteins, SREBP-1 and SREBP-2, thereby increasing gene transcription of the lipogenic enzymes involved in fatty acid and cholesterol synthesis, respectively.

25.5.2 Glucagon

Glucagon stimulates hepatocytes and adipocytes to release glucose and fatty acids, respectively, into the circulation. In the liver, glucagon stimulates both glycogenolysis and gluconeogenesis while inhibiting the pathways of fuel storage (e.g., glycogen and fatty acid synthesis). In adipocytes, glucagon stimulates lipolysis and release of free fatty acids and glycerol into the circulation.

25.5.3 Epinephrine (Adrenaline)

The synthesis of epinephrine from tyrosine in the adrenal medulla is stimulated by stress, endurance exercise, and hypoglycemia. Epinephrine acts through the same G-protein, cAMP-dependent protein kinase signaling pathway as glucagon and has similar effects in both liver and adipocytes. Unlike glucagon, however, epinephrine also acts on muscle cells. Since muscle cells lack glucose 6-phosphatase, the epinephrine-stimulated breakdown of glycogen results in enhanced glycolysis.

25.5.4 Hydrocortisone

Hydrocortisone, a glucocorticoid synthesized by the adrenal cortex, stimulates fuel mobilization from liver, muscle, and adipocytes. However, unlike glucagon and epinephrine, hydrocortisone acts primarily by regulating gene transcription and mediates longer-term metabolic changes during starvation, sepsis, and stress.

25.5.5 Adipocytokines

Adipose tissue is more than a site for triacylglycerol storage; it is also an endocrine organ. The hormones and cytokines secreted by adipocytes include leptin, adiponectin, adipsin, interleukin-6, and tumor necrosis factor α (TNFα). Production of the various adipocytokines varies with a person's energy status (e.g., normal weight or obese) and the anatomical location of the adipose depot (e.g., visceral or subcutaneous).

Leptin signals energy sufficiency and acts to inhibit further lipid storage in adipocytes and stimulate lipolysis of intracellular TAG. It also decreases appetite. Adipsin (acylation-stimulating protein) has effects opposite to those of leptin; adipsin stimulates glucose uptake and increases the activity of diacylglycerol acyltransferase, thus causing adipocytes to retain fatty acids in the form of TAG.

Adiponectin, another adipokine, increases fatty acid oxidation in both muscle and liver. Increased plasma levels of adiponectin are correlated with higher levels of HDL-cholesterol and lower plasma levels of TAG.

25.5.6 Exercise

In addition to the actions of hormones, sustained physical activity exerts major effects on the regulation of energy metabolism. There are many long-term metabolic adaptations to aerobic exercise, including increased muscle mass, resulting in an increase

in basal energy expenditure, and up-regulation of mitochondrial energy metabolism. Exercise may also increase total energy expenditure by enhancing peroxisomal proliferation (and thus oxidation of long-chain fatty acids not directly coupled to ATP synthesis) and increasing the expression of uncoupling proteins in mitochondria.

One important regulator of muscle metabolism is AMP-kinase, which is activated when ATP depletion results in increased intracellular levels of AMP. AMP kinase inhibits acetyl-CoA carboxylase and lowers the level of malonyl-CoA in the cytoplasm, thereby stimulating fatty acid oxidation by increasing the activity of carnitine palmitoyl transferase-1. AMP-activated protein kinase also increases glucose uptake into the muscle by insulin-independent recruitment of GLUT4 glucose transporters to the plasma membrane. This phenomenon explains why glucose uptake by skeletal muscle is greatly increased during exercise, when there is no increase in the insulin level. Exercise also renders muscle cells more sensitive to insulin, in part by stimulating intramuscular accumulation of TAG, and removing potentially deleterious fatty acid metabolites.

25.6 CONDITIONS WHERE NORMAL METABOLIC INTEGRATION IS IMPAIRED

25.6.1 Obesity

Obesity is the accumulation of excess adipose tissue. It is commonly assessed by calculating the body mass index (BMI), defined as weight (kg)/height $(m)^2$. A BMI of 25 or greater is considered overweight, and a BMI in excess of 30 is considered obese. For a 5ft 4in.-tall woman, overweight would therefore be $>$ 145 lb and obesity $>$ 175 lb. BMI is applicable to virtually all adults, except for highly trained athletes such as body builders who have an unusually large muscle mass.

Obesity is the result of a chronic imbalance between energy intake and energy expenditure. One pound of adipose tissue represents approximately 3500 kcal [454 g $\times$ 9 kcal/g (for TAG) $\times$ 0.85 (the fraction of adipose tissue that is triacylglycerol)]. A person who consumes a caloric excess of 100 kcal/day will therefore gain 10 lb/year. Conversely, a person with a 500-kcal/day caloric deficit will lose approximately 1 lb/week. It should be noted that a person who utilizes 2000 kcal/day cannot be expected to lose more than 4 lb of adipose tissue per week even if he or she is fully fasting. More-rapid weight-loss programs actually represent loss of water and some muscle protein rather than the desired loss of adiposity. Most medical recommendations for weight loss suggest maintaining a healthy diet with a deficit of 500 to 1000 kcal/day.

Although there are certainly wide variations in body structure and metabolism between individuals, the current epidemic of obesity in the United States and many other countries is due more to environmental than to genetic factors. The efficient storage of excess calories as triacylglycerol may have been advantageous for our distant ancestors who were physically very active and for whom food scarcity was often the norm. However, in the current context of sedentary lifestyles and in environments

where there is an overabundance of calorically dense foods containing large amounts of fats and sugars, efficient fuel storage in the form of fat can result in obesity and its undesirable medical and social sequelae.

25.6.2 Type I Diabetes Mellitus

Type I diabetes is caused by absent or insufficient insulin production. Although the most common cause is autoimmune destruction of the β-cells of the pancreas, it can also result from chronic pancreatitis. The resulting insulin deficiency has been described as "starvation in the midst of plenty," with metabolic pathways active in fasting mode despite high plasma levels of nutrients in the blood. A lack of insulin results in fasting hyperglycemia with both overproduction of glucose by the liver and underutilization of glucose by both muscle and adipocytes. The high rate of gluconeogenesis is often accompanied by extensive ketogenesis and severe ketoacidosis. Chronic hyperglycemia results in damage to many organs, including the eyes, kidneys, blood vessels, and nerves.

Diabetes affects lipid and protein metabolism as well as that of glucose. The high glucagon/insulin ratio stimulates adipose triacylglycerol hydrolysis and increases the plasma free fatty acid concentration; increased uptake and reesterification of fatty acids in the liver results in hypertriglyceridemia. At the same time there is increased catabolism of muscle proteins and hyperaminoacidemia.

Currently, the only effective treatment for type I diabetes mellitus is insulin therapy. The hormone is currently provided either by injection or using an insulin pump. Dosage and timing must be adjusted to a person's food intake and exercise, so as to maintain normoglycemia. Determination of glycosylated hemoglobin (HbA1c) is used to measure the efficacy of glucose control.

25.6.3 Insulin Resistance and Type II Diabetes

Most people with diabetes mellitus have type II diabetes rather than type I, and it is type II diabetes that is now reaching epidemic incidence in many countries. Type II diabetes mellitus is characterized by insulin resistance rather than primary insulin insufficiency. A person has insulin resistance when larger-than-normal amounts of insulin are required to support insulin-dependent metabolic processes. Insulin resistance is also commonly seen in the obese and in those with the metabolic syndrome. In most instances of insulin resistance, insulin secretion is not impaired and insulin receptors are functional. Although people in the early stages of insulin resistance can maintain normal blood glucose concentrations by increasing their insulin secretion, this compensation often becomes inadequate and they eventually progress to hyperglycemia and eventually to type II diabetes.

Like type I diabetes, type II diabetes is characterized by hyperglycemia, hypertriglyceridemia, hyperaminoacidemia, and elevated levels of free fatty acids. The high levels of free fatty acids in the blood are the result of increased TAG lipolysis by adipocytes. Elevated free fatty acid levels, in turn, result in increased TAG synthesis by the liver and export of TAG-rich VLDL particles. Unlike people with type

I diabetes, those with type II diabetes usually do not develop ketoacidosis, in part because – at least in the early stages of the disease – the liver is less insulin resistant than skeletal muscle or adipocytes.

Obesity often leads to insulin resistance in muscle. In people with insulin resistance, the muscle cells do not sufficiently up-regulate the acyltransferases involved in triacylglycerol synthesis to cope with the increased availability of free fatty acids. As a result, a high intracellular concentration of metabolic intermediates inhibits glucose uptake and glycolysis by muscle cells, and higher levels of insulin are required for glucose utilization. Although the mechanisms have not been fully elucidated, the relatively insulin-resistant state associated with obesity may be the result of an imbalance in adipokine production as well as elevated plasma levels of free fatty acids released from the excess adipocyte stores.

Exercise stimulates both TAG and glycogen synthesis in skeletal muscle and improves insulin sensitivity in both normal-weight and obese persons. Among the mechanisms involved are up-regulation of GLUT4 transporters and induced expression of diacylglycerol acyltransferase. In fact, moderate exercise and weight-loss regimes are often sufficient to preclude the need for pharmacological intervention in people with milder forms of type II diabetics. Type II diabetes can also be treated with a variety of drugs that stimulate insulin secretion (e.g., sulfonylureas) or increase insulin sensitivity (e.g, thiazolidinediones), or reduce hepatic gluconeogenesis (e.g., metformin). Many cases of type II diabetes, however, eventually progress to the point of pancreatic β-cell failure and dependence on exogenous insulin.

25.6.4 "Starvation Diets"

Weight-reduction plans usually involve a balanced diet that provides a reduced caloric intake and maintains the body's normal adaptations to the fed and fasted states. Under certain conditions, however, it is preferable to adopt a weight-reduction regimen that resembles starvation.

25.6.4.1 Protein-Sparing Modified Fasts (PSMF). Adaptation to starvation involves increased ketone utilization by the brain which decreases—but does not prevent—depletion of muscle protein. PMSF diets are designed to mimic starvation but prevent muscle loss. They usually provide 400 to 800 kcal/day of protein, with essentially no carbohydrates or fat, and are reserved for the morbidly obese (BMI $> 40\,kg/m^2$) who are under medical supervision. The very low caloric intake maximizes weight loss, whereas the provision of exogenous protein provides substrate for gluconeogenesis, thus minimizing muscle wasting.

25.6.4.2 Diets with Only Minimal Carbohydrate. A number of popular low-carbohydrate diets utilize a variation of the PSMF in that the person consumes relatively unlimited protein and fat, but carbohydrate intake is strictly limited. This regimen results in an initial rapid loss of water weight; however, subsequent weight loss depends on maintaining an energy deficit. For some people the loss of appetite that accompanies ketosis aids compliance. Very low carbohydrate diets can be helpful

for insulin-resistant type II diabetic persons since there is a decreased demand for insulin; once weight loss is achieved, the person's insulin sensitivity often improves. However, very low carbohydrate diets are not recommended for long-term use because they restrict intake of fruits, vegetables, legumes, and dairy products, all of which provide essential nutrients.

25.6.5 Kwashiorkor and Marasmus

Kwashiorkor is a form of protein–calorie malnutrition that is caused by dietary protein deficiency and is often exacerbated by infection. The classic presentation, particularly in poorer countries, is a young child who has been weaned to an adult diet that lacks sufficient protein to sustain healthy growth. The characteristics of kwashiorkor include growth failure, edema, fatty liver, and "flaky paint" patches of skin. Because of the low protein intake, there is a deficiency of amino acids for synthesis of serum albumin and other plasma proteins, resulting in edema and the characteristic swollen abdomen and limbs. The situation is made worse by the availability of ample dietary carbohydrates, which stimulate insulin secretion and thus inhibit mobilization of amino acids from skeletal muscle. This dietary carbohydrate also provides substrate for fatty acid synthesis, which in the absence of adequate protein synthesis results in fatty liver and hepatomegaly.

The clinical manifestation of a diet deficient in both protein and energy is marasmus, which results in severe muscle wasting and marked growth retardation. Marasmus is the form of malnutrition that occurs when an infant does not receive adequate breast milk or formula. The factors that determine whether an older child develops kwashiorkor or marasmus are complex and not fully elucidated.

25.6.6 Hypercatabolic States

Sepsis, trauma, and burns result in hypercatabolic states, characterized by markedly increased fuel consumption; a negative nitrogen balance in which excretion of nitrogen—mostly as urea—exceeds nitrogen intake; fat mobilization; and marked catabolism of muscle proteins. Hydrocortisone is the main mediator of these changes. Hyperglycemia is a common finding in hypercatabolic states because, even though the insulin level may not be depressed, the metabolic effects of insulin are overcome by increases in the serum levels of the insulin-counterregulatory hormones.

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